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POSTERS

Table of Contents

Abstracts submitted to the 41st FEBS Congress, which was planned for Kuşadası, Turkey from 3rd to 8th September 2016, and accepted by the Congress Organizing Committee are published in this Special Issue of *The FEBS Journal*. Unfortunately, the Congress was cancelled by FEBS after the excellent scientific programme was compromised by an insufficient number of confirmed speakers, and so the authors of these abstracts were not able to present their work at the event*. Late-breaking abstracts and abstracts withdrawn after Congress cancellation are not included in this issue.

About these abstracts

Abstracts submitted to the Congress are **not peer-reviewed**. In addition, abstracts are published as submitted and are not copyedited prior to publication.

We are unable to make **corrections of any kind** to the abstracts once they are published.

Indexing

Abstracts published in *The FEBS Journal* Special Issue for the 41st FEBS Congress will be included individually in the Conference Proceedings Citation Index published by Web of Science.

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* An optional closed online presentation opportunity of short duration on the Congress website was offered after Congress cancellation and may be taken up by some abstract authors.

** Each poster has been given a unique number beginning with the letter P; the next part relates to the session in which the poster will be presented.

01.01	DNA replication and recombination: Novel aspects
01.02	RNA biology, biogenesis and processing
01.03	MicroRNAs and noncoding RNAs
02.01	Nuclear architecture
02.02	Proteins in action
02.03	Autophagy: Regulation mechanisms
02.07	Extracellular matrix and metalloproteinases
02.08	Plant biochemistry and molecular biology
02.09	Developmental biology
02.10	Cell cycle and circadian clocks
03.01	Systems biology
03.03.2	Computational biology
03.03.3	Structural biology: Membrane complexes and supercomplexes
03.04	Single molecule techniques - Applications in biology
04.01	Host–pathogen interactions
04.02	Mechanisms of pro-inflammatory diseases
04.03	Biochemical mechanisms in tolerance and autoimmunity
04.04	Molecular mechanisms of inflammation
05.01	DNA repair and cancer
05.02	Epigenetics and cancer
05.03	Stem cells and cancer
06.01	Novel signaling pathways controlling the cardiac function
06.02	Cardiac regeneration: Programming human heart cells
07.01	Developments in biomaterials and tissue engineering
08.01	Functional genomics and proteomics
08.02	Personalized medicine
09.01	New optical methods for studying neuronal structure and function
09.02	Mechanism of neurodegenerative diseases
09.03	Aging
09.04	Chemical and biochemical aspects of oxidative stress
EDU	Education, Training, and Career Planning in Molecular Life Sciences
MISC	Miscellaneous

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POSTERS

Sunday 4 September 12:30–14:30

DNA replication and recombination: Novel aspects

P-01.01.1-001
The role of the N363S polymorphism of the human glucocorticoid receptor gene (NR3C1) in Turkish patients with major depressive disorder (MDD)

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Glucocorticoid receptor (GR) is one of the involved receptors and its gene has been recognized as a candidate gene for major depressive disorder and bipolar disorder. The involvement of the GR gene (NR3C1) locus on human chromosome 5q31-q32. The N363S (rs6195) is located within exon 2 and changes the second base of codon 363, leading to asparagine to serine substitution in the transactivation domain of GR. In this study, we aimed to examine the role of *NR3C1* N363S polymorphisms in genetic susceptibility to MDD development in a Turkish population. A total of 100 consecutive unrelated adult patients with documented medical records of MDD were from outpatient Psychiatry Clinic in Gaziosmanpaşa University Research and Training Hospital, Tokat, Turkey by referral from the treating physician, after obtaining initial verbal consent to participate in the study. In addition, 100 control subjects from the same area as the patients, and comprising blood donors, healthy volunteers were enrolled this study. DNA was isolated from peripheral blood samples and the N363S variant was screened by the RT-PCR technique. 99 out of the 100 MDD patients were found to be AA genotype at the N363S (AA genotype frequency 0.99; A-allele frequency 0.995). Also, 1 out of the 100 MDD patients was found to be AG genotype (AG genotype frequency 0.01; G-allele frequency 0.005). No homozygote for the rare G-allele was seen. Significantly more frequent occurrence of allele-A in N363S polymorphism was observed in the group of the patients with MDD in comparison with the control group (OR: 4.061, 95% CI: 0.449–36.660, χ^2: 1.823, p: 0.177). For genotype AG versus AA, no significant correlation was demonstrated between patients and the control group with respect to the investigated SNP (OR: 0.242, 95% CI: 0.027–2.208, χ^2: 1.846, p: 0.1742). This study was supported by the Gaziosmanpaşa University Scientific Research Fund (GOÜ BAP2013/8).

P-01.01.1-002
Generation of targeted insertion in the Klf5 gene of mouse myoblasts (C2C12 cells) using CRISPR/Cas9 system

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Klf5 is a zinc finger transcription factor that is expressed in early embryonic stem cells as well as adult somatic epithelial tissue. The function of Klf5 is diverging in a context dependent manner in cells and tissues. During development, Klf5 has a role in the maintenance of undifferentiated state in embryonic stem cells. Moreover, Klf5 is also acting on cellular processes such as cell migration, apoptosis, inflammation, angiogenesis and differentiation. Previous studies showed a novel role for Klf5 as a regulator of proliferation and differentiation in skeletal muscle stem cells. Detecting Klf5 at the protein level harbored technical obstacles. Commercially available antibodies exhibited low affinity, low specificity and failed to recognize post-translationally modified forms that are directly relevant to the function. Thus, these obstacles prevent further functional protein studies such as western blots, protein co-immunoprecipitation and chromatin immunoprecipitation (ChIP) assays. Therefore, we used CRISPR/Cas system to establish a stable cell line which carry V5 epitope tag into the N-terminal of Klf5 gene. Insertion into the target side of Klf5 gene via CRISPR-Cas9 system provided an opportunity to overwhelm the above mentioned obstacles. V5 epitope tag would not interfere with the function of the Klf5 and also enable us to recognize endogenous Klf5 via anti-V5 antibody in the mouse myoblast cell lines (C2C12). We confirmed the targeted insertion into the exon 1 of the Klf5 gene both at the DNA and protein levels.

P-01.01.1-003
Cloning and expression of sumo fusion human carbonic anhydrase II

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Carbonic anhydrases (CAs, EC 4.2.1.1) are zinc metalloenzymes that catalyze reversible hydration of CO_2. The enzymes have five genetically distinct classes in organisms all over the phylogenetic tree (α-, β-, γ-, δ-, and ζ-families). The catalytic CAs are included in various physiological reactions, including respiration, pH regulation, Na^+ retention, calcification, tumorigenesis, electrolyte secretion, gluconeogenesis, ureagenesis and lipogenesis. Rapid, efficient and cost-effective protein expression and purification are required for the production of the therapeutic proteins.

The aim of the research is to high amount express and purify of the carbonic anhydrase II by using SUMO-fusion expression system. In this study experimentally the recombinant gene for human carbonic anhydrase II was cloned into a pET-SUMO plasmid vector with an kanamycin-resistance gene and expressed in Escherichia coli BL21 (DE3) cells. The culture was grown at 37°C in the presence of kanamycin (50 μg/ml) until it reached an OD_{600} of 0.5. Isopropyl-β-D thiogalactopyranoside (IPTG) was added into the culture at a final concentration of 1 mM for protein expression. After expression, the SUMO moiety was cleaved by the highly specific and active SUMO (ULP-1) protease at the carboxyl terminal, producing a native protein. SDS-PAGE and Western blot analyses illustrated that the molecular mass of human CA II enzyme was determined ~30 kDa and after the enzyme purification with Probond™ affinity column. Activity of the fusion enzyme was determined as ≥7000 EU/mg with esterase activity. These data suggest that spesific activity is fairly well than the others.

P-01.01.1-004
Role of C-terminal domain dynamics in RecA protein activities

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The conformational dynamics of structural domains plays an important role in functioning of many proteins. The RecA proteins from *E. coli* are known to be the central catalyst of homologous recombination and repair in bacteria. It forms a helical filament on ssDNA capable to bind homologous dsDNA and catalysis of the exchange of the complementary strand. Significant mobility if its C-terminal domain has been observed experimentally by cryo-electron microscopy. However its potential significance for RecA protein activities still remains unclear.

In this work we investigated this question by construction of a mutant RecA protein with artificial disulfide bridge between central and C-terminal domains. The wild type protein has no disulfide bonds, and therefore its native mobility can be restored *in vitro*, by addition of β-mercaptoethanol.

Our data suggest that the S-S bridge decreases both the rate of ATP hydrolysis *in vitro* and the *E. coli* resistance to UV *in vivo*. Thus, our experimental results indicate that the flexibility of the C-terminal domain significantly affects recombination activity of RecA protein *in vivo* and *in vitro*.

P-01.01.1-005
MRC1 regulates the stability of S-phase checkpoint arrest

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Hydroxiurea (HU) is an inhibitor of Ribonucleotide reductase – the enzyme that catalyzes the process of free dNTPs synthesis in living cells. Treating cells with HU causes diminishment of the nucleotide pool. As a result, single-stranded DNA regions are generated, which leads to S-phase checkpoint activation. The progression of replication forks is blocked and the completion of DNA replication is prevented. This results in S-phase cell arrest.

Nevertheless, our results demonstrate that after prolonged HU treatment, the *Saccharomyces cerevisiae* cells seam to escape the arrest and continue the progression of their cell cycle. We show that when cells re-enter the cell cycle, Mrc1, but not Ctf4 is detached from chromatin. Our data also shows that meanwhile, Rad53 checkpoint activity is diminished in order to allow S-phase checkpoint escape and completion of the cell cycle. Moreover, cells not only continue the cell cycle, but steadily surmount in the presence of HU. All this data indicates that cells have made the decision to compromise S-phase checkpoint and to adapt to the novel environmental conditions in order to survive.

As both Mrc1 and Ctf4 are known to be responsible for polymerase and helicase harmonization during replicative arrest, our data indicates that Mrc1 has a more specific role in the process of adaptation. Our data demonstrates that Mrc1 is a leading protein to regulate the stability of S-phase checkpoint arrested replication forks.

Sunday 4 September 12:30–14:30

Nuclear architecture

P-02.01.1-001
Proteins with Zinc finger associated domain (ZAD) are involved in organisation of chromatin architecture

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Zinc finger domain is the most common DNA binding domain in metazoa. Almost 100 Drosophila proteins with C2H2 zinc fingers also have Zinc finger associated domain (ZAD). Several proteins with ZAD (Zw5, Pita and ZIPIC) were found to interact with CP190 and act as insulator proteins. For some of the ZAD-containing proteins (for example, Weekle and Grauzone) it was shown that their ZAD domains can form dimers with each other. The ability of these proteins to dimerize appears to be especially important in the light of the model suggesting that DNA-binding insulator proteins can support genome looping and organization of chromatin structure via interaction with each other.

In this work we aimed to understand the role that ZAD-mediated protein-protein interactions play in maintenance of DNA loops, focusing on proteins: Zw5, Pita, ZIPIC and CG6808.

First, we performed co-precipitation and yeast two hybrid assays to confirm dimerization of isolated ZADs *in vitro*. We observed that only ZADs from the same protein can specifically interact with each other (homodimerization) and they are unable to interact with ZADs from different proteins (heterodimerization). We confirmed homo- but not heterodimerization of ZADs *in vivo* with coimmunoprecipitation experiments in S2 cells.

Furthermore, we found that ZAD proteins can support long-distance interactions in transgenic constructs in flies. Using model system with CG6808 protein, we demonstrated that ZAD is essential for these interactions. Proteins without ZAD cannot maintain loop formation.

Finally, analysis of ChIP-seq experiments for Zw5, Pita, ZIPIC and CG6808 revealed that binding sites of ZAD proteins often overlap with regions of inter-chromosomal contacts known from Hi-C experiments.

We conclude that ZAD-containing proteins can support long-distance genomic interactions and dimerization of ZADs is necessary for these interactions.

This study was supported by the Russian Science Foundation (project №14–24–00166).

Sunday 4 September 12:30–14:30

Developmental biology

P-02.09.1-001
Effects of haloperidol and clozapine treatment on plasma concentration of thyroid hormones in Rats

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Over the years a large body of clinical knowledge has accumulated on pharmacological effects of drugs on thyroid function. Antipsychotics are administered over long periods in humans; therefore their possible adverse side effects should be taken into consideration. The aim of this study is to evaluate the effects of haloperidol and clozapine on plasma T3 and T4 concentrations in adult male Wistar rats. Fifty rats aged between 14 and 15 weeks (270 ± 30 g) were divided into five groups (n = 10 in each group), and drugs were administered each day intraperitoneally (IP) for 28 days. The first group was a sham group. The other four groups were considered as low and high treatment doses of the drugs. After a one-week habituation period, animals was administered haloperidol (0.05 mg/kg, n = 10 and 2 mg/kg, n = 10) and clozapine (0.5 mg/kg, n = 10 and 20 mg/kg, n = 10). The rats were anesthetized with ether, and bloods were collected by direct cardiac puncture 24 hours after the last injection. The T3 and T4 plasma concentration levels were analyzed with chemiluminescent immunoassay. Statistical analysis was performed with IBM SPSS v20.0. Kruskal-Wallis and Bonferroni tests were used. T4 plasma concentration levels significantly differ between sham (median=8.25 mg/kg) and haloperidol (2 mg/kg) (median=7. 00 mg/kg), haloperidol (0.05 mg/kg) (median=7.70 mg/kg) and clozapine (20 mg/kg) (median=8.32 mg/kg), haloperidol (2 mg/kg) (median= 7.00 mg/kg) and clozapine (20 mg/kg) (median= 8.32 mg/kg) groups ($p < 0.05$). However, no significant differences between the groups regarding to T3 plasma levels were observed. In conclusion, haloperidol and clozapine increased the T4 plasma concentrations, but didn't have any significant effect on T3 plasma concentrations.

P-02.09.1-003
Isolation of lipase producing strains of bacillus obtained from olive wastewater and screening for substrate specificity

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Lipases are part of the family of hydrolases that act on carboxylic ester bonds. The physiologic role of lipases is to hydrolyze triglycerides into diglycerides, monoglycerides, fatty acids, and glycerol. This versatility makes lipases the enzymes of choice for potential applications in the food, detergent, pharmaceutical, leather, textile, cosmetic, and paper industries. Limitations of the industrial use of these enzymes have mainly been owing to their high production costs, which may be overcome by molecular technologies, enabling the production of these enzymes at high levels and in a virtually purified form.

In this work, wastewater samples of an olive factory from Yusufeli (Artvin, Turkey) were collected carefully. After a centrifugation period of samples, supernatants were applied to a 0.45 μm filter and incubated on LB agar medium for 24 hours. Based on differencies of colony morphologies, 13 isolates were selected and purified for identification. 16S rDNA analyses revealed that the isolates belong to the genus *Bacillus*. The isolates named as, *Bacillus* sp. L1, *Bacillus* sp. L2, *Bacillus* sp. L3, *Bacillus* sp. L5, *Bacillus* sp. L6, *Bacillus* sp. L7, *Bacillus* sp. L8, *Bacillus* sp. L9, *Bacillus* sp. L10, *Bacillus* sp. L11, *Bacillus* sp. L12, *Bacillus* sp. L13, *Bacillus* sp. L15.

Lipase activity assay was carried out by Rhodamine B. All of the 13 strains exhibited lipase activity. For determining the substrat specificity of isolates, 5 different substrates were used; 4-nitrophenyl-butyrate, 4-nitrophenyl-caprylate, 4-nitrophenyl-laurate, 4-nitrophenyl-myristate, 4-nitrophenyl-palmitate. Results were measured spectrophotometrically at 405 nm. All of the strains hydrolyzed 4-nitrophenyl-butirat, while there was no activity with 4-nitrophenyl-palmitate. *Bacillus* sp. L3 was the most efficient strain that hydrolyzed all of the substrates. The gene encoding for lipase of *Bacillus* sp. L3 will be cloned and expressed for more analyses and industrial applications.

P-02.09.1-004
Some quantitative aspects of hair follicle layers differentiation

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In the course of stable hair growth the differentiation of hair bulb cambium cells to several layers with dissimilar cytochemistry and morphology takes place. This means the activation of different genes in the cells of different layers. Depending upon the hair diameter some layers may be absent (medulla in the thin hairs). The hair diameters of the Carpet sheep breeds vary widely even within the same square mm of the skin. We compared the different layers thicknesses proportions for the follicles with varying hair diameters. The follicle layers were measured on 155 microphotos of transverse histological sections of the follicles made under the standard magnification. All follicles belonged to the same skin biopsy. The measurements were made at the levels just below the fissure separating the hair and inner root sheath appeared. The empirical regressions of the layers thicknesses and of ratios of different layers against hair diameters were counted. The computer model was made on the basis of these regressions which allowed to obtain the absolute parameters of the layers as well as ratios of these parameters for every chosen hair diameter. Using this model we found an essential trend in changing the proportions in relative layers dimensions as we choose the follicles with more and more thick hair. When we change the follicles with 30 mcm hair diameter for those with the hair diameter 100 mcm the ratio of hair medulla diameter to hair diameter increases from 0.07 to 0.76. The ratio of hair diameter to the diameter of inner root sheath increased from 0.70 to 0.84. It means that the thicker is the hair the higher proportion of cells produced by cambium are spent to build innermost layers (medulla layer within the hair or hair within the complex – inner root sheath + hair). These data may throw some light on positional information mechanism of layers differentiation.

P-02.09.1-005
Optimization of Klebsiella pneumonia GST for pulp bleaching

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Lignin is a heterogeneous polymer that constitutes 30% of woody plant cell walls. Microorganisms that degrade lignin are fungi, actinomycetes and to a lesser extent, bacteria. In case of industrial applications, the use of fungi is not feasible due to the structural hindrance caused by fungal filaments, requirement of particular culture conditions such as humidity, aeration which are not compatible with industrial processing environments. Bacteria are worthy of being studied for their ligninolytic potential due to their immense environmental adaptability. Environmental concerns and increasingly stringent emissions standards have led the pulp and paper industry to devise ways to decrease the level of chlorinated lignin residues in its effluents through both production process changes and improved treatment technologies. Bleaching with the enzymes is the most promising because the enzymes may be very efficient, and can be used under industrial conditions.

The main objective of this study was to investigate the adequency of *Klebsiella pnuemonia* GST (Glutathione-S-transferase) pretreatment for bleaching of calabrian pine kraft pulp. For this purpose the following conditions were investigated: enzyme loadings from 3 to 10 U/g pulp basis and the consistecy of the pulp was between 3 and 10%. Enzyme at the desired concentration was added to the pulp and the mixture was incubated at 40°C for 2 hours. After the enzymatic pretreatment to determine the optimum conditions the kappa number of all reactions were analyzed according to TAPPI standarts.

As a result of this study we determine the optimum conditons as 5% pulp consistecy, 8U/g enzyme for pulp treatment. After the enzymatic treatment carried out under optimum conditions we are planning to submit a short bleaching sequence and analyze for physical properties such as viscosity and brightness. Owing to this bleacing sequence we are going to able to compare the enzymatic and chemical treatments of pulp in bleaching prosess.

P-02.09.1-006
Biochemical characterization of lipase from Bacillus subtilis strain A10 from olive waste water

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Lipases (triacylglycerol acyl hydrolases, EC 3.1.1.3) are regarded as mild and environment-friendly biocatalysts for triacylglycerols hydrolysis. In addition to this hydrolytic reaction, they also catalyze reverse reactions of esterification, transesterification, and interesterification in non-aqueous environments. Substrate, stereo-, regio- and enantio- specificities, and chiral selectivity are certain unique attributes of lipases that make them industrially attractive. These properties are often exploited in the manufacturing of detergent formulations, synthesis of fine chemicals, useful esters and peptides, food processing, paper manufacturing, degreasing of leather as well as in bioremediation.

In this study, lipase from *Bacillus subtilis* strain A10 is partially purified and characterized. *Bacillus subtilis* strain A10 is isolated from olive factory from Soke (Aydin, Turkey) and identified with 16s rRNA analysis. Lipase activity is screened on petri supplemented with Rhodamine B. Bacteria was grown in LB medium supplemented with 1% olive oil (vol/vol) for 48 hour at 37°C. After incubation, cells were harvested by centrifugation at 11,000 rpm for 5 minutes, resuspended in 50 mM Tris–HCl (pH 8.0) buffer, followed by sonication with Sartorius Labsonic M to release intracellular proteins. Q-sepharose is used as ion-exchange column chromatography for lipase purification. Effects of temperature on activity and stability were determined spectrophotometrically using p-nitrophenyl laurate as the substrate. Effects of pH on activity and stability were also determined. The effects of various metal ions and other reagents on the hydrolytic activity were assayed at 37°C.

The enzyme was active and stable in the broad pH range of 5.0–10.0 and temperature range of 24–50°C. *Bacillus subtilis* strain A10 have high lipolytic activity. After cloning this enzyme to an expression vector and detailed characterization, this may suggests its usefulness in industrial applications.

P-02.09.1-007
Investigation of PIN1 as a nuclear factor one binding partner

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The Nuclear Factor One (NFI) proteins are important regulators of gene expression in the developing embryo and in adult stem cell niches. This transcription factor family has four members: NFIA, NFIB, NFIC, and NFIX. NFI proteins bind a consensus sequence on gene regulatory regions as homo or heterodimers. Each member of NFI family has a highly conserved N-terminal DNA binding and dimerization domain and a diverse proline rich C-terminal transcriptional activation/repression domain. As knockouts of NFI genes display distinct developmental phenotypes, we hypothesized that specificity of NFI protein function may arise from their interactions with binding partners. A yeast-two hybrid screen identified protein interacting with never in mitosis A1 (PIN1) as a potential NFIB interactor. PIN1 is a ubiquitously expressed protein that specifically recognizes and binds to a phospho-serine or a phospho-threonine followed by a proline (pS/pT-P motif), and catalyzes isomerization of peptidyl-prolyl bonds. Interestingly, both N-terminal and C-terminal domains of four NFI isoforms contain several conserved putative pS/pT-P motifs and some of these are reportedly phosphorylated. We looked for NFI PIN1 interactions *in vitro* by GST-pulldown and co-immunoprecipitation assays. While GST-PIN1 fusion protein interacts with all of four NFI isoforms, it binds NFIB most strongly, NFIA and NFIC moderately, NFIX most weakly. Moreover, deletion of the C-terminal domain leads to loss of NFI affinity for PIN1 implicating this domain in NFI-PIN1 interactions. Co-immunoprecipitation assays where we co-expressed various epitope tagged NFI and PIN1 proteins in HEK 293T cells showed that PIN1 precipitates NFIB, as well as other NFI isoforms and NFIB can, in turn, precipitate PIN1. We are currently carrying out site-directed mutagenesis on NFIB to identify the specific residues that PIN1 recognizes. We will further explore if this interaction regulates NFI function during embryonic development.

P-02.09.1-008
Protein degradation in Atlantic salmon *Salmo salar* L. skeletal muscles during smoltification

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Atlantic salmon *Salmo salar* L. parr smoltification involves morphological, behavioral, physiological and biochemical changes

that pre-adapt migrating fish to the life in seawater. Among others, smoltification induces intense growth of fish that enter the ocean at a size where risk of predation is significantly reduced. Skeletal muscle growth depends on a tightly controlled balance between protein synthesis and degradation. Protein synthesis driven by hormone regulation is well studied in smoltified Atlantic salmon; while less is known on protein degradation occurring via a number of pathways including cytosolic ubiquitin-proteasome system and calcium dependent calpains. The aim of this study was to compare calpain and proteasome enzymatic activities in the skeletal muscles of *S. salar* parr, pre-smolts and smolts.

Calpain and proteasome activities were determined by casein or Suc-LLVY-AMC hydrolysis in the skeletal muscles of *S. salar* from Indera river (Kola peninsula, Russia).

Our results demonstrated the significant differences in studied protease activity levels between parr and smolts. Calpain and proteasome activities in *S. salar* smolt muscles showed a significant drop compared with that of parr. The negative correlation between proteases activity levels in the muscle tissue and overall fish growth rate was shown. So, our data indicated life stage specificity in skeletal muscle protein degradation capacity in migrating fish. We suppose that intense muscle growth in *S. salar* pre-smolts is supported by various mechanisms including accelerated muscle protein accretion through the reduction of protease activities.

Obtained results enhance our knowledge in the mechanisms of Atlantic salmon smoltification. The work was supported by the Russian Scientific Foundation, project no. 14-24-00102.

P-02.09.1-009
The sociodemographic characteristics of the pregnant women who double and triple prenatal screening test

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Double and triple prenatal screening tests which are applicable during first and second trimesters of pregnancy predict existent abnormalities at early stage. The aim of this study is to investigate the relationship between positive results of double and triple tests, further confirmatory tests during prenatal phase, postnatal status of babies and maternal age.

In this study, double and triple test results of pregnant women who were admitted to Meram Faculty of Medicine during 2009–2013 period were scanned from archive and test results indicating risk were detected. From these results, those which were above cut-off values for Down syndrome, Trisomy 18, open spina bifida were determined. A questionnaire was carried out with voluntary participants by reaching to these individuals. Positive-negative result ratio of all double and triple test results and sociodemographic features such as age, occupation, presence of consanguineous marriage were investigated. All data from archive and answers from survey questions were assessed statistically.

Participants of the study were 18 to 46 years old and their average age was 29.89 $\pm$ 6.56. 219 ofthem (69.30%) were under 35 years of age whereas 97 of them (30.70%) were above 35 years of age. Number of pregnancies were scaling between 1 to 13 with an average of 2.56 $\pm$ 1.56. 207 of 316 mothers (65.50%) were not undergone amniocentesis, whereas 6 babies with chromosomal abnormalities were detected among 109 mothers who were undergone amniocentesis.

In conclusion, there may be regional, sociological and such that reasons for those who were not undergone amniocentesis despite positive double and triple test results. 6 (5.50%) chromosomal abnormalities were detected among pregnancies with increased risk assessment with positive double and triple results.

P-02.09.1-010
The effects of oil on the growth and development of amphibians

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Currently, the pollution of ecosystems by oil and oil products is increasing everywhere. The oil gets into water and ground during oil production, transportation and accidents. As a result, terrestrial animals and hydrobionts are exposed to oil contamination. Thus, populations of animals decline. It can be assumed that the most sensitive to the effects of pollutants are animals in early stages of development. Amphibians have established themselves as the most convenient bioindicator species. Since lake frog *(Rana ridibunda)* and green toad *(Bufo viridis)* are the bioindicator species in Kazakhstan, the study of the effects of oil on their larvae was carried out.

We used water-soluble fraction of the oil from Zhanazhol field (Aktobe region) in our test. The larvae of control group were kept in pure water, and larvae of test groups – in aquariums with 0.05, 0.5 and 1% concentrations of the oil fractions. The concentrations were chosen in accordance with the level of pollution of Kazakhstan's water bodies with oil. Mortality of larvae, morphometric parameters and morphogenesis were studied.

It was found that high mortality of larvae is the most visible reaction when exposed to oil. This indicator rose noticeably depending on the doses (0.05, 0.5% and 1%) in both species with percentages 16%, 51% and 78% in *R. ridibunda* and 22%, 61% and 83% in *B. viridis*, respectively, while in the control group it was about 10%. Furthermore, delayed larval development was detected. Thus, the larvae from the control and 0.05% oil group reached Gosner stage (Gs) 36, tadpoles from 0.5% and 1% groups were at Gs-32 and Gs-29, respectively, by the 30th day of life. Moreover, behavioral abnormalities (sluggish movements) and decreased sensitivity to mechanical stress (touch) were observed under the influence of high concentrations of oil fractions.

Thus, oil in low concentrations alters the growth and development of tadpoles of anurans, and causes their increased mortality in high concentrations.

P-02.09.1-011
Effect of catechin loaded PLGA nanoparticles on glioma cell line

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Catechin is an important plant polyphenolic compound. Catechin has been observed to have major biological effects including antioxidant, anti-mutagenic, anti-carcinogenic, anti-viral. But, Catechin is susceptible to change in conditions such as pH, temperature,light therefore It quickly undergoes degredation resulting in loss of activity. Becasue nanoparticles can increase solubility and enhance its absorption. Nanoparticular carrier system has been seen that to be one of the most suitable. In this study, Poly Lactide Co Glycolic Acid as polymer was preferred for the preparation of nanoparticle due to its low toxicity, biodegradability and biocompatibility.

Catechin, Poly Lactide Co Glycolic Acid as polymer, Dicholorometan as organic solvent and pure water were used for nanoparticle synthesis. Due to high hydrophilic property of its, Catechin-loaded PLGA nanoparticles were done with double emulsion solvent evaporation method (w/o/w). Particle synthesis was made through sonicator. Finally, the mean particle size, the zeta potential of particles was determined by a dynamic light scattering technique.

The aim of study was to produce Catechin-loaded PLGA nanoparticles and research its effect on glioma cell line. As a result of, changed parameters weren't significant effect on particle size while these parameters were significant effect on entrapment efficiency. Due to the controlled release speficitaion of nanoparticular carrier system, encapsulated Catechin showed longer antioxidant activity prolong time than free Catechin.

Catechin loaded PLGA nanoparticles were successfully synthesized. However, A low entrapment efficiency was obtained with Catechin. With the results obtained, Our data indicate that nanoparticles can prevent Catechin against the oxidation/degradation and also be a basic strategy for both enhancing its bioavailability. Effect of encapsulated catechin on glioma cell line still has been conducted.

P-02.09.1-012
Decreased level of H1t histone variant in testicular biopsies of infertile men with spermatogenesis failure

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Histone H1t is a linker histone which binds to DNA and contribute in chromatin condensation as well as regulation of specific genes through spermatogenesis. Replacement of this histone H1 subtype and hyperacetylation of histone H4 tail, facilitate the replacement of histones with sperm chromatin condensing proteins of TNPs and PRMs.

Ethical approval and informed patient consent was gained from 12 infertile men referred to Royan Institute. Testicular biopsies were collected from patients through assisted reproductive techniques (ART) procedure. Based on pathological results samples were classified into the following three subgroups: obstructive azoospermia (as positive control), complete maturation arrest and Sertoli cell only syndrome (negative control). Chromatin of tissues evaluated for presence/absence of histone H1t protein in regulatory regions of *TNPs* and *PRMs* genes using ChIP-Real Time PCR.

Results showed lower incorporation of H1t protein on regulatory regions of *TNPs* and *PRMs* genes in two spermatogenic failure group versus positive control.

In this study, it can be concluded that the decreased levels of H1t histone variant in testis tissues and failure in chromatin condensation have significant association with male infertility.

P-02.09.1-013
Serum Dickkopf-1 levels in obese children and adolescents

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Introduction: The basic interactions between obesity and bone is complex and not well known. Recent research findings suggest that obesity is detrimental to bone health despite potential positive effects of mechanical loading conferred by increased body mass on bones. The Wnt/β-catenin pathway is essential for normal osteogenesis. Serum Dickkopf-1 (Dkk-1) is one of the most important inhibitors of the Wnt//β-catenin pathway.

The aim of this study was to investigate the serum Dkk-1 levels in obese and non-obese children and adolescents.

Materials and Methods: The study included 30 obese children and adolescents (14 males and 16 females) aged from 7 to 17 years and 30 healthy normal-weight controls (13 males and 17 females) aged from 6 to 17 years. Serum Dkk-1 levels were measured by ELISA method using commercially available kit.

Results: Body mass index of the obese children was significantly higher than that of non-obese children ($p = 0.000$). However, there was no significant difference between DKK-1 levels of the groups. (These results are preliminary and the study is continuing).

Discussion and Conclusion: Our result showed that serum Dkk-1 levels were not changed in obese children and adolescents.

P-02.09.1-014
Transcriptional regulation of CDO by nuclear factor one proteins

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Nuclear Factor One (NFI) transcription factors play important roles in regulation of central nervous system development. Three of the four members of NFI family, *NFIA, NFIB,* and *NFIX* are expressed in neural progenitors, as well as neurons and glia in the embryo. Inactivation of these genes in mice show that they function in development of neocortex and hippocampus in the forebrain, cerebellum, spinal cord and precerebellar nuclei of the hindbrain, regulating neurogenesis, gliogenesis, as well as neuronal migration, axonal outgrowth and guidance. All three neural specific NFIs are expressed in precerebellar neuroprogenitors, however, only deletion of *NFIB* leads to a delay in development of precerebellar neurons. Investigation of misregulated genes in $NFIB^{-/-}$ precerebellar neuroprogenitors identified *cell adhesion associated, oncogene regulated* (*CDO*) as a potential downstream target of NFIB. Interestingly, this gene has been reported to be upregulated in $NFIA^{-/-}$ hippocampus as well. CDO, a cell surface glycoprotein of the Ig superfamily, has been found to regulate neurogenesis *in vivo*, is highly expressed in the developing brain and can induce neural differentiation by promoting heterodimerization of basic helix loop helix transcription factors with E proteins. Bioinformatic analysis of the 5 kb human *CDO* promoter region identified five NFI binding sites: one cluster in the first 1 kb region, another in the 3.5 kb upstream region. Electrophoretic mobility shift and supershift assays showed that NFIB binds to all five sites. Furthermore, NFIB, along with the other neural NFIs, inhibits the proximal *CDO* promoter driven luciferase activity by up to 85% in HEK293T cells. Preliminary data indicate that NFIs bind to sites in both clusters in human neural stem cells (hNSCs) suggesting that these sites are functional *in vivo*. We are currently investigating this possibility through NFI overexpression and silencing experiments that will examine regulation of *CDO* in hNSCs.

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P-02.09.1-015
In vivo imaging and safety evaluation of Lys-graft-p (HEMA) nanoparticle as drug delivery system

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Nanoparticles are widely used for several biological applications such as targeted drug delivery, imaging, and biosensors. The aim of the study was to determine safety and biodistrubition of Lys-graft-p (HEMA) nanoparticle.

Surfactant free emulsion polymerization and grafting to lysine with p (HEMA) polymer was carried out. Cytotoxicity of nanoparticle was determined by the WST-1 assay on HEK 293 (human embryonic kidney) cell line. Genotoxicity was evaluated by Ames test in *S.typhimurium* TA98, TA100, TA 1535 and TA 1537 strains. *In vivo* single dose toxicity test, the up and down procedures was used according to the OECD Guideline 425. For hemolysis test, the hemoglobin released from damaged red blood cells by exposure to nanoparticles was determined under the ASTM standard practice F 756-00. Imaging of nanoparticle for biodistribution was acquired using the *In vivo* Imaging System (IVIS). The protocol was approved by the Ege University, Local Ethical Committee of Animal Experiment (25.11.2015, 2015-090).

According to our test results, lys-graft-p (HEMA) was showed no cytotoxicity, genotoxicity and hemolytic effect. Also no lethality was observed with oral doses of nanoparticle. It is determined that nanoparticle was not hemolytic according to hemolysis test. The *in vivo* biodistribution of the nanoparticle was recorded at specific time points with IVIS imaging system. The nanoparticle doses did not cause any death during the treatment.

Limited studies in this subject show that need for many studies on polymer sciences in particular the toxicity, effects and especially biodistribution of polymeric nanoparticles. These results support to safety and biocompatibility of test materials. This study has been an important step in this research area. In conclusion, this polymeric nanoparticle can be used as drug delivery systems.

P-02.09.1-016
The DRD2/ANKK1 and BDNF gene variants in early-onset schizophrenia

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Schizophrenia is a neurodevelopmental disorder in which hallucinations, delusions, delusional thoughts, speech disorders and motor abnormalities are observed. If symptoms start before the age of 18, it is called early-onset schizophrenia (EOS). Dopamine receptors (DRD) are G-protein coupled receptors functioning in memory, learning, motivation, cognition, and neuroendocrine signaling. Brain-derived neurotrophic factor (BDNF) is a growth factor supporting neuronal survival, new neuron growth and differentiation. The aim of this study is to investigate BDNF and DRD2/ANKK1 gene variants in EOS development.

In this study, 111 EOS patients and 138 healthy controls were used. Genomic DNA extraction was performed from peripheral blood leukocytes. DRD2/ANKK1 Taq1A (rs1800497) and BDNF Val66Met (rs6265) polymorphisms were determined by real-time polymerase chain reaction (RT-PCR). Positive and Negative Syndrome Scale (PANSS) was used to determine EOS severity.

For DRD2/ANKK1 rs1800497 polymorphism, there was a significant difference in the genotype frequencies between patients and controls for the co-dominant model ($p = 0.05$, OR = 1.723; 95% CI: 0.996–2.98). However, no significant relationship was observed in the genotype frequencies of BDNF Val66Met polymorphism between EOS patients and controls ($p = 0.489$).

These results indicate that, DRD2/ANKK1 rs1800497 genotypes may affect EOS development. However, BDNF Val66Met polymorphism may not be associated with EOS. Lack of association of BDNF Val66Met polymorphism may be due to limited number of patients. Our findings need to be confirmed by further studies.

P-02.09.1-017
Laccase-catalyzed decolorization of Burdirect Black Meta Konz (C.I. Direct Black 38)

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Various dyes used in the textile industry are discharged in large quantities to the receiving environment in the manufacturing process. This is the beginning of a process that is difficult to compensate for environmental and human health. Therefore, contaminated areas should be cleaned. In addition, technologies with high polluting potential should be integrated with biological approach and thereby the impurities consisting of dyes should be reduced. In this experiment; Burdirect Black Meta Konz (C.I. Direct Black 38) was intended to decolorization using laccase. Firstly, enzymatic decolorization of the dye was determined using spectrophotometry. The wavelengths of maximum absorption of Burdirect Black Meta Konz (C.I. Direct Black 38) was determined between 200 and 800 nm. Then, optimization studies have been done. For optimization studies; dye concentration, laccase activity, pH, buffer concentration, temperature, mediator effect, mediator concentration and time parameters were determined. Lastly, in optimal conditions, ATR-FTIR and GC-MS analyzes of ensuring decolorization of dye were analyzed. Decolorization of Burdirect Black Meta Konz (C.I. Direct Black 38) was performed successfully and the absence of any metobolite in the decolorization medium has been provided by ATR-FTIR and GC-MS analyzes. Assessing in terms of application, it can be easily applied by provided the reaction conditions in textile factories. Laccase is a tool of decolorization of dyes in environmental friendly process.

P-02.09.1-018
Gene expression profile of protamines in male infertility

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Introduction: During spermiogenesis, the protamine proteins play an integral role in spermatid chromatin compaction and

thus for the development of spermatids into mature sperm able to fertilize the oocyte.One of the causes of male infertility is in fact impaired sperm fertilization capacity due to sperm chromatin abnormalities and aberrant protamine replacement.Recent research has focused on protamine biology,including protamine gene and protein structure,mechanisms of protamine expression regulation and involvement of the protamines in male fertility.Various studies reported abnormal expressions of protamine (PRM) genes in sperm of infertile men.The aim of the study is to investigate the gene expression of PRM1, PRM2 and their relationship with defective spermatogenesis.

Materials and Methods: This study has been performed on 50 infertile and 3 fertile Turkish men.Total RNA was extracted from the sperm pellet using Trizol reagent.After RNA extraction and cDNA synthesis,real-time quantitative polymerase chain reaction (RT- QPCR) was used to determine the expression of PRM1 and PRM2.

Results: Distinct levels of spermatozoal PRM1 and PRM2 mRNA were found in infertile patients compared to fertile control groups.We found that the mRNA levels of PRM1 was reduced in 23 (%47), and the mRNA levels of PRM2 was reduced in 33 (%64) out of 50 infertile patients.In the current study,we found statistical significant association between the PRM1 expression and infertility ($p < 0.05$).Although PRM2 gene expression was decreased in most of infertile patients compared to fertile control groups,the differences between the groups were statistically insignificant ($p > 0.05$).

Discussion: The results of the study suggested that, the protamine expressions which were associated with spermatogenesis may be important in infertility treatment. Further studies are required in a large series of different populations to clarify the role both PRM1 and PRM2 themselves and their mRNA expression on male fertility.

P-02.09.1-019
Muscle-specific genes expression in young Atlantic salmon (*Salmo salar* L.)

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The study was conducted to characterize the processes of muscle growth in Atlantic salmon (*Salmo salar L.*) of different ages inhabited rivers Indera and Varzuga (Kola Peninsula, Russia) in summer and autumn. The expression levels of genes myosin heavy chain *MyHC*, myostatin (*MSTN*), and myogenic regulatory factors (MRF – *MyoD*, *Myf5*, *myogenin*) in white muscle were studied in salmon parr of age groups 0+, 1+, 2+ in June and October. The changes in expression levels of MRFs, *MyHC* and *MSTN* indicating the extent of hyperplasia, hypertrophy, and restriction of muscle growth at different ages of parr were revealed. The pattern of age-related changes differed between seasons. Especially, the expression of genes *MyoD*, *myogenin* and *MyHC* peaked in yearling parr (1+) in summer, that indicated the high rate of hyperplastic and hypertrophic muscle growth in yearlings (1+). At the same time, the *MSTN* expression level, the negative regulator of muscle growth, was highest in parr at age 1+. Possibly, it is the necessary regulation mechanism to attenuate hyperplasia and hypertrophy and control muscle growth. In autumn, the expression level of *MyHC* and *myogenin* were higher in salmon of age 0+ and 1+ then in 2+, indicating the higher intensity of hypertrophy in parr at both first ages in comparison to 2+. There was no differences in expression level of *MyoD*, *Myf5* and *MSTN* between age groups in autumn. Moreover, the expression levels of genes studied were lower in autumn than in summer. Thus, it indicated the decrease of muscle protein synthesis and muscle growth rate in autumn. These findings expand knowledge on age- and season-related features of muscle development in young Atlantic salmon in their natural habitat.

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P-02.09.1-020
LMP2 and LMP7 gene polymorphisms in the southeastern Anatolia population of Turkey

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Introduction: The low molecular weight polypeptide 2(*LMP2*) and low molecular weight polypeptide 7(*LMP7*) genes are located in the class II region of the Major Histocompatability Complex (MHC) locus on chromosome 6. These genes encode peptides forming the large components of the proteosome complex which degrades short-lived cytoplasmic proteins. Due to the significant role of *LMP* products antigen presentation, these genes can be accepted as strong candidates of susceptibility factors for different diseases. Population genetic studies can also contribute to understanding of the possible role of *LMP* gene polymorphisms. The aim of this study was to determine the allele and genotype frequencies of the *LMP2* and *LMP7* gene polymorphisms in Southeastern Anatolia population and to compare these with the frequencies in other populations previously reported.

Material and Methods: A total of 110 healthy and unrelated individuals participated in this study. Polymorphism analyses were done by Polymerase Chain Reaction (PCR)–Restriction Fragment Length Polymorphism (RFLP) method and allele/genotype frequencies of *LMP2* and *LMP7* genes were determined.

Results: A deviation from the Hardy-Weinberg equilibrium ($\chi 2 = 17.97, p < 0.05$) was found for the genotype distribution of *LMP2* gene polymorphism, while the *LMP7* genotypes found to be distributed ($\chi 2 = 0.43, p > 0.05$).

Discussion: Available allele frequency data for different populations were used to calculate genetic distances and to construct a neighbor-joining tree. Among the included populations, Nahuas (Mexico) population was found to have the lowest genetic distance from the Southeastern Anatolia-Turkey population.

Conclusion: It can be concluded that, more studies using different types of genetic markers are needed to clarify the filogenetic relationships of Southeastern Anatolia population with other populations and also the number of population studies on *LMP2* and *LMP7* genes should be increased to understand their effects as a genetic marker.

P-02.09.1-021
Investigation of *in vitro* antioxidant activity of quercetin loaded PLGA nanoparticles

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Antioxidant compounds in food play an important role as health-protecting factors. One of the most well known antioxidant Quercetin is a flavonoid which has great antioxidant activity that can be used against various diseases and has various

pharmacological effects. But its usage is restricted because of low aqueous solubility, poor bioavailability, poor permeability and instability in physiological medium. These problems can be overcome with encapsulation of quercetin into nanocarriers such as biodegradable PLGA based nanoparticles. Polymeric nanoparticles which have 1–1000 nm particle size and providing controlled released of biological active agent are prepared by using biodegradable and biocompatible polymers.

In this study, encapsulation of Quercetin molecules into PLGA nanoparticles was carried with using the single emulsion (w/o) solvent evaporation method. Size measurements of the obtained nanoparticles were performed by Zetasizer and their size were found 882.1; 189.25; 436.9 nm respectively. The morphological features were examined by SEM images. Antioxidant activities of Q5, Q7 ve Q10 nanoformulations have been investigated by DPPH (2.2.a-Diphenyl-1-picrylhydrazyl) and NO (nitric oxide) methods.

It is thought that the nanoparticular formulations that is developed in this study can be useful model for the other antioxidant molecules and will provide a significant contribution to the food and pharmaceutical industry.

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P-02.09.1-022
The effect of environmental enrichment on spatial memory and certain NMDARs, and 5HT2A expressions in rat pups

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Introduction: The aim of the study was to investigate the effect of environmental enrichment exposed during whole childhood on spatial learning and memory and certain NMDARs, and 5HT2A in the hippocampi of pups.

Materials and Methods: Four-weeks old, male, weaning rats were randomised into 2 groups as enviromental enrichment (EE, n = 12) and standard cage control (SCC,n = 12) groups. EEG housed in an enriched environment and SCCG were kept in standard cages for 8 weeks. Following the experiment the rats were trained and tested in the Morris Water Maze (MWM), open field test (OFT) and forced swim test (FST) in order to assess the neurobehavioural effects of EE. NR2A, NR2B, 5HT2A protein levels were analyzed by western blotting from hippocampi of rats.

Results: The positive effect of EE was seen at the learning phase in the MWM as 'latency to locate the hidden platform' between groups thoughout the 4 training days showed that EEG located the hidden platform significantly earlier than SCCG on days 2, 3 ($p = 0.006$, $p < 0.0001$). Also EEG significantly spent lower time in the outer zone of the maze on days 2, 3 which was the sign of low anxiety level ($p = 0.011$, $p = 0.049$). The parameters of OFT which indicated increased locomotion, exploration and low anxiety were significantly higher in EEG ($p < 0.05$), in FST comparison of groups showed no difference ($p > 0.05$). The levels of NR2B and 5 HT2A were significantly increased as compared to SCCG as well ($p < 0.0001$, $p = 0.003$).

Discussion & Conclusion: These findings showed that exposure to EE throughout the whole childhood causes several neurobehavioural effects like increased exploration and low anxiety. These effects may lead to improvement in speed of learning. Increase in the NR2B and 5HT2A concentrations which are the receptors that are related to learning and memory in the hippocampi accompanied these changes which may be basis of the neurobehavioural improvements or may provide contribution to positive neurobehavioural effects.

P-02.09.1-023
Effects of monosodium glutamate exposure during prepubertal term on several biochemical parameters in rats

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Monosodium glutamate, which is commonly used in processed foods as flavor enhancer, is considered 'generally recognised as safe' by FDA; however many studies have revealed the negative effects of MSG.We aimed to evaluate the effects of MSG in childhood on several serum parameters.

Sixty-six rats, (4 weeks old) were divided into 3 groups as Control (CG,n = 22; 11 + 11, male+female), Experiment 1 (MSG-low dose, E1G, n = 22; 11 + 11, male+female) and Experiment 2 (MSG-high dose, E2G, n = 22; 11 + 11) groups. MSG was administered at 25 mg/kg/d to E1G, 2.5 g/kg/d to E2G for 6 weeks by oral gavage. The rats were sacrified and blood samples were collected from aorta. The blood samples were centrifuged, the serum samples were separated and glucose, ALT, total protein, albumin, creatinine, cholesterole and triglyceride levels were analysed by Beckmann AU 5800 autoanalyser.

Level of total protein was significantly increased in E1G and E2G groups when compared to CG ($p < 0.05$). Level of albümine was also increased in both EGs but significant difference was seen in E2G as compared to CG. Creatinine levels were significantly increased in EGs when compared to CG ($p < 0.05$). Although the glucose levels in both EGs were increased, the increase in E2G was statistically significant ($p < 0.05$). The ALT levels of in EGs were also increased but the significant increase was seen in E2G ($p < 0.05$).

The effect of MSG seem to be dose dependent and especially effect on carbonhydrate metabolism. Increasing doses caused increase in glucose level, and tendency to glucose intolerance. Increasing doses of MSG also caused increase in creatinine and urea. Another apparent effect of MSG was detected on ALT activity. In conclusion the negative effect of MSG on glucose level, liver and kidney functions depends on daily dose intake. Consumption of MSG seem to be inevitable it has to be restrained in children otherwise early metabolic problems may be future problems for these children.

P-02.09.1-024
The effects of artificial food dyes on oxidative stres status in adult female rats

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Food dyes are used to impart, preserve or enhance the colour of food. Recently the use of artificial food dyes were increased. The aim of the this study was to investigate effects of artificial food dyes on oxidative stress status.

A total of 18 adult female rats were categorized three groups as: G_1 (n = 6) was fed basal diet and served as control, G_2 (n = 6): basal diet + Amaranth (0.5 mg/kg) + Sunset Yellow FCF (2.5 mg/kg) + Allura Red AC (7 mg/kg) + Tartrazine (7.5 mg/kg) + Brilliant Blue FCF (12.5 mg/kg) + Ponceau 4R (4 mg/kg) +Azorubine (4 mg/kg) +Indigotine (5 mg/kg) + Erythrosine (0.1 mg/kg) andG_3 (n = 6): basal diet + Amaranth (15 mg/kg) + Sunset Yellow FCF (250 mg/kg) + Allura Red AC

(700 mg/kg) + Tartrazine (750 mg/kg) + Brilliant Blue FCF (600 mg/kg) + Ponceau 4R (70 mg/kg) + Azorubine (400 mg/kg) + Indigotine (500 mg/kg) + Erythrosine (10 mg/kg). Artificial food color mixture were administered to G_2 and G_3 and drinking water was applied simultaneously to G_1 by oral gavage per day for 3 weeks. After application all rats were sacrificed, the total oxidant (TOS)/antioxidant (TAS) capacity were analyzed in rats' brain, liver, kidney homogenate and serum with Rel TOS-TAS Diagnostics Assay kit.The statistical analysis was carried out by using Kruskal Wallis test.

TAS and TOS levels in liver homogenate were not found significantly different between all groups ($p > 0.05$). In serum and kidney and brain homogenate, TAS levels were not significantly different between all groups. TOS levels in G_3 were higher than G_1 and G_2 in serum and kidney and brain homogenate ($p < 0.05$).

Exposure to synthetic food colors may increase oxidative stres in vitale organs such as brain, kidney in female rats. These alterations differ according to organ and dose.

Sunday 4 September 12:30–14:30

Systems biology

P-03.01.1-001
Metabolic network-based analysis of probiotic cheese starter cultures

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Parallel with increasing trends on healthy eating habits, consumption of prebiotics and probiotic microorganisms have been popular due to their benefits on human health. Functional dairy foods such as probiotic yoghurt and cheese are the most common foods including probiotic microorganisms. Due to some considerations such as standardization and quality in bulk production, starter cultures are used in industrialised fermentative food production to start fermentation. Starter culture basically refers the microorganisms which induce and maintain fermentation of the fermentative foods and starter cultures including probiotic microorganisms are called as probiotic starter cultures.

In this study, probiotic cheese starter cultures as a microbial community were investigated using computational systems biology tools. A metabolic network model of probiotic cheese starter culture was reconstructed using microbial community network modeling approach. Literature-based genome-scale metabolic models of commonly used lactic acid bacteria were used for the microbial community metabolic model.

The microbial community metabolic model simulated metabolic interactions of the microorganisms in the probiotic starter culture. Metabolic flux values computed by the metabolic network model also predicted the metabolic pattern of the glycolysis (conversion of lactose), lipolysis (conversion of fat) and especially amino acid catabolism which are associated cheese flavor metabolism.

Simulations obtained by metabolic network-based analysis of cheese starter cultures can also be used for other fields like genetic engineering, upstream processing of the functional cheese production.

P-03.01.1-002
ER quality control protein network in CF to modulate F508del-CFTR rescued

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Background: The most common disease-causing mutation (F508del, present in ~85% of CF patients) leads to CFTR misfolding which is recognized by the endoplasmic reticulum (ER) quality control (ERQC) resulting in ER retention and early degradation. It is known that the CFTR traffic from the ER is mediated by specific sorting motifs that include the 4 retention motifs AFTs (arginine-framed –RXR- tripeptides) and of the diacidic (DAD) exit code that controls the interaction with the COPII machinery.
Aim: Here, we aim to identify traffic factors that regulate CFTR exit from the ER at these specific QC checkpoints.
Methods: We performed pull-down assays with synthetic peptides that mimic mutated or non-mutated CFTR protein at the AFT regions and co-immunoprecipitation of F508del-CFTR (with and without mutated AFT motifs) as well as of wt-CFTR (with and without abrogation o the diacidic code (expressed in CFBE cells) to identify and isolate the factors that interact specifically with each of these variants. The respective protein profiles were analysed by LC-MS/MS and proteins showing differential interactions (with the two sets of peptides or between CFTR interactors) were selected and isolated.
Results and Discussion: A high number of interacting proteins was identified, with the majority corresponding to proteins localized to the ER or involved in cytoskeleton regulation. Several of these interactors were novel, i.e., not previously directly associated with CFTR regulation.

The identification of the specific CFTR interactors/regulators, and its validation which is in progress, will likely identify novel therapeutic targets that could be ultimately used to the benefit of CF patients.

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P-03.01.1-003
Determining the oncogenic effect of Dvl in CML

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Wnt/β-catenin signaling pathway aberrancy has been reported in both solid tumors and hematopoetic malignancies including chronic myeloid leukemia (CML). Dishevelled (Dvl) proteins act as scaffolds essential for Wnt signaling and are important in β-catenin regulation. Elevated expression was shown in various tumors; however a role for Dvl in CML biology has not been previously reported. We aimed to study the potential oncogenic

effect of Dvl proteins in CML using the Philedelphia chromosome positive blastic cell line K562. Dvl1, 2, and 3 genes were silenced by siRNA expression vectors; followed by the mRNA expression analyses of hematopoesis, stem cell, leukemia and Wnt signaling related 144 genes, by a custom designed PCRarray. The effects of Dvl gene silencing on Wnt signaling was examined by western blot and immunoflourescence staining. Cell proliferation was analyzed by MTT assay. Effects of Dvl silencing on Tyrosine kinase inhibitor (imatinib mesylate) sensitivity was assessed by flow cytometric analyses. We've shown that silencing of Dvl genes reduce cell proliferation in K562 cells. In addition Dvl gene silencing increases cell sentivitiy to apoptosis. The mRNA expression of F2R, CDNK1A, JUNB, LRP6, NXN and TERT genes were upregulated; and LEF1, CYLD, CSNK1E, CCDN1, SMAD1, VANGL2, RUNX2, BRD7, and NKD2 mRNA expressions were downregulated. At the protein level, we have also shown that the total β-catenin protein was not altered; whereas β-catenin target protein c-myc was downregulated. Also phosphorylation levels of LRP6 increased after silencing Dvl expression. Noncanonical Wnt pathway proteins pJNK was up regulated and RhoA was down regulated. Silencing Dvl genes reduced proliferation of cells and sensitized cells to imatinib. We suggest that activated Dvl's might have a role in aggressive expansion of myeloid cell lineages, therapy resistance and blastic crisis of CML. We propose that Dvl proteins may be a potent target for novel treatment strategies in CML.

P-03.01.1-004
Circulating betatrophin levels are associated with the apnea-hypopnea index and lipid profile in subjects with obstructive sleep apnea

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Background: Obstructive sleep apnea (OSA) have increased risk of the adverse medical outcomes of this condition include insulin resistance, metabolic syndrome, cardiovascular disorders. Betatrophinis a novel protein that There is an association between betatrophin expression and serum lipid profiles, obesity, metabolic syndrome, and diabetes mellitus. We aimed to investigate the association between OSA and betatrophin in OSA and control subjects.

Methods: 90 patients with suspected OSA had polysomnography to determine the Apneae and hypopneae Index (AHI). We measured fasting plasma glucose, fasting serum insulin, plasma Betatrophin, leptin, adiponectin, and full lipid profile. Patients were classified on the basis of the AHI, as control or OSA.

Results: 38.8% of subjects had normal polysomnography, 61.2% had OSA. The level of betatrophin, leptin and adiponectin was higher in subjects with OSA (256.59 ± 29.35 μg/ml, 374.20 ± 37.93 μg/ml, 17.86 ± 2.63 μg/ml, respectively) compared to the controls (141.86 ± 26.20 μg/ml, 205.53 ± 14.75 μg/ml, 7.52 ± 1.02 μg/ml, respectively). Betatrophin expression was positively correlated with AHI level ($r = 0.413$, $p = 0.002$). The TG level was significantly higher and HDL-C level lower in OSA subjects compared to the controls (244 ± 20.33 vs. 138 ± 14.89, 37.21 ± 1.26 vs. 43.78 ± 1.62 respectively).

Conclusion: Our results showed that circulating betatrophin levels were significantly increased in patients with OSA and that there was a high correlation between betatrophin level and AHI, TG, and leptin. The results suggest a complex interaction between OSA and betatrophin, leptin, adiponectin and the lipid profile. This complex interaction requires further investigation.

Keywords: Betatrophin, leptin, Adiponectin, OSA, AHI, Lipid Profile

P-03.01.1-005
Relation between vitamin D levels and E23K polymorphism in KCNJ11 gene in gestational diabetic individuals

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Introduction: Gestational diabetes mellitus (GDM) confers 4-to 7-fold greater risk of incident type 2 diabetes mellitus (Type2 DM). The KCNJ11 gene has a key role in insulin secretion and is a substantial candidate gene for Type2 DM. Therefore, E23K mutation in KCNJ11 gene increases the risk of Type2 DM and considered to be associated with GDM. Furthermore, there is an increasing interest in association between vitamin D and GDM.

Materials and Methods: Seen from this aspect, we aimed to investigate the relations between E23K polymorphism in KCJN11 gene and vitamin D in pregnant with GDM and healthy pregnant as an evaluation of retrospective data.The present study was conducted on 91 pregnant (24–28 gestational weeks) individuals who are admitted to Selcuk University Faculty of Medicine, Endocrinology Polyclinic. Vitamin D levels of 54 GDM patients and 37 healthy pregnant, who had E23K polymorphism been genotyped at KCJN11, were evaluated. GDM individuals were defined as "Patients group" and healthy pregnant were defined as "Control group". A p value < 0.05 was considered statistically significant.

Results: According to data, we did not determine significant relations between E23K polymorphism and vitamin D levels (x±SE) in our GDM population ($p = 0.485$). D vitamin levels (mg/dl) were; 19.44 ± 3.16 in G/G genotype; 20.55 ± 2.22 in G/A genotype and 8.98 ± 3.12 in A/A genotype, respectively. Interestingly, GDM patients with A/A genotype have the lowest vitamin D levels that are below the reference range of GDM. We did not find a statistically significant difference in comparing the levels vitamin D (mg/dl) of the patients [15.71 (13.01–18.97)] and control group [(19.39 (15.58–24.14)] according to descriptive statistics results ($p = 0.157$).

Conclusion: Although vitamin D values are the highest in the G/A genotype, and the levels in A/A genotype are below the reference range, there are no statistically important differences according to genotypes E23K polymorphism in KCNJ11 gene.

P-03.01.1-006
Association between TSH status and genotypes of E23K polymorphism in KCNJ11 with gestational diabetic individuals

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Introduction: Thyroid disorders especially subclinical hypothyroidism are common in pregnancy. Maternal hypothyroidism has been associated with increased risk of congenital malformations and respiratory distress in newborns. An impaired glucose tolerance disease which is diagnosed during pregnancy called as gestational diabetes mellitus (GDM) can influence the secretion of TSH. The KCNJ11 gene, has a key role in insulin secretion and is a substantial candidate gene for Type2 DM. Therefore, E23K mutation in KCNJ11 gene increases the risk of Type2 DM and also considered to be associated with GDM.
Materials and Methods: The present retrospective study is aimed to investigate the relations between E23K polymorphism in KCJN11 gene and TSH levels in pregnant with GDM and healthy pregnant. The present study was carried out on 104 pregnant (24–28 gestational weeks) individuals who are admitted to Selcuk University Faculty of Medicine, Endocrinology Polyclinic. TSH levels of 60 GDM patients and 44 healthy pregnant, who had E23K polymorphism been genotyped at KCJN11, were evaluated. GDM individuals were defined as "Patients group" and healthy pregnant were defined as "Control group". Statistically significant was considered as $p < 0.05$.
Results: TSH levels were 1.63 (1.43–1.87) μIU/ml in patients and 1.87 (1.65–2.13) μIU/ml in control group. We did not determine significantly difference between the groups ($p = 0.165$). In both groups TSH levels were in reference range.TSH levels (μIU/ml) were; 1.68 (1.43–1.97) in G/G genotype; 1.82 (1.59–2.07) in G/A genotype and 1.5 (1.13–1.99) in A/A genotype, in patients group ($p = 0.186$). In control group, TSH levels were 2.26 0.18 in G/G genotype, 1.97 0.17 in G/A genotype, 1.70 0.45 in A/A genotype, respectively ($p = 0.313$).
Conclusion: TSH levels are lower in GDM's pregnant than healthy pregnant, but there are no statistically important differences according to genotypes of E23K polymorphism in KCNJ11 gene in both groups.

P-03.01.1-007
Analysis of protein and mRNA expressions of NQO1 and GST-pi enzymes in liver, colon and prostate cancer cell lines to study drug and carcinogen metabolism

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Phase II xenobiotic metabolizing enzymes convert parent compounds into more hydrophilic metabolite by catalyzing conjugation reactions including glutathione and amino acid conjugation, glucuronidation, sulfation and acetylation. This study was aimed to describe the best cell line model for studying phase II xenobiotic metabolizing NQO1 and GST-pi enzymes. For this purpose, mRNA and protein expression of NQO1 and GST-pi enzymes were analyzed in HT29 and SW620 (colon); HEPG2 and HUH7 (liver); PNT1A and PC3 (prostate) cell lines by qRT-PCR and Western blotting techniques, respectively. Protein expression analysis revealed that NQO1 protein was expressed in all cell lines and relative protein expression is highest in the HEPG2 (100%) and PNT1A (97%) while HUH7 (31.9%) showed relatively low expression of NQO1. In addition, NQO1 mRNA expression was relatively high in HT29 (1.68 fold) and PNT1A (1.92 fold) when compared with liver cell line HEPG2 (1.00 fold). GST-pi protein expression was found very high in HUH7 (100%) while there was no expression in HEPG2. GST-pi mRNA expression was relatively higher in PNT1A (3.56 fold) and HT29 (2.47 fold) when compared with HUH7 (1.00 fold). According to these results, choosing the best cell line as model depends on the purpose of the research. For studying metabolism of a chemical by NQO1 and GST-pi or effect of a chemical on translational regulation of these enzymes, it is better to consider protein expression of the cell lines for choosing best model. However, if the aim is to study effect of a chemical on transcriptional regulation of these enzymes, it is better to choose a cell line that expressing highest mRNA of gene of interest. In conclusion, considering both mRNA and protein expression levels together, the best model cell lines for studying phase II xenobiotic metabolizing NQO1 and GST-pi are HT29 and HUH7, respectively.

P-03.01.1-008
Drug repositioning strategies applied on a group of cancer

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Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. Number of new cases is increasing worldwide. On the other hand, investments for cancer treatment in pharmaceutical industry have also gradually increased to fulfill demand. Investing on R&D takes billions of dollars and 9–14 years to bring a new drug into the market. Therefore, purposing of new therapeutics has great impact. Computer-based analysis of omics data based on the cancer datasets provides significant potentials for investigations. This study aims to determine metabolic changes and target drugs among different cancer types based on transcriptomics datasets.

20 transcriptome datasets belonged to six different cancer types including prostate, breast, colorectal, pancreas, thyroid and lung cancer were acquired from Gene Expression Omnibus. Each dataset was statistically analysed independently to identify differentially expressed genes (DEGs with p-value<0.05). All datasets were normalized and implemented in the affy package of R/Bioconductor. Afterwards, DEGs were integrated into Human Metabolic Model to find reporter metabolites and moreover, drugs were determined via DGIdb drug-gene interaction database. Functional enrichment analyses were carried out by using DAVID.

Consequently, metabolic and signaling pathways, biological processes were investigated and provided the information to define alterations on different biological states in various cancers. Common and distinct molecular signatures based on DEGs, metabolites and drugs were discussed among six types of cancer. The drug candidates were also compared with FDA approved cancer drugs. Reporter metabolites were confronted with drugs to chase metabolic response of patients which may substitute drugs. As a conclusion, this systematic study was designed for drug repurposing enables to predict novel drug targets among six important cancer types.

P-03.01.1-009
Investigation of hematological values of pregnant women with HELLP syndrome

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Object: HELLP syndrome is a life-threatening complication of high degree preeclampsia during pregnancy. Abnormal changes in blood composition that occur during pregnancy are known to affect the mother and fetus health. In this study, we aimed to investigate some haematological values in women with HELLP syndrome.
Method: This study included 15 pregnant women who were diagnosed with HELLP syndrome and 20 healthy pregnant women in the same age group were included. The volume of neutrophils, leukocytes, monocytes, basophils, eosinophils, erythrocytes, hemoglobin, mean erythrocytes volume, white blood cell count, mean platelet volume, platekrit, erythrocyte distribution volume and platelet volume of distribution in whole blood samples with EDTA taken from all pregnant women were measured. Statistical analysis was performed with SPSS 20.
Results: While Neutrophils, mean erythrocytes volume, and platelet volume of distribution in the HELLP group significantly higher than the control group (p values are respectively 0.016, 0.044 and 0.048), pletekrit and platelet values were significantly lower (p values are respectively; 0.000, 0.000). In addition no differences was found in erythrocytes, lymphocytes, monocytes, eosinophils in white blood cell, hemoglobin and mean platelet volume values between groups.
Conclusion: In our study, we found some hematologic values were significantly higher in pregnant women who were diagnosed with HELLP syndrome. It is believed in distressed pregnancy, such as HELLP, investigation, routinely examination and monitoring of hematological parameters, is important for prevention of deficiencies both mother and fetus healty.

P-03.01.1-010
The studies on the pancreatic cells' surface glycoconjugates profiles in rats fed with high fat with lectin labelling methods by flouresans microscopy

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In this study, the backbone of the cellular adhesion-recognition mechanism, located in the cell membrane. The study material selected pancreas tissue, has a privileged structures.

The pancreas is one of the main organs to aid in digestion. The pancreas functions as an exocrine gland and role in digestion. In addition, the pancreas also functions as an endocrine gland, secreting several hormones into the blood that control the blood levels of glucose and other nutrients. Due to the pancreas have been selected for this unique feature. Thus, different types of cells in the same sample will be able to study the structure of the surface glycoconjugates. Generally researches about determination of carbohydrates in the cell, glycoproteins or/and glycolipids are cut with enzymes. Next step, the oligosaccharide mixture obtained, than establishing the complete structure of oligosaccharides and polysaccharides requires determination of branching positions, the sequence in each branch, the configuration of each monosaccharide unit, and the positions of the glycosidic links. This is a more complex problem than protein and nucleic acid analysis. These processes are indispensable for the understanding of the chemical structure of the sugar. Whereas in cells using labeled lectins specific sugars, it is possible to accurately determine.

In this study, was used Triticum vulgaris (WGA) labeled with Fluorescein (FITC). Thus, the cells located on the cell surface [GlcNAcβ1-4GlcNAcβ1-4GlcNAc] and Neu5Ac (sialic acid) for WGA sugar residues were investigated. According to preliminary results of this study, WGA labeled with FITC is specifically binding of these sugars. When this study is completed, the differences of sugar on the surface of different type of cells in the pancreas can be distinguished in micrographs. Thus, in the cells of the pancreas, the sugar units involved in adhesion-recognition can be possible to determine specifically.

P-03.01.1-011
Literature supported gene network of colorectal cancer

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Large scale gene networks could be topologically analyzed in order to obtain possible global system-level structure Cancer gene co-expression networks can have lower connectivity as compared to normal samples. Using colorectal tissue gene expression datasets, we observed that tumor specific networks are less connected than normal networks. Functional enrichment analysis suggested that cell cycle genes and methylation-associated cell adhesion genes can specifically play a role in the connectivity loss of carcinoma samples. Literature confirmation provided a gene network including significant genes playing roles in the intersections between cell cycle, cell adhesion, and cell skeleton dynamics. This network can provide novel insight to our understanding of the molecular mechanisms of colorectal cancer.

P-03.01.1-012
TF-miRNA circuits specific to epithelial cancers

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Cancer is the most common cause of death in the world but there are still a lot of uncertainties about the exact mechanism taking roles in regulation of it. Cancers can be classified according to cell type; in which they start. Carcinomas are the most prevalent types of cancer and start in epithelial tissues. They are also named as epithelial cancers (ECs) and make up about 85 out of every 100 cancers. Over the past few years, many studies are concentrated on miRNAs, which have emerged as important regulators of gene expression like transcription factors (TFs). TFs are regulators at transcriptional level while miRNAs are post-transcriptional regulatory key-elements. Otherwise the transcription of mRNAs and miRNAs are known to be regulated by TFs and TFs are the targets of miRNAs. Therefore, it is crucial to characterize the relation of TFs, miRNAs and their targets by building circuits in diseases such as in ECs.

For this study, miRNA and mRNA expression studies including epithelial tumors and normal samples searched in GEO and Array Express microarray databases. 4 mRNA studies and 4

miRNA study, which were designed for 4 different ECs (breast, lung, ovary and colorectal) were selected to be analyzed. Differentially expressed (DE) mRNAs and miRNAs between epithelial tumors and normal samples were extracted ($p \leq 0.05$, 2 fold change). Among DE genes, transcription factors and miRNAs were identified and listed for epithelial tumor vs. normal comparison.

Circuit analysis resulted with remarkable circuit, which was common for all the types of ECs that includes KLF4 transcription factor and hsa-miR-145.

In the literature hsa-miR-145 and KLF4 are known as important regulators in different types of cancer, which indicated that the motifs involving TFs and miRNAs might be useful for understanding the regulation of ECs.

As a conclusion finding out new and common circuits may aid us in predicting new or alternative diagnostic and/or prognostic biomarkers for ECs.

P-03.01.1-013
The investigation of applicable 8-hydroxyquinoline-5-sulfonic acid ligand for removal of iron (III) ions from human plasma

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Introduction: Iron overload toxicity is beginning to be taken seriously in medicine around the world. Currently applied method for treatment is chelation therapy. In this study, it will try to carry out the synthesis of low cost ligands which can be used remove Fe^{3+} ions from iron overload patient plasma *in vitro*. Therefore,8-Hydroxyquinoline-5-sulphonic acid (8-HQ-5-SA) is used as a complexing agent. The cytotoxic effects of 8-HQ-5-SA chelator against human endothelial cell lines were evaluated using MTT assay.

Materials and Methods: In this study, 8-HQ-5-SA ligand formed complexes by reaction with high concentrations of iron at physiological pH at room temperature.The complex formed at the rate of 1/3.The resulting complex was solved in plasma even at concentration and obtained in 95 percent yield. 8-HQ-5-SA and iron (III) complex was characterized by FT-IR, magnetic susceptibility, elemental analysis and UV-Vis spectrophotometers.The remaining metals were determined by ICP-MS.

Results: UV-VIS measurements showed that amount of complexation increase depending on time.Uv-Vis was measured 220,255,314,367 nm for 8-HQ-5-SA; 205,207 nm for Fe (NO3)3 and 215, 238,325,314,352,390 nm for complex.ICP-MS showed that concentration of iron (initial concentration is 50 ppm) decreased to 3 ppm after complexation.FT-IR show that the main peaks schift to lower after complexlation. MTT assay and the results showed the lowest cytotoxic effect.

Discussion and Conclusion: Previously, 8-HQ-5-SA ligand was used in many studies for different purposes in the complexation of metals, but the therapeutic use of 8-HQ-5-SA ligand for iron accumulation in the body is investigated for the first time.ICP-MS, FT-IR, UV-Vis and magnetic susceptibility results showed that Fe ions which prepared in specific concentrations inplasma highly formed complex with 8-hydroxyquinoline-5sulfonic acid.Theseresults and cytotoxic effect indicate that using of 8-HQ-5-SA for this aim in further studies is appropriate.

P-03.01.1-014
Comparative genome wide gene expression analyses of primary MSC and MSC lines

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Mesenchymal stem cells (MSCs) are multipotent stromal cells that can differentiate into a variety of cell types which are used in cell therapy. Although they are the most attractive cell type for cell therapy studies, primary MSCs lose their differentiation potential with increasing time in culture and passage so they are of limited use. Due to this disadvantage, MSC lines are more suitable for *in vitro* researches owing to their immortality.

In this study we compared primary bone marrow-derived MSC (BM-MSC) with bone marrow derived MSC line (RCB2153) in terms of cell characteristics and gene expression profiles to determine the functional differences among MSCs types. Firstly, MSCs were identified by using CD29, CD70, CD90 and CD105 as positive markers and CD34 as a negative marker. Gene expression profilling was investigated using Affymetrix HG-U133-Plus2 arrays.

The significant GO biological process terms and KEGG pathway enrichment analyses of the identified DEGs were performed using DAVID ($p < 0.001$, Fold change$\geq$2). The analysis showed similar pathway clustering in both cell types. The resulting quantitave transcriptome of 754 genes were identified that differentially expressed in MSC line versus primer MSCs (538 up-regulated and 216 down-regulated). Functional classification of changed genes was mainly clustered in cell cycle, cell death and mismatch repair. KEGG pathway analysis revealed that the genes were significantly enriched in pathways including "Cell cycle, DNA replication and Focal adhesion" pathways.

In conclusion, our results indicate that MSC lines can be used instead of primary MSCs. These quatitative results provide an important basis to adapt cell lines to more closely resemble physiological conditions as oppossed to animal experimentation. This could help to minimize the use of animals in research.

P-03.01.1-015
Association between loss of 18q21, gain of 20q13.33 and progression in sporadic colorectal cancer

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Colorectal cancer (CRC) is one of the most diagnosed cancer and the third leading cause of cancer deaths throughout the world. Identifying of copy number variation (CNV) profiles between early and late stage cancers can be useful to understand the progression and aggressiveness of cancer. The main goal of this study was to construct a comprehensive insight of association between CNV and sporadic CRC stages in order to identify novel candidate targets which may contribute to tumor progression.

Affymetrix 6.0 GeneChip SNP arrays were used for characterization of CNVs in tumor and matched normal formalin-fixed, paraffin-embedded (FFPE) tissues from 5 stage I, 17 Stage II and

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25 Stage III samples. Paired CNV analyses were performed using Partek Genomic Suite 6.6 and genomic segmentation algorithm was performed using a minimum of 10 markers per segment, a signal-to-noise ratio of 0.3 and the cut-off value for the gain and loss was set of 2 ± 0.3. The adjusted p-value ≤0.05 were considered to be significant.

Whole genome CNV analysis revealed that amplification of 20q13.33 with 4 genes was found the most frequent (76.5%) in stage II tumors. The most frequent (36%) amplifications were 13q12.2 and 7p22.2 in stage III tumors. While deletion of chromosome 18q21.2 in stage III with a frequency of 36% was found the most frequent loss, deletion of 18q21.1 was seen the most frequent (64.7%) in stage II tumors. Two tumor suppressor genes SMAD2 and SMAD4 which are found in these deletion regions were common genes between stage II and Stage III.

Our results showed that gain of 20q13.33 might have a significant role in the progression of cancer. Loss of 18q21 comprising two tumor suppressor genes is also another important finding. 18q21 loss can be a significant prognostic value in colorectal cancer even though validation of target genes requires additional study and larger sample size. This work was supported by TUBITAK project no:109S477.

P-03.01.1-016
Meta-analysis based miRNA signature discriminates cervical cancer from normal samples

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Gynaecological cancers are common problems in female health. Squamous cell carcinoma (SCC) is a type of these malignancies. This tumor type is derived from pre-cancerous lesions, which is called cervical intraepithelial neoplasia (CIN). CIN is classified as CIN1, CIN2 and CIN3 according to their dysplasia grade in the cervical tissues. miRNAs are small non-coding RNAs that were shown to have important roles in the development and progression of various cancers. The aim of this study is identifying miRNAs, which are playing a part in progression of cervical lesions by a ranking based meta-analysis approach.

Two mRNA and three miRNA expression studies, which include normal, CIN1, CIN3 and SCC samples were selected from ArrayExpress and Gene Expression Omnibus (GEO) databases. Three miRNA studies were combined with ANOVA dependent ranking based meta-analysis program which was developed in our laboratory to find out a miRNA signature that can discriminate CIN1, CIN3 and SCC samples from normal samples. The top five miRNAs with the highest ranks in meta-list were selected for further analysis. Predicted targets of these miRNAs were identified by miRDB target prediction tool. Additionally two mRNA datasets were selected for miRNA-target validation studies. Common genes, which were obtained from meta-miRNA targets and differentially expressed genes between normal and CIN1, CIN3 and SCC groups from two independent studies, were identified and they were subjected to pathway enrichment analysis.

Pathway enrichment analysis that was performed with 338 common genes showed that these targets were significantly enriched ($p < 0.05$) in especially cell proliferation, cell survival and cell cycle pathways, which are the key players of cancer development and progression.

The meta-analysis results together with validation analysis of their targets may point out the potential roles of miRNAs as biomarkers for the diagnosis and the treatment of cervical cancer.

P-03.01.1-017
The hypoglycaemic and regenerative activity of Thymbra spicata in alloxanized-diabetic rats

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Thymbra *spicata* (Labiatae), a carvacrol and thymol containing plant, is one of the medicinal herbs used by diabetic individuals to reduce blood glucose in Turkey. We investigated the hypoglycaemic and anti-lipemic effects of the aqueous extract prepared from dried leaves and flowers of this plant in alloxanized-diabetic rat model. Rats were divided as: Diabetic control (group 1), diabetic+glibenclamide (group 2), diabetic+plant extract (group 3), untreated control (group 4) and control+plant extract (group 5) groups (n = 6 for each group). Serum glucose, lipid levels and body weight changes were mesasured and pancreas and liver histology of the rats were examined. Each rat in all groups were administered the plant extract (30 mg), and the reference drug Glibenclamide (2 mg/kg) by gastric gavage every day for 8 weeks.

In group 1, blood glucose, serum ALT, AST, triglyceride, cholesterol and LDL cholesterol levels increased while body weights decreased. In group 2, serum glucose, ALT, AST, triglyseride and HbA1c levels decreased compared to group 1 while cholesterol and LDL levels were high. In group 3, serum ALT, AST, trigliserit, cholesterol, LDL levels decreased significantly but serum glucose and HbA1C were higher compared to group 4. Body weights increased except Group 1 and HDL levels were not altered. Histologically degenerative changes observed on pancreas of Group 1 were decreased in groups 2 and 3. There was no difference on liver histology of the groups.

In conclusion, *Thymbra spicata* showed a protective and regenerative effect on diabetic pancreas. The hypo-lipidemic effect of the plant extract was also more effective than glibenclamide possibly due to the flavonoids, saponins and triterpenoids contents in the extract. Its hypoglycaemic and protective activity should be tested for different doses and extract preparations and for longer periods. Our study suggests that *Thymbra spicata* is an excellent candidate for future studies on diabetes mellitus.

P-03.01.1-018
Genome scale comparison of perturbations in the nitrogen and phosphate metabolism of streptomyces coelicolor by integrating transcriptome data with a curated metabolic network

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Streptomyces species are soil-dwelling, gram positive, filamentous bacteria with a high G+C content genome. They have very complex regulatory systems and metabolic switches that change the metabolism between primary and secondary metabolism. Pharmaceutically important compounds are produced during the secondary metabolism which is often observed when the culture has entered the stationary phase in a batch culture growth system. Complex regulatory network of *Streptomyces* species can be simplified with different approaches of systems biology (genome scale metabolic models, omics technologies etc.) and the results may suggest ways of increasing production levels. In this study, a genome scale metabolic network of *S. coelicolor* was integrated

with three different transcriptome data sets from the public Gene Expression Omnibus database: time dependent data of ΔphoP mutant, ΔargR mutant and wild type strain. The dynamic data spanned both primary and secondary phases of the metabolism. Statistical results of transcriptome data were used for reporter metabolite analysis and reporter pathway analysis, which identify the metabolites (or pathways) with a significant coordinated transcriptional change in response to gene deletion perturbation in phosphate and nitrogen metabolisms. Further, the production of actinorhodin, a pharmaceutically important compound, was modeled in the two deletion strains by calculating the metabolic fluxes subject to transcriptional level constraints on enzyme-coding genes. The metabolic switch from primary to secondary metabolism was highlighted in terms of the activity of pathways and fluxes as a result of the computational analyses in this work, leading to a better understanding of the role of phosphate and nitrogen metabolisms in increasing production levels.

P-03.01.1-019
Antioxidant and toxicological in-vivo and in-vitro examination of licorice extract

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Introduction: As a member of legume family Licorice (*Glycyrrhiza glabra* L.) has been widely used by human kind for many years as food constituent. Especially by folks in rural sites licorice consumed widely. Beside food constituent Licorice has been used for medical purposes as well. Licorice found effective with scientific datas on peptic skin infections, ulcers, inflammation, eczema, Alzheimer disease, liver disease, and cancers. It also has been used as natural sweetener and food additive for preparing candies, chewing gum and beverage since ancient times. Like all other medicine it has not been free of adverse event or toxicological effects.

Material and Methods: Alcoholic extracts of plant obtained by maceration process. For *in vitro* examination of anti-oxidant profile of licorice DPPH free radical scavenging, ABTS cation radical scavenging and cupric ion reducing antioxidant capacity assay applied. Application of extract made by oral route to rats for a week. Anti-oxidant profile has been evaluated by Myeloperoxidase (MPO), Arylesterase (ARES), Total Oxidative Stress (TOS) and Total Antioxidant Status (TAS) of serum levels. Determination of toxicological effects ALT, AST, LDH and ALP values studied. Histological investigation applied on liver and kidney tissues.

Results and Discussion: Results compared with control and standarts. Antioxidant potential of licorice has been observed by in-vitro assays. Serum MPO and ARES values also compared with in-vitro results and correlation between them has evaluated. Toxicological investigations made after evaluation of AST, ALT, LDH and ALP values.

Conclusion: *In vitro* assays has showed that licorice has potential anti-oxidant effect. Investigation revealed that a mild toxic effect of licorice by biochemical tests. Toxicological profile compared with control group and ALT, AST values found slightly decreased and a mild elevation has been seen in LDH and ALP values. For further and detailed investigation is needed.

P-03.01.1-020
On the applications of a metabolic network model of mesenchymal stem cells

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Mesenchymal stem cells (MSCs) have several applications in tissue engineering and regenerative medicine. MSCs can be very useful in stem cell therapy, because they can be isolated bone marrow or adipose of an adult. These cells have also been used as gene or protein carriers. Therefore, maintaining them in a desire metabolic state has been the subject of several studies.

Here, we have used a genome scale metabolic network model of bone marrow derived MSCs for exploring the metabolism of these cells. Then, we try to validate the computational results by experimenal tests.

We analyzed metabolic fluxes in order to increase stem cell proliferation using the metabolic model. Consequently, the experimental results were in consistency with computational results.

In the present work, the applicability of the metabolic model was successfully approved. Therefore, this metabolic model can be useful in biomedical researches of stem cells.

P-03.01.1-021
QTL analysis for body weight and fatness in BXD recombinant inbred mouse strains

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Genetic variation in body weight and composition is under the influence of many genes and have different genetic architectures. In the present study, the genetic factors contributing to body weight and fatness were examined under energy rich feeding conditions. Growth traits, lean and fat weight, fat mass gain were analyzed to map QTLs in a set of BXD RI strains. Genome-wide analyses were revealed several genomic loci that control body weight and associated bodily changes in a sex and age-specific manner. The genetic data provided evidence for significant QTLs on chromosome (Chr) 4, 5, 14, and 16. Most likely candidate genes within or near the regions with the highest significance levels were identified. The genes 1700048F04Rik, Gbe1, A830060N17, and four genes Cenpc1, Stap1, Uba6, Gnrhr for example, are suggested as most likely positional candidates accounting for the QTL effects on Chr 5 for fat mass, on Chr 16 for fat mass gain and on Chr 16 for lean weight, and Chr 16 for body weight, respectively. Our results showed that body composition and fatness are highly complex that many genetic factors regulating and suggested candidate genes, which may help for studies of human fatness.

P-03.01.1-022
Preliminary look to some nutrients involved in neurotransmitter synthesis and premenstrual symptoms

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Premenstrual syndrome is the most common disorder among reproductive women. The premenstrual symptoms could be

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related to serotonergic and GABA systems in response to hormonal changes. The nutrients involved in neurotransmitter synthesis may be the cause of relationship between diet and premenstrual syndrome. Therefore, the aim of this study was to investigate the effect of various nutrients and premenstrual syndrome.

This study was conducted to 29 healthy women aged 20–37 years. Participants were asked to fill in Premenstrual Assessment Form. Dietary intakes (three days in each phases) were recorded during premenstrual, menstrual and postmenstrual phases. Energy, protein, amino acids, iron, calcium, and magnesium intakes were estimated. Statistical analyses were performed using the SPSS software. Friedman tests were conducted and differences were considered to be statistically significant for p-values lower than 0.05.

60.9% of the participants reported premenstrual symptoms and premenstrual symptoms related nutrient intake were increased in these women. It was determined that energy ($p = 0.03$) and protein ($p = 0.001$) intakes were higher in the premenstrual phase. During premenstrual phase; tyrosine ($p = 0.002$), isoleucine ($p = 0.001$), leucine ($p = 0.001$), lysine ($p = 0.008$), methionine ($p = 0.008$), cysteine ($p = 0.008$), tryptophan ($p = 0.000$), and glutamic acid ($p = 0.005$) intakes were higher than other phases. Likewise, iron intake was higher on premenstrual phase ($p = 0.054$). On the other hand, intake of other potential premenstrual syndrome related nutrients like fat, cholesterol, calcium, magnesium, and vitamin B6 were not significantly different within the menstrual phases.

Amino acids including tyrosine, tryptophan, glutamine, and vitamin B6 are involved in neurotransmitter synthesis and might be related to premenstrual symptoms. Consequently, elevated intakes of dietary protein and some amino acids during premenstrual phase may be related to premenstrual syndrome symptoms.

P-03.01.1-023
Dietary fatty acid pattern and preferences are not associated with fatty acid transporter CD36 in diabetic patients

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Long chain fatty acid transporter known as FAT or CD36 has been figured out prominently in the fields of diabetes and food choice. Therefore, this study was conducted to determine the relationship between dietary fatty acid pattern, fatty food preference and blood CD36 and insulin sensitivity in patients with type II diabetes compared to the control group.

The study was conducted to 38 patients at the age of 30–65 years of whom recently diagnosed type II diabetes mellitus who applied to Kırıkkale University Hospital at Department of Endocrinology and compared with 37 healthy subjects. In the study, the personal information was recorded, fasting CD36 was analyzed, and dietary fatty acid pattern, and fatty food preferences were estimated from dietary recalls and food frequency questionnaires.

At the end of the study, there was no significant difference between the groups in terms of nutrition preferences and dietary fatty acid pattern ($p > 0.05$). However the olive oil intake and daily amount of fat was significantly higher in healthy subjects ($p < 0.05$). Furthermore, carbohydrate intake was high in diabetic patients ($p < 0.05$). Additionally, there was not any significant difference between blood CD36 levels and dietary fatty acid pattern and fatty food preferences ($p > 0.05$).

These results reveal that there is not any significant difference observed between groups in terms of fatty food preferences and dietary fatty acid pattern. But amount of fat and carbohydrate intake, and oil type might be important for pathophysiology of the diabetes mellitus. These results suggest that, role of CD36 in dietary fat intake, olive oil and fat preferences should be more studied to reveal its role in not only in diabetes but also in obesity.

Sunday 4 September 12:30–14:30

Host–pathogen interactions

P-04.01.1-001
Peculiarities of the potexvirus optical activity

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Until now far UV CD spectra of only two potexviruses were published. The *Papaya mosaic virus* (PapMV) spectrum, measured by Leclerc and co-authors contained no obvious anomalies and was similar to the spectrum of isolated PapMV coat protein (CP). But measured 30 years earlier by Homer and Goodmanfar UV CD spectrum of *Potato virus X* (PVX) itself had anomalous character and differed strongly from the spectrum of isolated PVX CP. In the present work we measured far UV CD spectra for two more members of Potexvirusgenus: *Alternanthera mosaic virus* (AltMV) and *Potato aucuba mosaic virus* (PAMV) and their free CPs. The AltMV virion and AltMV CP spectra were similar to each other and to the spectra of PapMV and its CP. The PAMV spectrum resembled the PVX spectrum in anomalously low ellipticity of the negative band at 208 nm, but in contrast to PVX, did not have additional peak at 228 nm. Homologous modeling showed that CP of the three viruses is very similar in the core structure, and the observed difference may be explained by differences in disordered parts of proteins. Possible reasons of potexvirus structural variability are discussed and it is suggested that the intravirus potexvirus CPs may assume different conformations in different virions of the same preparation or even along the length of one virus particle. This work was supported by the Russian Science Foundation (grant 14-24-00007).

P-04.01.1-002
Elucidating the mechanism by which plant derived small molecules affect virulence determinants of the genus Pectobacterium

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Pectobacterium (formerly *Erwinia*) causes soft-rot on fruits, ornamentals and vegetables produced worldwide. Being a major threat for potato and ornamental plant industries, efforts were dedicated to control the disease in fields and in storage, with limited degree of success. We focused on the potential of plant derived molecules (mainly phenolics), to control the disease.

The antimicrobial potential of different phenolics was tested on *Pectobacterium* in search of possible mode of action. In this respect, biofilm formation, exoenzyme activity, gene expression and virulence on its natural host (potato, cabbage, calla lily) were performed. Also computational approach to show interaction between phenolic compounds and target protein was carried out using docking tools.

The virulence determinants of *Pectobacterium* were significantly impaired, at compound concentrations that did not affect bacterial cell growth. These observations suggested a mechanism which specifically interferes with bacterial virulence. Since, these virulence determinants in *Pectobacterium* are controlled by quorum sensing (QS), we focused on the effect of phenolics on the QS system in pectobacteria. The study revealed an inhibiting effect of the tested compounds on the expression level of central QS system and controlled genes, using qRT-PCR. Also, there was a prominent reduction in the level of QS signal molecules N-acyl-homoserine lactone (AHL) accumulation. In addition infection capability was also practically blocked, which was completely recovered by application of exogenous-AHL. These results were supported by a potential interaction of plant phenolics with QS targets, as shown by molecular docking tool.

Collectively, results suggest the potential interference of phenolic compounds with QS central components (ExpI/ExpR proteins). Moreover, it holds potential for future development of control measures against *Pectobacterium*, and possibly other pathogens with similar mode of virulence.

P-04.01.1-003
Does yeast virus possess specificity towards replication of satellite virus?

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Saccharomyces cerevisiae has been a key experimental organism for the study of infectious diseases, including double-stranded RNA (dsRNA) viruses. The L-A dsRNA virus family of *S. cerevisiae* is widely distributed in nature. Several versions of L-A virus are described and new ones continue to be discovered. Some *S. cerevisiae* strains along with L-A dsRNA possess smaller dsRNAs, called M satellites. These dsRNAs encode a sole secretable protein, known as K1, K2, K28 and K-lus toxin. L-A genome encodes the Gag major structural protein and Gag-Pol fusion protein, formed by ribosomal frameshifting. Gag-Pol has transcriptase and replicase activities are necessary for maintenance of both L-A and M satellite dsRNAs. So far, it's not known whether certain L-A virus has evolved to maintain a distinct type of satellite dsRNA or this phenomenon lacks inherent specificity.

We developed universal strategy to obtain full length L-A and M dsRNA genomes from *S. cerevisiae*. Complete viral dsRNA genomes can now be cloned, as evidenced by L-A-28 dsRNA, analyzed and sequenced directly from any yeast strain by means of enzymatic manipulations on total or fractioned RNA content. We have identified previously undescribed L-A variant from different yeast strains specifically associated with certain type of M satellites. Moreover, we identified for the first time full 5′-UTR and 3′-UTR sequences of M2 satellite. Highly conserved sequence regions along with variable fragments were discovered at protein level, revealing clear trend to form clusters among different L-A Gag-Pol proteins. The obtained data suggest that each L-A virus variant can specifically maintain a distinct type of satellite dsRNA.

P-04.01.1-004
Physic-chemical characterization of PLGA adjuvants for immunization per os

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Antibodies against diphtheria toxin play the most important role in the immunity against *Corynebacterium diphtheriae*. All current diphtheria vaccines have parenteral route of administration. Undoubtedly, oral administration of antigens would be the most patient-friendly way of immunization. However, the efficacy of free antigens oral administration is limited by their degradation in the gastrointestinal tract and poor absorption by M-cells.

Biodegradable and biocompatible polymers, like poly (D,L lactide-co-glycolide) (PLGA), are widely used for the design of mucosal immunizing agents. Importantly, that the way of particle preparation plays an important role in PLGA biodegradation and antigen release.

The aim of this work was to characterize the main physic-chemical properties of two types of PLGA particles: with immobilized antigen (PLGA 1) and with encapsulated antigen (PLGA 2).

We have prepared two types of PLGA particles containing EGFP-SbB proteins (non-toxic recombinant fragment B of DT fused with eGFP). The antigen loading efficiency of particles was determined based on the ratio of protein concentration in solution before and after loading and shown better results for PLGA 2 particles (PLGA 1 – 72.05%, PLGA 2 –90.02%). The flow cytometry results demonstrated that 99% of PLGA1 particles conjugated with eGFP-SbB, and only 92.2% of PLGA 2 particles conjugated with protein.The particle sizes had the slight difference by the results of two different techniques (NTA – number based, the software tracks individual particles; DLS –scattering intensity weighted), however demonstrate similar patterns. DLS data showed that the mean PLGA 1 particles size was 203.3 nm and PLGA 2 – 211.6 nm. NTA data also showed that mean PLGA 1 particles size a little smaller than PLGA 2 (183.8 nm and 192.8 nm respectively).

Demonstrated differences in the properties of synthesized particles may have an influence on the immunogenicity of the used for oral immunization antigen.

P-04.01.1-005
A suitable system for studying the functionality of a Plasmodial protein in mammalian cell lines

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Malaria is one of the most significant causes of morbidity and mortality worldwide. In *P. falciparum* phospholipid biosynthesis has an essential role in the synthesis of membranes. The most prevalent way of *de novo* biosynthesis is Kennedy-pathway, where the reaction catalysed by CTP:phosphocholine cytidylyltransferase (CCT) (CTP+ChoPàCDPCho) is rate-limiting. Because of the persistent need of Plasmodium for membrane synthesis during its life cycle, *de novo* phospholipid biosynthesis emerges as a target for new generation antimalarial drugs.

CHO-MT58, a mutant cell line was proved to be an appropriate tool for investigating intracellular function of CCT. In this cell line, the endogenous CCT activity decreases dramatically at 40°C, blocking membrane synthesis and ultimately leading to apoptosis.

We have studied the rescuing potential of *Pf*CCT in CHO-MT58 cells with the isogenic CHO-K1 cells as a control. Cells after transient transfection were incubated at 40°C and then analysed by FACS using the fluorescence of EGFP fused to *Pf*CCT. The proportion of cells undergoing apoptosis was determined by propidium-iodide staining.

We have demonstrated for the first time that heterologously expressed *Pf*CCT is able to complement endogenous CCT activity in mammalian cells. Thus, a suitable system has been established for functional investigation of structural elements of *Pf*CCT.

In order to reveal the role of different protein sequences in enzymatic function, we redesigned the structural gene of *Pf*CCT obtaining a modular system where different domains are easy to be removed or exchanged. Here we designed a series of different truncation and deletion constructs to reveal the role of Plasmodium specific sequences. In parallel, heterologous expression experiments of different constructs in the mutant CHO-MT58 and the wild type control cell lines are performed to validate the reported model system.

P-04.01.1-006
Host-pathogen interactions: is there a relationship between TLR 4 polymorphisms and tuberculosis in a group of Turkish patients?

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Introduction: Tuberculosis (TB) is a global health problem and according to World Health Organization (WHO) each year more than 2 million individuals die from TB and each year20,000 cases of TB are notified in Turkey. Malatya is the third largest city in East Anatolian Region of Turkey and TB incidence rate is higher (28.5/100,000) comparing to the general population of the country. For this reason it is important to determine the factors that lead to TB in this population. Disease agent can stay in the latent phase for long periods of time after infecting the individuals. While some infected individuals show the symptoms some others never do and even 90% of these never develop clinical disease. Various mechanisms take place during the host response to infectious agents. Toll-like Receptor (TLR) genes are shown to be candidate genes in these responses.

Materials and Methods: In this study 49 TB patients and 50 healthy controls were included. TLR 4 genotyping for rs4986790, rs4986791 was performed by using a commercial TaqMan SNP Genotyping Assay kit. Data were summarized by count and percent. Hardy-Weinberg equilibrium was tested by chi-square distribution with 1 df. Differences between groups due to allelic and genotypic distributions were analyzed by Pearson's exact or Fisher's exact tests. In all comparisons significance level was considered to be 0.05.

Results: The single nucleotide polymorphisms (SNPs) which were subject of this study haven't been screened in Turkish population earlier. No significant association was found between TB and the SNPs we screened in our group of patients.

Discussion and Conclusion: Unlike other populations results we couldn't find a significant association between the disease and the genotypes of our patients. The study should be performed in bigger populations in order to confirm the results.

P-04.01.1-007
Lytic action of bacteriophages as a tool for the obtaining of images

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Obtaining of images by different types of bacteria now became a very special branch of skill at the interface between science and art. However the authors did not found any scientific article, where bacterial lawn was used as the background and the image was formed by the lytic action of the virus (bacteriophage). Whereas the mentioned approach could be used not only with artistic aims but for the practical use. The aim of this work was to demonstrate a possibility to obtain the image on the bacterial lawn by the lytic action of the bacteriophage.

The bacterial lawn was obtained by the standard metod using the 1.5% agar with the nutrient medium and the 0.7% agar containing *Escherichia coli* culture. Stencils with the preparation of the bacteriophage T4 were applied. Samples were incubated during the twenty-four hours at +37°C. After that stencils were removed and the samples were stained by *C*oomassie blue R-250 or fuchsine (with further fixation by the 7% acetic acid).

Several approaches to obtain the image by the lytic action of the virus were applied. First of all stencils made from printing paper and filter paper were compared. It was demonstrated, that the use of filter paper stensil allows to obtain more accurate and controllable images, than the use of the printing paper stensil. In the next series of the experiment the possibility of the reversed stencil use (where the image is formed not by the lytic zone but by the zone of bacterial growth) was demonstrated. Also the possibility of the partial staining of the obtained image was explored. It gives an opportunity to obtain polychrome images using available colorants.

Summarizing the above it should be noted, that it was the first time when the graphical image was obtained by the lytic action of the virus on bacteria. This approach could be used not only for the artistic aims but as well for the practical use, for example, for the restriction of the action of microorganisms in out-of-the-way places.

P-04.01.1-008
Searching for novel serodiagnostic markers of Lyme borreliosis in Latvia

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Lyme disease is the most common vector-borne disease in the temperate zone of Northern Hemisphere and an important emerging zoonosis in Europe, North America and Far Eastern countries. It is caused by the spirochetes of Borrelia burgdorferi *sensu* lato complex. Because of remarkable heterogeneity of Borrelia

burgdorferi the identification and characterization of possible antigens is essential for the improvement of current laboratory diagnostics for Lyme disease and vaccine development.

In this study, several recombinant B. burgdorferi outer surface proteins have been obtained and their antigenic properties have been evaluated in an effort to characterize novel immunodominant antigens. Because B. afzelii and B. garinii are the most prevalent species in Latvian ticks, proteins with conserved domains were included in this study. A panel of serum samples of Lyme disease patients with early and disseminate disease stage was used. The controls were matched by age and sex to the patients and represented the same geographic area.

The results show that proteins of several B. burgdorferi gene families have properties with respect to their candidacy as a subunit assay for a novel Lyme disease immunodiagnostic. Especially, the difference in their size in a range on the Western blot assay may provide good discrimination between protein bands. However, they have potential for diagnosis if used in combination with other antigens but not as a "stand alone" test.

In conclusions, this study showed the existing challenges in serological testing of early Lyme disease. The conservation of the sequence of antigen between species of B. burgdorferi complex is essential for the most successful serodiagnostic marker candidate. The presence of homologous proteins in Treponema species could lead to the cross reactivity in syphilis patients, and should be carefully evaluated.

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P-04.01.1-009
Comparative proteomic studies of ESBL producing *E. coli* isolated in Republic of Georgia

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Antimicrobial resistance is one of the greatest challenges in modern medicine. There is a pressing need for better understanding of the specific mechanisms that contribute to resistance to optimize existing therapies. In 2013 in Georgia extended-spectrum beta-lactamase (ESBL)-producing *E. coli* strain was isolated from the post-surgical sample obtained from gallbladder of the patients with chronic calculous cholecystitis which belongs to the sequence type 23 (ST23) complexes with CTX-M 55 gene. Is this strain characterized by other differences on a proteome level? Are antibiotics against which the strain is resistant inducing the changes in bacterial proteome? The present work was aimed (i) to study the differences on a proteome level (i) between *E. coli* 92-1917/13-G and ATTC *E. coli*-reference strain and (ii) to compare the proteomes of 1917 strain at two conditions: with and without antibiotics. 1917 strain was grown in the presence of three antibiotics: Rocephin (Ceftriaxone), Fortum (Ceftazydym) and Claforan (Cefotaxime sodium) together. Proteomic expression was analyzed using two-dimensional gel electrophoresis and mass spectrometry. Significant differences were found for several proteins, including putative ABC trnsporter arginine protein 2, cystine-binding periplasmic protein, FKBP-type peptidyl-prolyl cis-trans isomerase, Outer membrane protein A, D-galactose binding periplasmic protein and some others. The importance of these differences for anti-microbial resistance will be discussed.

P-04.01.1-010
Molecular characterization of resistance and virulence features in *Staphylococcus aureus* clinical strains isolated from cutanaeus lesions in patients with drug adverse reactions

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Patients treated with epidermal growth factor inhibitors often experience cutaneous adverse reactions. However, the infectious complications of these toxic effects and the contribution of specific pathogens, such as the community emergent methicillin resistant *Staphylococcus aureus* strains. The present study was aimed to identify the types of SCCmec and virulence genes profile in clinical *S. aureus* isolated from cutaneous lesions of different severity degrees in patients with dermatologic toxic effects.

This study was conducted on a total of 42 *S. aureus* clinical strains isolated in 2016 from acneiform reactions pustulae and periungual lesions in patients with drug cutaneous adverse reactions. Multiplex PCR was performed on genomic DNA from isolates in order to identify the SCCmeccentral elements and the virulence genes: bbp (bone bound sialoprotein), ebpS (elastin-binding protein), fnbB, fnbA (fibronectin-binding proteins), fib, clfA, clfB (clumping factors A and B), cna (collagen-binding protein), luk-PV (Panton-Valentine leucocidin), hlg (haemolysin), tst (toxic shock toxin).

The MRSA phenotype was genetically confirmed by the presence of mecI gene in case of 19.04%, mecA in 14.28%, SSCmec type IVd element in 11.90%, ccrB2 in 7.17% and SCCmec types I, III, IV in 4.76% of the studied *S. aureus* strains. Regarding the virulence genes encountered in *S. aureus* strains, the most frequent was clfA (90.47% of the isolates), followed by clfB (88.09%), fib (35.71%), hlg (16.66%) and bbp (14.28%).

These results confirm the high prevalence of mec I and SSCmec type IV elements, usually encountered in community-acquired MRSA strains, in cutaneous isolates from patients with dermatologic toxic effects. More data on the virulence and genetic background of these local strains are needed to appropriately assess the risk of such infections and avoid the inappropriate administration of beta-lactams.

P-04.01.1-011
Analysis of toxicogenic properties of *Staphylococcus aureus* strains isolated from cows with subclinical form of mastitis in the central area of Russian Federation.

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Staphylococcus aureus is the main causative agent of mastitis. The ability of *S. aureus* to cause the disease is determined by two main components: a variety of toxins and factors of resistance to antibiotics. The aim of this work is to determine virulence genes (mecA, mecC) and genes of toxins (phenol soluble modulines,

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pore-forming toxins and enterotoxins), which are present in *S. aureus* strains isolated from clinically healthy cows.

Staphylococcus strains were isolated from cow's milk. Disk diffusion method was used to determine the sensitivity to antibiotics. PCR analysis was used for detection of mecA, mecC genes and genes of toxins.

Investigated strains were resistant to oxacillin (14%), vancomycin (8%). It was found that all strains, which contain mecA and mecC genes, showed resistant to more than 5 antibiotics. It was determined that among the investigated strains 13% contained mecA, 13% - mecC, 9% contained both mecA and mecC. Some strains contained genes of Panton-Valentine leukocidin (PVL) or alpha-hemolysin and several strains contained both types of genes. Enterotoxin A (SEA) gene was detected in 26.7% of cases, SED - 5%, SEG - 10%, SEI - 35%. Genes of staphylococcal toxins B, C, E, H were not found. The presence of phenol soluble modulin biosynthesis genes was determined: genes of alpha peptide synthesis were found in 93% of strains, beta peptide toxin genes in 73%, delta toxin gene in 100%.

It was determined, that clinically healthy animals are carriers of *S. aureus* strains that cause mastitis. High level of *antibiotic resistance was found in* strains containing mecA and mecC genes. The major part of the strains carried genes of phenol soluble modulin biosynthesis. The role of phenol soluble modulins as well as of PVL and alpha-hemolyzin in the development of mastitis is not completely clear. We conclude that pore-forming toxins have dominant role in the latent form of mastitis.

P-04.01.1-012
Impact of lactoferrin on the hydrophobicity and adherence to the inert substratum of *Staphylococcus aureus* strains isolated from patients with cutaneous drug reaction

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Skin healing is a complex biological process that requires the involvement of different cell types and humoral effectors. One of the main factors are aggravating and delaying the healing process is represented by the *supra*-infection with pathogenic or opportunistic microorganisms that grow in specialized consortia embedded in a self-produced extracellular polymeric matrix, called biofilms, which are extremely resistant to any antimicrobials and host immune response. Lactoferrin (LF) is an iron-binding glycoprotein which promotes skin healing by enhancing the initial inflammatory phase, but also by inducing an over-abundant immune response. The aim of this study was to investigate the influence of LF, one of the main components of innate, humoral anti-infectious immunity on some microbial features, involved in the first steps of the infectious process, such as hydrophobicity and adherence of *Staphylococcus aureus* strains isolated from maculo-pustular lesions in patients with adverse reactions to epidermal growth factor inhibitors. For hydrophobicity measurement the bacterial suspensions were grown in the presence or absence of LF, and then, the "microbial adherence to hydrocarbons test" (MATH) was performed. The capacity to develop biofilms on inert substrata and the influence of LF on this feature was spectrophotometrically quantified using an adapted microtiter method, after crystal violet staining. Our results showed that LF decreased the hydrophobicity and limited the biofilm development of all 42 *S. aureus* tested strains, in a dose and time dependent manner. The decreasing effect on the microbial hydrophobicity was accompanied by a lowering effect on the adhesion of microbial strains to the inert substratum. In conclusion these observations indicate that LF exhibits a wound pro-healing effect, by limiting the microbial colonization and biofilm formation and thus, the occurrence of infectious complications of skin lesion.

P-04.01.1-013
Host-specificity determinants of bacteriophage vB_EcoM_FV3

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Medical and nanotechnological potential of bacterial viruses (bacteriophages or phages) includes strategies for detecting bacteria, curing bacterial diseases, decontaminating surfaces, or for drug delivery to the specific targets. Knowledge of phage host-specificity determinants is crucial for the success of phage applications. The aim of this work was to identify host cell receptors for *Escherichia* virus vB_EcoM_FV3 (later, FV3) attachment. FV3 belongs to the group of virulent enterobacterial phages that are proposed as therapeutic agents.

Since FV3 was isolated on *E. coli* strain K-12, we could use the single-gene knockout mutants of *E. coli* K-12 strain BW25113 (Keio collection) for the identification of FV3 receptor. Eleven mutants of the genes involved in lipopolysacchride (LPS) core biosynthesis, and 10 mutants lacking outer membrane proteins (OMP) were tested using spot assay of decimal dilutions of phage suspension. In addition, shiga toxin-producing E. coli (STEC) representatives belonging to five O-serogroups O26, O103, O111, O145, and O157 have also been tested for their susceptibility to FV3.

While outer membrane proteins are known to frequently serve as phage receptors, all OMP mutants tested and BW25113 were similarly susceptible to phage FV3. Four mutants of the genes affecting LPS inner core synthesis (*waaC*-, *waaF*-, *waaG*-, and *waaP*-) were resistant to FV3, whereas mutants with impaired outer core LPS were sussceptible. Also, out of five STEC strains tested, only that belonging to O111-serogroup showed negligible susceptibility to FV3.

Taken together, our data suggest that the LPS inner core with at least one glucose moiety added, and a particular O-antigen can serve as receptors for FV3 attachment, which is likely governed by the combined activity of phage-coded tail fiber adhesin and a tail spike protein. This research was funded by a grant (No. MIP-002/2014) from the Research Council of Lithuania.

P-04.01.1-014
Moleculer detection of enterotoxigenic and methicillin resistance *Staphylococcus aureus* isolates in water buffalo milk and dairy products

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Staphylococcus aureus is a gram-positive foodborne pathogen that causes a variety infectious in human and animals, including endocarditis and osteomyelitis. Raw milk and milk products are

considered vehicles of *S.aureus* intoxication in humans throughout the world. The objective of the present study was to assess the presence of enterotoxigenic and methicillin-resistant *S. aureus* in water buffalo milk and dairy products. A total of 100 water buffalo milk and 100 dairy products (50 water buffalo cream and 50 water buffalo cheese) were collected from different dairy farms, smallholders and local bazaars in Samsun, Turkey. All samples were analyzed using the standard procedure EN ISO 6888-1 and isolates were confirmed for the presence of the 16S rRNA and *nuc* gene by polymerase chain reaction. *S. aureus* was identified in 30 of 100 water buffalo milk (30%), 9 of 50 water buffalo cream (18%), and 17 of 50 water buffalo cheese (34%). A total of 99 isolates were confirmed as *S. aureus* by PCR. Genotypic methicillin resistance was evaluated using PCR for the *mecA* gene. Out of 99 isolates, 9 (9%) were found to be methicillin resistant (*mecA* gene positive) by PCR. The enterotoxigenic *S. aureus* was identified in 12 out of 99 (12%) isolates by the mPCR technique. Five isolates produced staphylococcal enterotoxins SEA (5/12; 41.6%), two isolates produced SEC (2/12; 16.6%), one isolate produced (1/12; 8.3%) SED, one isolate produced (1/12; 8.3%) SEE and three isolates produced SEC+SED (3/12; 25%). None of samples were positive for SEB. In conclusion, the presence of enterotoxigenic and methicillin-resistant *S. aureus* in milk and dairy products is of significant for public health concern and also these enterotoxin genes *sea* and *sed* are predominant toxins that can cause staphylococcus intoxication in humans.

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P-04.01.1-015
Identification and biochemical characterization of an immune modulating protein from *Helicobacter pylori*

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Helicobacter pylori is able to achieve persistent infection with minimal immune response. The first line of defence during *H.pylori* infection is through gastric epithelial cells which present Toll like receptors (TLR). A family of bacterial proteins which share homology with the Toll/IL-1 receptor (TIR) domain were identified. The structure of BtpA from *Brucella* showed that bacterial TIR proteins (BTP) mimick human TIR domain proteins and act on MyD88 signaling pathways to suppress TLR signaling. *H.pylori* might also produce a similar protein.

A putative *H. pylori* TIR protein was found based on sequence homology and the corresponding gene; hp1437; was cloned in fusion with an N terminal cleavable 6his-tag. The recombinant protein, 6his-1437 was purified using nickel affinity chromatography. 1437 was subjected to limited proteolysis and the bands were analyzed by peptide mass fingerprinting (PMF). Oligomerization of 1437 was investigated by *in vitro* pull-down and size-exclusion chromatography.

1437, a 239 amino acid protein, has a predicted C terminal TIR domain similar to other BTPs and sequence alignments verified the presence of TIR domain signature regions. Recombinant 6his-1437 was produced with a yield of 10 mg/l culture. A structurally stable 25 kDa fragment was obtained from limited proteolysis which contained the TIR domain as verified by PMF. *in vitro* pull down assays showed 1437 interacts with itself forming dimers as shown by size-exclusion chromatography.

TIR domain proteins function by interacting with themselves and other TIR domains. Our results showed that 1437 also form dimers, supporting that it is a BTP. Current research is focused on solving the structure of 1437 and investigating its interaction with MyD88. 1437 might play a direct role in reduced immune response against *H.pylori* by binding to MyD88 analogous to other BTPs. Further characterization of 1437 will provide the first solid evidence of presence of a TIR domain protein in *H.pylori*.

P-04.01.1-017
Lipopolysaccharides with different lipid A acylation status from *Vibrio cholerae* and *Campylobacter jejuni* contribute differently to IL6 production by bone marrow-derived macrophages

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Lipid A is a biologically active part of lipopolysaccharide (LPS) from Gram-negative bacteria that is responsible for the activation of the innate immunity through interaction with Toll-like receptor 4 (TLR4) and subsequent production of proinflammatory cytokines. Bacteria frequently transform their lipid A so that its recognition by TLR4 is not sufficient for induction of effective antibacterial immune response.

We compared biological activity of various LPS from pathogenic bacteria *Vibrio cholerae* and *Campylobacter jejuni*. We purified R-form LPS for each strain by hydrophobic chromatography. The biological activity of LPS preparations was evaluated by their ability to activate production of proinflammatory cytokine IL6 by bone marrow-derived macrophages from C57BL/6 mice, using TLR4-deficient macrophages to control for specificity of TLR4 signaling. LPS from *E. coli* and inactive LPS from *F. tularensis* were used as positive and negative controls.

LPS from *V. cholerae* demonstrated biological activity similar to that of LPS from *E. coli*, consistent with the presence of highly acylated lipid A in both strains. However, the former was a slightly weaker activator than the latter, because lipid A from *V. cholerae* had on average shorter acyl chains. Lipid A from *C. jejuni* had on average longer acyl groups than in *E. coli*, while degree of acylation was lower, and as a result its lipid A displayed significantly lower biological activity.

Our study demonstrates importance of functional groups of lipid A in the ability of LPS to activate production of IL6 by macrophages. In line with our previous reports, we confirmed a direct correlation between biological activity of various LPS species with their lipid A acylation status: the biological activity increases with increase in the length and in the number of the acyl chains. Excess proinflammatory cytokine production through TLR4 activation can cause sepsis, while inefficient activation may result in the failure to clear bacteria.

P-04.01.1-018
Development of a biosensing system for *E. coli* cells based on antimicrobial peptide cecropin

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Introduction: Antimicrobial peptides are cationic peptides which recognize bacteria specifically. Cecropin antimicrobial peptide family is specific for *E. coli* cells and interacts with the outer membrane proteins of them. Herein this study, we used Cecropin A peptide as a recognition element of constructed biosensing system. We designed a genetically encoded fusion protein system consisting of yellow fluorescent protein (YFP) and Cecropin A peptide. We investigated changes on fluorescent intensities of YFP as a function of *E. coli* cells.
Objectives: The aim of this study is developing a new detection system for real time and easy analysis of bacteria. The major objective of this work is also creating a model, simple detection system for pathogenic bacteria.
Materials & methods: The gene encoding fusion biosensor protein CEC-YFP was cloned, produced in *E. coli*, and then it was purified by using IMAC.
Results: A significant increase in fluorescent intensities of YFP was observed depending on the increasing concentrations of *E. coli* cells. Biosensing protein exhibited a logarithmic response range toward *E. coli* cells from $1X10^3$-$12X10^3$.
Conclusion: The developed biosensor is a promising detection system that could be an alternative to time consuming and expensive polymerase chain reaction (PCR) based methods. The cecropin peptide needs living *E. coli* cells for interactions. So, developed biosensor has the potential to classify *E. coli* cells based on their viability. Moreover, this system is a unique model for the detection of living pathogenic bacteria.

P-04.01.1-019
Cytotoxicity induced by Clostridium perfringens phospholipase C requires internalization and activation of multiple signaling pathways

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Clostridium perfringens phospholipase C (CpPLC) is the most toxic extracellular enzyme produced by this bacterium and it is an essential virulence factor in the pathogenesis of gas gangrene. CpPLC may lead to cell lysis at concentrations that causes extensive degradation of plasma membrane phospholipids. However, at sublytic concentrations it induces cytotoxicity without causing evident membrane damage. The results of this work demonstrated that the cytotoxic effect of CpPLC requires its internalization and the activation of the MEK-ERK pathway. CpPLC internalizartion occurs through a dynamin-dependent mechanism and in a time progressive process: first, CpPLC colocalizes with caveolin both at the plasma membrane and in vesicles, and later it colocalizes with early and late endosomes and lysosomes. The results also showed that CpPLC requires endocytosis in order to activate MEK-ERK, because treatment with the dynamin inhibitor, dynasore, prevents CpPLC endocytosis, ERK 1/2 activation and cytotoxcity. Cholesterol sequestration as well as inhibition of actin polymerization also prevents CpPLC internalization and cytotoxocity, involving endocytosis in the signaling events required for CpPLC cytotoxic effect. Once internalized, CpPLC induces reactive oxygen species production through the activation of PKC, MEK/ERK and NFκB dependent pathways. Inhibition of either of these signaling pathways prevents CpPLC's cytotoxic effect. In addition, it was demonstrated that NFκB inhibition leads to a significant reduction in the myotoxicity induced by intramuscular injection of CpPLC in mice. These data provide new insights about the mode of action of this bacterial phospholipase C, previously considered to act only locally on cell membrane. Understanding the role of these signaling pathways could lead towards developing rational therapeutic strategies aimed to reduce cell death during a clostridial myonecrosis.

P-04.01.1-020
Apoptosis induced by Clostridium perfringens phospholipase C is mediated by reactive oxygen species

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Bacterial phospholipases are lipolytic esterases surface associated or secreted by a wide variety of bacterial pathogens. *Clostridium perfringens*, the most broadly distributed pathogen in nature, secretes a prototype phospholipase C (PLC), also called α-toxin, which plays a key role in the pathogenesis of gas gangrene. This toxin causes death to cultured cells and extensive myonecrosis when injected intramuscularly in experimental animals. The results of the present study showed that *C. perfringens* PLC (3–5 ng/ml) induces morphological and biochemical changes characteristic of apoptosis in cultured cells, as determined by scanning electron microscopy. Nuclei condensation and fragmentation were observed by fluorescence microscopy and a typical ladder fragmentation pattern of genomic DNA was detected by DNA in agarose gels. Cell death was prevented by the caspases inhibitors Z-DEVD-fmk and Z-VAD-fmk. *C. perfringens* PLC induces oxidative stress in cultured cells as determined by fluorescence microscopy and flow cytometry using the membrane permeable probe DCFDA. Different antioxidants including the gluthation precursor NAC, several iron chelators and the free radical scavengers tiron and edaravone prevent cell death induced by *C. perfringens* PLC in cultured cells or in mice challenged intramuscularly with 1.2 μg of that toxin. Thus, this work provides compelling evidence that superoxide, hydrogen peroxide, and the hydroxyl radical are involved in the cytotoxic and myotoxic effects of *C. perfringens* PLC. Furthermore, the data demonstrated that edaravone, a clinically used hydroxyl radical trap, reduced the myonecrosis and the mortality caused by *C. perfringens* in a murine model of gas gangrene, induced by intramuscular bacterial injection of 10^8 bacteria. This knowledge provides new insights for the development of novel therapies to reduce tissue damage during clostridial myonecrosis.

P-04.01.1-021
M-COPA, a novel Golgi disruptor, suppresses apoptosis induced by Shiga toxin

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Shiga toxin (Stx)-producing *Escherichia coli* (STEC), including O157, O104 and O111, causes diarrhea and hemorrhagic colitis in the gut. When Stx traverses the epithelium and passes into the circulation system, it occasionally causes systemic complications such as encephalopathy and hemolytic-uremic syndrome sometimes resulting in the death of the infected patients. Therefore, the development of an antidote to prevent the lethal effects of Stx is urgently required. We previously reported that Stx induces rapid apoptosis in THP1 cells and CD77 synthase transfected U937 cells. Upon Stx treatment, apoptosis inhibitory proteins were rapidly downregulated, and proteasome inhibitors prevented the reduction of them and the progression of apoptosis. In an effort to develop novel antidotes against Stx, we found that 2-methylcoprophilinamide (M-COPA), a compound under development as an anti-cancer agent, suppresses Stx-induced apoptosis.

THP1 cells and U937/CD77 synthase cells were treated with Stx1 and Stx2 in the presence of test compounds, and the progression of apoptosis was measured by propidium dye exclusion assay. The morphology of the cells treated with Stx and M-COPA was observed by immunofluorescence microscope.

M-COPA suppressed Stx1/2-induced apoptosis in a dose-dependent manner and at 10 μM apoptosis was almost completely suppressed. M-COPA also suppressed the reduction of anti-apoptotic proteins, FLIP and Mcl-1, indicating that M-COPA interferes with an event upstream of the degradation of anti-apoptotic proteins. In the M-COPA-treated cells, the structure of Golgi apparatus was seriously affected and the localization of Stx to this organelle was completely abolished.

These results indicate that M-COPA disrupts Golgi structure and inhibits retrograde transport of Stx as does brefeldin A. This implies that inhibition of retrograde transport by chemical compounds such as M-COPA may be a novel strategy to suppress the fatal effects of Stx.

P-04.01.1-022
Study of lectins from *Photorhabdus luminescens* to reveal their function in *P. luminescens* life cycle

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Lectins are ubiquitous proteins able to recognize mono- and oligosaccharides with high specificity and low affinity. Lectins do not have any catalytic activity, unlike enzymes, and they are not products of the immune system in contrast to antibodies. Lectins play a crucial role in cell interactions on molecular level showing their importance in various physiological and pathophysiological processes as well as both mutualistic and parasitic interactions between microorganism and hosts.

Photorhabdus luminescens is a Gram-negative bacterium from the family Enterobacteriaceae. The bacteria have a complex life cycle that involves mutualistic and pathogenic interaction with two different invertebrate hosts. It is highly pathogenic towards insect larvae. In addition, *P. luminescens* lives in the intestine of infective juveniles of nematode *Heterorhabditis bacteriophora*, together forming an effective entomopathogenic complex.

We have identified several soluble lectins produced by *P.luminescens*. In this study, we focus on 4 proteins from *P. luminescens*, which show a high sequence homology with each other. A wide range of methods was used for structural and functional studies of *Photorhabdus* lectins, e.g. surface plasmon resonance, isothermal titration calorimetry, analytical ultracentrifugation and X-ray crystallography. All lectins from *P.luminescens* recognize l-fucose and d-mannose. Despite being closely related, they differ in fine binding specificities. To determine their biological function, knock-out mutants of *P. luminescens* are being prepared to study its interaction with axenic nematodes and insect larvae.

Sunday 4 September 12:30–14:30

DNA repair and cancer

P-05.01.1-001
Escherichia coli AlkA and AlkB proteins repair of 1,N6-α-hydroxypropanoadenine and 3,N4-α-hydroxypropanocytosine *in vivo* and *in vitro*

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coli 3-methyladenine DNA glycosylase II (AlkA) is a DNA repair enzyme that removes lesions in DNA *via* the base excision repair pathway. It can excise a variety of alkylated DNA lesions including 3meA, 7meA, 3meG and εA. *E. coli* AlkB dioxygenase and its human homologues are repair enzymes that remove alkyl lesions from bases *via* an oxidative mechanism restoring native DNA structure. They belong to the superfamily of 2-oxoglutarate and Fe (II) dependent dioxygenases. Both AlkA and AlkB are induced within *E. coli* system of adaptive response to alkylating agents (Ada response).

1,N^6-α-hydroxypropanoadenine (HPA) and 3,N^4-α-hydroxypropanocytosine (HPC) are formed in reaction of adenine and cytosine with acrolein (ACR). ACR is a mutagenic agent originated from different sources including cigarette smoke, exhaust fumes and overcooking. It is also generated endogenously during oxidative stress as a by-product of lipid peroxidation.

The test system used comprised the pIF101-106 plasmids bearing the lactose operon of CC101-106 origin which allowed to monitor Lac^+ revertants, that arose by particular base substitution. To avoid ACR cytotoxicity, we generated *in vitro* HPA lesion in plasmid DNA. ACR modified plasmids were introduced into *wt*, *alkA* and *AlkB* bacterial cells, and mutants (Lac^+ revertants) were selected. The observed differences of mutation frequencies indicate that both of the lesions are mutagenic. HPA causes mainly A→T and A→G substitutions, whereas HPC – C→T transitions followed by C→A transversions. For the first time, we have shown that AlkA glycosylase, together with AlkB dioxygenase, efficiently repairs HPA and HPC *in vivo*. These lesions are new, unpublished so far, substrates for AlkA and AlkB enzymes. As AlkB and AlkA act, respectively, on ssDNA and dsDNA together they constitute a complete defense against acrolein adducts to adenine and cytosine.

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P-05.01.1-002
Investigating the effects of Hericium erinaceus extracts on telomerase activity in MCF-7 cells

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Hericium erinaceus (Basidiomycetes) class, is an edible mushroom widely grows on dead or decaying trees especially in Japan and China.

The aim of this project is to determine the effects of *Hericium erinaceus* extracts on telomerase activity in MCF-7 estrogen receptor positive breast cancer cell lines. Firstly, we determined the effects of six different *Hericium erinaceus* extracts (Ethanol, Ether, Ethylacetate, Ethanol-water, Methnol-water, Water) on cell viability in MCF-7 cells by WST8 method. Cell culture experiments were carried out with different doses and incubation times of *Hericium erinaceus* extracts to evaluate the dose response of extracts. Water extract was found to be the most effective extract among others in the inhibition of cell viability. The $IC_{(50)}$ dose of *Hericeum erinaceus* water extract was detected as 250 μg/ml at 72 hours in MCF-7 cells. After incubation with 250 μg/ml water extract for 72 hours, cells were collected and effects of water extract on telomerase activity were analyzed.

Hericium erinaceus showed no significant effect on telomerase activity in MCF-7 breast cancer cells. Therefore, it is contemplated *that* water extract of *Hericium erinaceus* shows its cytotoxic effects through other mechanisms in MCF-7 cells.

P-05.01.1-003
Modulation of DNA-dependent protein kinase by a reactive nitro-benzoxadiazole compound

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Abstract: The expression and activity of DNA-dependent protein kinase (DNA-PK) is related to a DNA repair status in the response of cells to exogenous and endogenous factors. Recent studies indicate that EGFR is involved in modulating DNA-PK. It has been shown that a compound NSC 228155 (NSC), bearing a nitro-benzoxadiazole (NBD) scaffold, enhances tyrosine phosphorylation of EGFR and triggers downstream signaling pathways. Here, we studied the behavior of DNA-PK and other DNA repair proteins in prostate cancer cells exposed to compound NSC. We showed that both the expression and activity of DNA-PKcs (catalytic subunit of DNA-PK) rapidly decreased upon exposure of cells to the compound. The decline in DNA-PKcs was associated with enhanced protein ubiquitination, indicating the activation of cellular proteasome. However, pretreatment of cells with thioglycerol abolished the action of compound NSC and restored the level of DNA-PKcs. Moreover, the decreased level of DNA-PKcs was associated with the production of intracellular hydrogen peroxide by stable dimeric forms of Cu/Zn SOD1 induced by compound NSC. Our findings indicate that reactive species of oxygen and electrophilic intermediates, generated and accumulated during the redox transformation of NBD compounds, are primarily responsible for the rapid modulation of DNA-PKcs functions in cancer cells.

Keywords: DNA-PKcs; DNA repair; hydrogen peroxide; SOD1; nitro-benzoxadiazole; chemiosensitization; protein targeting; prostate cancer.

P-05.01.1-004
Doxorubicin induces multidrug resistance in MDA-MB-231 breast cancer cell line

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Breast cancer is the major disease of women in developed countries occuring predominantly after the age of 65. Triple negative breast cancer (TNBC) is a typical subtype of epithelial breast cancer which lacks estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) all together. Although various researches have been focused on characterizing TNBC and enlightening different molecular markers with the aim of improving the overall outcome, currently the sole affective therapy action for TNBC is chemotherapy. Thus chemoresistance is the main clinical challange and accounts for 90% of failures in terms of treating the disease. Multidrug resistance (MDR) is defined as simultaneous resistance towards the drugs which do or do not demonstrate structural resemblance and have different effects on their molecular targets. P-glycoprotein (P-gp) is a membrane protein coded by ABCB1 (MDR-1) gene. P-gp is an ATP-dependent pump which pumps a wide range of drugs out of the cells including chemotherapeutic agents such as doxorubicin (DOX) and pactilaxel.

In the present study, TNBC cell line MDA-MB-231 was treated with increasing doses of DOX, cell viability was examined with SRB assay and development of MDR was investigated through MDR assay and RT-PCR. Results demonstrated that cell viabiliy decreased significantly with the treatment of higher doses. MDR was shown to be increased when cells were treated with 50, 200 and 800 nm of the drug respectively along with 15 μM of P-gp inhibitor verapamil. RT-PCR results were obtained to be consistent with MDR assay results and indicated increased MDR-1 gene expression with the treatment of DOX. Especially after 400 nM of DOX treatment, MDR-1 was overexpressed to be 59 fold when compared to control.

In conclusion, it was demonstrated that MDA-MB-231 cells have shown to display elevated resistance to higher doses of DOX.

P-05.01.1-005
Targeting DNA damage response pathway in cancer cells under heat stress and the mechanical effect of ultrasound

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Ultrasound (US) has been widely utilized for diagnosis and therapy in many medical fields. The biophysical modes of US are divided into three classes, thermal, cavitation and non-thermal non-cavitation effects. In clinical use for cancer therapy, the thermal effect was utilized for hyperthermia therapy with focusing US on cancer to rise the temperature from 41 °C to 44 °C, or

further which could induce thermal ablation of cancers. Cavitation leads to a variety of mechanical stress such as shear stress, shock wave, high pressure, and chemical stress such as free radical formation, both of which have been inferred to act simultaneously on all biological materials. It has been indicated that US induces cell killing, cell lysis, loss of viability, and loss of clonogenicity. Recently, we found that heat stress as well as US without thermal effect induce not only DNA single-strand breaks but also DNA double-strand breaks, a most cytotoxic region of DNA, in chromatin DNA detected by both gammaH2AX staining and neutral comet assay. In response to the stresses which induce DNA damage, the DNA damage sensor protein kinase, ataxia telangiectasia mutated (ATM), ATM and Rad3 related (ATR), and DNA-dependent protein kinase (DNA-PK) become activated form to initiate signal transduction pathways activating cell-cycle checkpoints, DNA repair, and apoptosis. The molecules consisting of DNA damage response pathway were expected as therapeutic targets because defects in the response to DNA damage agents can be lethal. This work was designed to explore the possible therapeutic targets of the molecules in DNA damage response pathways for future US-aided therapy. Finally, several kinases (e.g., checkpoint kinase) on DNA damage response pathway seems to be the targets for hyperthermia and US therapy.

P-05.01.1-006
Biotechnological synthesis of new nucleosides based on 2-aminopurine with an amino acid fragment at C6 position

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Based on the recently synthesized (*S*)-(2-aminopurin-6-yl) amino acids (Gly, Ala, Val, Phe, Pro), we obtained a series of novel modified nucleosides using the transglycosylation reaction. For the first time, it has been demonstrated that the corresponding nucleobases are good substrates for the genetically engineered recombinant *E. coli* purine nucleoside phosphorylase (conversion to nucleosides reached 90–98%). Nucleosides, such as ribosides, 2-deoxyribosides, and arabinosides were obtained in high yields (70–88%). It has been found that yield in the transglycosylation reaction does not depend on the structure of the amino acid fragment. The nucleosides synthesized are considered as potential inhibitors of intracellular adenosine deaminase (AD), the increasing activity of which is observed in hepatitis, cirrhosis, hemochromatosis, obstructive jaundice, prostate and bladder cancer, hemolytic anemia, rheumatic and typhoid fever, gout, and Cooley's anemia. Cytotoxicity of the synthesized nucleosides was tested in the Jurkat (model of human T-lymphoblastic leukemia) and EL-4 (model of mice T-lymphoblastic leukemia) cell lines. The compounds studied did not exhibit cytotoxic activity compared to the activity of the known antitumor agent Nelarabin. The work was financially supported by the Russian Science Foundation (grant 14-13-01077).

P-05.01.1-007
DNA binding, DNA cleavage, antimicrobial activities, antimutagenic and anticancer studies of a Schiff base and its complexes

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Schiff bases are considered as favored and the most widely used ligands, due to their metal complexes having variety of applications as antibacterial and anticancer agents. The rational design and synthesis of new Schiff bases and their metal complexes have been drawing great interest because of their diverse biological and pharmaceutical activities. So, exploring and designing novel molecules that have biological activities and capable of interacting with nucleic acids has a great significance for disease defence and to discover new DNA-targeted anticancer drugs for chemotherapy. In this study, we report the synthesis and characterization of a novel Schiff base and its Ni(II) and Cu(II) complexes. The minimal inhibitory concentration (MIC) of the compounds was screened *in vitro* against bacteria and yeast cultures using broth micro dilution test. DNA binding and DNA cleavage activity of the compounds were investigated by UV-Vis spectroscopy and agarose gel electrophoresis. Antimutagenic activity of compounds were tested in the absence of microsomal enzymes (S9-). Also, cytotoxicity of the compounds against HepG2 cell lines was assayed by the MTT (3-(4,5-dimethylthyazolyl-2)-2,5-diphenyltetrazolium bromide) method.

Consequently, UV-Vis spectroscopy studies indicated that the compounds interact with Calf Thymus DNA (CT-DNA) via intercalative binding mode. DNA cleavage activity studies showed that the Cu(II) complex can effectively cleave pBR322 plasmid DNA. Compounds inhibited the base pair mutation with high inhibition rate in the absence of S9. Also, Schiff base complex had cytotoxic activity towards HepG2 cell line, that it was found to be more potent than the control cisplatin.

P-05.01.1-008
Single particle electron tomography of RNAP elongation complex, stalled at position +24

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Genome *in vivo* is constantly exposed to the damaging effects of the environment. Single-strand breaks (SSBs) are the most frequently occurring DNA lesions. Accumulation of unrepaired SSBs can interfere with the cells metabolism and increase genomic instability. *In vivo*, SSBs are repaired in specific pathway, but, in eukaryotic nuclei, DNA is organized in chromatin that could affect the accessibility of lesions to sensor proteins. Breaks in a template strand induce arrest of RNA polymerase II (PolII) *in vitro* and *in vivo* and can be revealed in a transcription-dependent manner.

Our recent biochemical studies identified two key intermediates formed during transcription through a nucleosome by RNAP that are nearly homogeneous, active and stable by biochemical criteria (complexes stalled after entering 24 or 42 bp into the nucleosome; EC+24 or EC+41, respectively).

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Hear we produced two complexes, both stalled in the +24 position, one without break in the DNA, and the other with introduced SSB at position +12 of a non-template DNA strand. Complexes were purified using affinity chromatography and applied to a carbon-coated, glow-discharged EM grid. Tomographic studies were performed at ±70° in a JEOL microscope at 200 kV accelerated voltage. Images were recorded using a Gatan CCD camera. Image analysis was performed using the IMOD software.

The resulting structure of the EC+24 complex with no break in DNA consist of two domains, connected by a single DNA string. The complex with a break introduced into the DNA has a more compact appearance and its two domains were connected by two DNA strings, thus forming an intranucleosomal DNA loop. Our data suggest that SSBs in a non-template strand can induce the formation of stable non-productive transcription intermediate. The inhibitory effect of SSBs onto transcription may suggest a possible mechanism for their recognition *in vivo* with a transcription-dependent pathway.

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P-05.01.1-009
Investigation of four colorectal cancer related single nucleotide polymorphisms in Turkish sporadic colorectal cancer cases

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Colorectal cancer (CRC) is one of the leading causes of cancer-related deaths in the developed countries. According to 2014 WHO report new incidence rate of CRC in Turkey is 6.5% among other cancer types. Owing to difficulty of the low allele frequency variations detection, genetic association profiles of CRC have not been entirely identified. Low allele frequency variations MLH1 −93G>A (rs1800734) promotor substitution, MLH1 415G>C (rs28930073) exonic substitution, MTHFR C677T (rs1801133) and APC 1307 T>A (rs1801155) were investigated in this study.

These 4 SNPs "rs1800734, rs28930073, rs1801133, rs180115" are located on 1p36.3, 5q21, 3p22 respectively. Colonoscopic investigations were performed on both cancer and control group. The 4 SNPs were genotyped using Kompetitive Allele Specific PCR technology in 1014 cases and 805 healthy controls. Statistical analysis was carried out with Cochran-Armitage chi-square test.

In this study these 3 of the 4 SNPs in MLH1, MTHFR genes were examined for the first time in Turkish sporadic CRC cases. Statistical analysis showed no significant association within our Turkish sporadic CRC population. Percentage of MLH1 −93AA genotype in group aged ≥ 65 was found to be 5.7% in cancer versus 2% in control group. Moreover APC 1307A, MLH1 415C alleles were detected only 1 and 3 allele respectively.

Previously, APC 1307A allele was determined in 53.3% of a Turkish cohort. However in the present study APC 1307A allele was detected on 1 allele only. Studies showed MLH1 −93 promoter variation as a risk factor for microsatellite instabile CRC but for the current study this data is not available. In spite of literature MTHFR C677T and MLH1 415G>C SNPs were not found to be associated with sporadic CRC in Turkish population. This research demonstrates that importance of population based studies in multifactorial disease.

P-05.01.1-010
Excision of damaged bases from transcription intermediates by Fpg/Nei superfamily DNA glycosylases

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Oxidative lesions are abundant due to constant presence of reactive oxygen species in living cells. Repair of oxidative base lesions is initiated by DNA glycosylases. For example, bacterial Fpg and Nei DNA glycosylases excise oxidized purines and pyrimidines, respectively, from DNA. Their human homologs, NEIL1 and NEIL2, have been reported to show preference towards oxidized lesions in DNA bubbles. From these observations, it had been hypothesized that NEIL proteins may be involved in the repair of lesions in DNA bubbles generated during transcription. However, it is not presently clear how NEILs would behave on bubbles more closely resembling transcription intermediates (e. g., containing the RNA strand), and bacterial homologs Fpg and Nei had never been investigated with bubble substrates.

We have studied excision of either 8-oxoguanine (8-oxoG) or 5,6-dihydrouracil (DHU) by *E. coli* Fpg and Nei and human NEIL1 and NEIL2 from single-strand oligonucleotides, perfect duplexes, bubbles with different number of unpaired bases (6 to 30), D-loops with DNA or RNA and from complexes with RNA polymerase.

Fpg, NEIL1 and NEIL2 efficiently excised DHU located inside a bubble. Fpg and NEIL1 was generally more active than NEIL2 in excision of 8-oxoG from ssDNA and bubbles. Nei, on the other hand, was active only on DHU located in dsDNA (either perfect duplex or DNA/DNA D-loop). Fpg and NEIL1 also have shown activity in D-loops with RNA. The presence of an additional unpaired 5′-tail of the third strand of D-loops didn't affect the glycosylases activity. The activity of Fpg was observed in pre-assembled transcriptional complexes with *E. coli* RNA polymerase and depended on the position of the lesion in the transcription bubble, possibly reflecting local accessibility of the lesion within the elongation complex.

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Repair of bulky lesions in DNA of mammalian cells: a properties analysis of model substrates, simulating various types of damaged DNA

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Nucleotide excision repair (NER) is a multistep process that eliminates a wide range of lesions in DNA, including UV photoproducts and base modifications by many carcinogenic and chemotherapeutic agents.

One of the advanced approaches to NER process investigation is based on reproducing the repair reaction by mixing protein extracts from mammalian cells with model linear DNAs, bearing lesions. Long linear DNAs (137 bp) containing efficiently recognized and processed by NER system lesions (fluoro-azidobenzoyl

photoactive lesion Fab-dC, nonnucleoside lesions nFlu and nAnt) in both strands have been synthesized.

We have demonstrated that DNAs containing closely positioned lesions in the both strands represent difficult-to-repair (Fab-dC/nFlu(+4), Fab-dC/nFlu(−3)) or unrepairable (nFlu/nFlu (+4), nFlu/nFlu(−3), nAnt/nFlu(+4), nAnt/nFlu(−3)) structures. Besides, it has been shown that model DNAs bearing 2 bulky lesions in opposite positions (Fab-dC/nFlu(0), nFlu/nFlu(0)) represent unrepairable structure as well. The model substrates with increasing distance between lesions in the duplex demonstrated the full recovery of substrate properties in NER process (Fab-dC/nFlu(+8), Fab-dC/nFlu(−10), Fab-dC/nFlu(−21), nFlu/nFlu (+8), and nAnt/nFlu(+8)), whereas the level of specific excision from nFlu/nFlu(−10), nFlu/nFlu(−21) and nAnt/nFlu(−10), nAnt/nFlu(−21) was approximately 50% of the nFlu/dG or nAnt/dG DNA respectively.

It has been shown that modified DNA-duplex (54 bp) with Fab-dC has decreased structurally dependent affinity for XPC–HR23B compared to duplexes containing lesions in both strands being analyzed (Fab-dC/dG, Fab-dC/nFlu(+4), Fab-dC/nFlu (−3), Fab-dC/nFlu(+8), Fab-dC/nFlu(−10), Fab-dC/nFlu(–21)) and increased compared to umDNA.

The data provide an argument that the NER system of higher eukaryotes recognizes and eliminates injured DNA fragments on a multi-criteria basis.

P-05.01.1-012
DNA binding, photocleavage and topoisomerase I inhibition of novel water soluble phthalocyanine compounds

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It is well known that DNA plays crucial role in the biological system because of including all the genetic information for cellular function. Therefore, the interaction of molecules with DNA has gained interest in the medicinal chemistry to explore new anticancer agent. Photodynamic therapy which is alternative cancer treatment method depends on free radicals and singlet oxygen to destroy tumor tissue via necrosis and apoptosis. Phthalocyanines (Pcs) are used for photodynamic therapy because of their absorption of high wavelength light ability and they have high triplet quantum state yields and long lifetimes in triplet states. Also they do not have any toxic effect without light. In this study the novel synthesized 4-[2-(2-morpholin-4-ylethoxy) ethoxy]phthalonitrile substituted zinc(II), manganese(II) and copper(II) phthalocyanines were used. The potential properties of phthalocyanine compounds for photodynamic therapy were purposed to reveal by the preliminary work. For this aim, the mode of DNA binding, photocleavage and topoisomerase I inhibition of these compounds were investigated.

4-[2-(2-morpholin-4-ylethoxy) ethoxy]phthalonitrile substitued zinc(II), manganese(II) and copper(II) phthalocyanine compounds have been synthesized. The interaction of novel Pcs compounds with calf thymus (CT) DNA was investigated by using UV-Vis spectroscopy, thermal denaturation studies and viscosity measurements. Additionally, DNA photocleavage and topoisomerase I inhibition studies were performed to pBR322 DNA by using agarose gel electrophoresis.

The interaction studies indicated that Pcs compounds powerfully bound via an intercalation mechanism with CT-DNA. These compounds showed efficiently DNA photocleavage under irradiation at 650 nm. The all of Pcs inhibited topoisomerase I in a dose-dependent manner.

All the experimental studies showed that Pc compounds might be used agents for photodynamic therapy.

P-05.01.1-014
Target search by base excision repair DNA glycosylases

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The problem of rapid target search in DNA is faced by transcription factors, restriction endonucleases, DNA repair enzymes and other sequence- or structure-specific DNA-binding proteins. Theoretically, the fastest target search in DNA can be achieved by combining one-dimensional diffusion along the DNA contour (processive search) and three-dimensional diffusion (distributive search). The balance between these search modes depends on many factors affecting DNA-protein interactions, such as the presence of mono- and divalent cations, competing proteins, crowding effect, etc. Presently, the mechanisms of target search are understood only for a handful of enzymes.

We have recently developed an assay to study target search by DNA repair enzymes, based on cleavage of oligonucleotide substrate containing two targets. Thus, the distance between the targets can be precisely controlled, and any modification can be introduced into DNA. Subsequently, the probability of correlated cleavage (P_{cc}) is estimated, reflecting the efficiency of enzyme transfer between the specific sites. In this work, we have investigated five repair enzymes: *E. coli* endonuclease VIII (Nei), its human homologs NEIL1 and NEIL2, and uracil-DNA-glycosylases (UNG) from *E. coli* and vaccinia virus.

As expected, P_{cc} of all enzymes depended on the ionic strength of the solution and the presence of Mg^{2+}. UNG from vaccinia virus was the most sensitive to these factors, raising questions about its proficiency as a suggested processivity factor of viral DNA polymerase. Nei, NEIL1 and NEIL2 showed a peak of P_{cc} at low but non-zero ionic strength indicating that nonpolar interactions contribute to binding of these proteins to nonspecific DNA. This conclusion was also supported by analyzing amino acid conservation in the catalytic core of Nei. Introduction of bulky fluorescent group between two specific sites greatly reduced the ability of glycosylases to slide along DNA.

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P-05.01.1-015
Does causes 1800 MHz magnetic field application KRAS and P53 mutations in colon?: occurences histopatologically and microbiologically changes in colon

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Introduction: The aim of this study was to determine effects of 1800 MHz magnetic field on the amout of colonic *Bacteroides* and *Fusobacterium* and colon histopathology in rats and the

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determination of kirsten rat sarcoma (KRAS) and p53 gene mutations in colon.

Materials and Methods: In this study, three groups were prepared as control,sham and electromagnetic field (EMF) group.1800 MHz radiofrequency (RF) radiation was produced by using an electromagnetic energy generator.The EMF group rats were exposed to electromagnetic field for 12 weeks as 45 minutes per day.At the end of experiments, rats were sacrificed under ethyl ether anesthesia and the rat colons were dissected.Fecal speciments were collected.Fecal DNA (for detection of *Fusobacterium* and *Bacteroides*) and colonic DNA (for detection of KRAS and p53 mutations) were isolated.RT-PCR tchnique was used for detection of bacterias and mutations.

Results: No any differences was observed histopathologically between control and sham groups.Erosions and partial losses were observed at mucosal epitelium in the EMF group.The corrupted gland structure, the mucosal edema and the inflammatory cell infiltration were observed.The amout of collagen was increased and fibrosis was detected in EMF group.Goblet cell number decreased statistically significant when compared to control and sham groups ($p < 0.05$).The amount of Fusobacterium increased significantly in EMF group compared to controls.The difference was not detected between groups in the amount of Bacteroides.All the samples analysed for KRAS and Tp53 mutations in the colon tissue were found to be wild type.No significant difference was observed between the control group and the EMF applied group.

Discussion and Conclusion: In conclusions,for 12 weeks 45 minute/day exposure to 1800 MHz EMF caused histopatological damage in rat colon.The amount of *Fusobacterium* is increased.EMF exposure did not caused to KRAS and p53 mutations in colon tissue.

P-05.01.1-016
Synthesis, antimicrobial activity, genotoxicity, DNA binding and DNA cleavage studies of new glycine methyl ester derivative Schiff base

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There has been an increasing focus on the binding study of small molecules to DNA during the last decades, since DNA is an important genetic substance in organisms. Therefore, the current growing interest in small molecules that are capable of binding and cleaving DNA is related to their utility in the design and development of synthetic restriction enzymes, new drugs, DNA agents, and also to their ability to probe the structure of DNA itself. In recent years, Schiff bases have found increased application in pharmaceutical research, organic synthesis, and bio-processes. Schiff bases are considered as favored and the most widely used ligands, due to their metal complexes having variety of applications as antibacterial and anticancer agents.

In this study, we report the synthesis and characterization of a novel glycine methyl ester derivative Schiff base. The minimal inhibitory concentration (MIC) of the compound was screened *in vitro* against bacteria and yeast cultures using broth micro dilution tests. Antimutagenic activity of compound was tested in the absence of metabolic activation. Also, DNA binding and DNA cleavage were investigated of compound by UV-Vis spectroscopy and agarose gel electrophoresis respectively.

Consequently, this compound differs significantly in its activity against tested microorganisms. This difference may be attributed to the fact that the cell wall in Gram-positive bacteria is a single layer, whereas the Gram-negative bacteria cell wall is a multilayered structure, and the yeast cell wall is quite complex. The compound inhibited the base pair mutation in the absence of S9 with high inhibition rate. UV-Vis spectroscopy studies of the interactions between the compound and calf thymus DNA (CT-DNA) showed that the compound interacts with DNA via intercalative binding.

P-05.01.1-017
Analysis of somatic SNVs in G4 motifs in pancreatic cancer

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To date a large number of the sequences in the human genome (G4 motifs) with the potential to form a spatial structure, G-quadruplexes is known. G4 motifs were found in the promoter regions of most of the known oncogenes. Recent experimental studies have shown that genome instability directly related to the non-canonical DNA structures, including G-quadruplexes. In this work we study the distribution of somatic SNVs within the G4 motifs in tumor samples with the aim to identify involvement of the motifs in the process of mutagenesis in pancreatic cancer.

Using the access kindly provided by the international ICGC consortium to the database, we analyzed 68 samples of Pancreatic Ductal adenocarcinoma and 27 samples of Pancreatic Endocrine neoplasms. We considered only the promoter regions as the richest with G-quadruplex motifs.

We found that quadruplex sequences have the ability to focus somatic SNVs. This could be explained by the errors of polymerase during replication through secondary DNA structures. Furthermore, the SNVs occur much more often in loops of G4 motifs than in G blocks, without changing the motive. In addition, T>G(A>C) and T>C(A>G) substitutions occur significantly more likely in loops which in turn stabilize the G-quadruplex structure. The cancer-related mutations tend to increasing the length of G blocks. The conservation of G4 motifs may indicate an important functional significance of G-quadruplex structures in human genome. Supported by project no. 16-14-10396 of the Russian Science Foundation.

P-05.01.1-018
Mean platelet volume values in patients with multiple myeloma

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Background: Multiple myeloma (MM) is a rare, leading to bone destruction and marrow failure, largely incurable malignant disease of plasma cells. Anemia (mostly normocytic normochromic) is seen in most patients. Mean platelet volume (MPV) is a laboratory marker of platelet function and activity, the most accurate measure of platelet size. The aim of this study was to investigate the mean platelet volume (MPV) values in this disease.

Materials and Methods: Whole blood samples were collected from 60 healthy controls and 105 patients with MM. The mean age for controls and patients were 54.5 ± 8.5 and 57.4 ± 8.1 years, respectively. MPV levels were calculated with

Abbott Cell Dyne heamotolgy analyzer. Statistical analysis was performed with SPSS v16.

Results: The mean of MPV values in patients with MM (7.24 ± 1.18) were significantly lower compared to control group (8.01 ± 1.34) ($p < 0.05$).

Conclusions: MPV is a component of the complete blood count test and a potential marker of platelet reactivity. Although clinical utility and validity of MPV have not been established yet, some authors argue its use in inflammatory disorders. According to this study's results, MPV values was detected to be decreased in patients with MM.

P-05.01.1-020
Apoptosis inducing effects of novel benzimidazole derivatives bearing pyridyl/pyrimidinyl piperazine moieties on A549 lung adenocarcinoma

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Cancer is a chronic disease in the world which is the second leading cause of death, after cardiovascular diseases. Benzimidazoles have been known to act as antiproliferative or anticancer agents in chemotherapeutic drug research area. In this regard we aimed to investigate the cytotoxic and apoptotic properties of novel benzimidazole derivatives bearing pyridyl/pyrimidinyl piperazine moiety against A549 lung adenocarcinoma cells.

A549 lung adenocarcinoma cell lines were used in the studies. The cytotoxic activities of the tested compounds were determined by MTT assay. Detection of apoptosis was performed using Annexin V-FITC apoptosis detection kit BD, Pharmingen according to the manufacturer's instruction. All measurements were performed on a FACS-calibur cytometer.

The IC_{50} values of the compounds were determined for A549 cell line. Compounds 4, 7 and 12 which were including 4-chlorophenyl, 4-nitrophenyl on pyridine ring; 4-fluorophenyl on pyrimidine moiety, had significant cytotoxic activity with IC_{50} values lower than 55.33 ± 23.40 μg/ml. Compound 12 showed the highest cytotoxic activity with a IC_{50} value of 16.67 ± 2.24 μg/ml, whereas cisplatin IC_{50} values were 14.0 ± 2.0 μg/ml μg/ml against A549 cells. Cytotoxic activity of compound 4 and 7 with a IC_{50} value were 55.3 ± 23.4 and 21.7 ± 8.4 μg/ml, respectively. Also, compound 12 showed the highest population of early apoptotic cells (39.4%) of the tested compounds which was 5.88-fold higher than for cisplatin. Compound 7 produced a comparable population of apoptotic cells with a percentage of 9.5%, respectively according to cisplatin's percentage of 6.2%.

It was determined that synthesized compounds 4, 7 and 12 had considerable anticancer activity against A549 cell lines compared to cisplatin. Compound 12 including 4-florophenyl on pyrimidine ring was the most cytotoxic compound against the A549 cell line. Our study results demonstrated that compound 7, 12 also induced apopototic pathway on A549 cells.

P-05.01.1-021
In vitro/*in vivo* antimitotic activity and structure-activity relationships of New Glaziovianin A Isoflavone Series

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Glaziovianin A (GVA), isolated from the leaves of the Astelia glazioviana, demonstrated cytotoxicity, disrupting microtubule structure and dynamics of HL-60 cells. The aim of the present work was to devise a concise synthetic route toward GVA and its derivatives in order to expand structure-activity relationship studies and to investigate their anti-mitotic effect.

A concise six-step protocol for the synthesis of GVA and its alkoxyphenyl derivatives 9 starting with readily available plant metabolites from dill and parsley seeds was developed. The sea urchin embryo tests confirmed that GVA directly affects tubulin/microtubule dynamics and structure. The B-ring substitution pattern of GVA derivatives exhibited strong effects on activity. According to the assay results, the anti-mitotic activity decreased in the following order: GVA > myristicin ≥ 3,4,5-trimethoxyphenyl = 4-methoxyphenyl > dillapiol > 3-methoxyphenyl> 3,4-dimethoxyphenyl > 2,3,4,5-tetramethoxyphenyl derivatives. A methylenedioxy moiety was essential for the activity of compounds substituted with four B-ring alkoxy groups. The MTS assay of the limited panel of cancer cell lines shows that GVA displayed the highest inhibitory activity, with IC50 values ranging from 0.27 (A375 cells) to 2.2 μM (MDA-MB-231 cells). Compounds, containing 3,4,5-trimethoxy and apiol-derived B-rings, respectively, were less active. Other isoflavones did not affect cancer cell growth up to 10 μM. Anti-proliferative effects of isoflavones 9 observed in both the sea urchin embryo model and human cancer cell lines correlated well. Importantly, none of the synthesized isoflavones 9 demonstrated cytotoxicity in human PBMCs, up to 10 μM.

In summary, GVA and its analogues were synthesized via a scalable six-step reaction sequence. The GVA and its analogues containing 3,4,5-trimethoxy and apiol-derived B-rings were found to be promising anti-mitotic microtubule destabilizing agents with low toxicity against human PBMCs.

P-05.01.1-023
Knock-down of anti-apoptotic Bag-1 modulated the regulation of cell survival pathways in breast cancer

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Bag-1 is a multifunctional protein which has interactions with a number of cellular proteins; nuclear hormone receptors, Bcl-2, Hsp70/Hsc70 family, growth hormone receptors, Raf-1, ubiquitin machinery and DNA to regulate cell survival. For this reason, Bag-1 is a critical molecular player in the regulation of cell survival signaling and apoptosis mechanism. Elevated expression levels of Bag-1 are associated with progression of cancer.

In the treatment of breast cancer, silencing tools as a promising combined therapy strategies in the presence of classical chemotherapeutics gain importance to investigate interaction networks of cell death and survival signaling pathways. Therefore,

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we aim to understand potential role of Bag-1 silencing in the treatment of breast cancer cells with apoptotic agents; cisplatin or paclitaxel.

Our results showed that, silencing of Bag-1 enhanced cisplatin or paclitaxel-induced apoptosis in MCF-7 cells by down-regulating antiapoptotic and upregulating proapoptotic Bcl-2 family proteins, changes on cell cycle, upregulation on subG1 phase, activating caspases and cleavage of PARP. In addition, knockdown of antiapoptotic Bag-1 has a suppressive role in PI3K and Akt signaling pathway in MCF-7 breast cancer cells through inhibition of Akt phosphorylation and downregulation on PI3K. Investigation targets of Akt pathway showed that mTOR cell survival pathway also affected through Bag-1 silencing. Bag-1 silencing inhibited mTOR signaling via downregulating both Rictor and Raptor proteins which are the members of rapamycin-insensitive mTORC2 and rapamycin-sensitive mTORC1 complexes, respectively.

Knockdown strategies of Bag-1 is important to enlighten the network interactions of Bag-1 and clarify its interaction partners in the cells. Therefore utilization of Bag-1 targeted strategies might further increase therapeutic efficiency of drugs through inhibiting cell survival machinery in the treatment of metastatic breast cancer.

P-05.01.1-024
Biological activity evaluation of new 3,5,6-trisubstituted triazine derivatives bearing different heterocyclic rings against lung cancer cell lines

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Cancer is one of the major death causing disease worldwide. Among the various cell types occurs on different organs, lung cancer is one leading cause of cancer death accounting for approximately 26% of all female and 28% of all male cancer deaths in 2013. The resistance development, cytotoxicity and inadequacy are the main encountered problems by the treatment with existing chemotherapeutic agents. Therefore, there is continuous need to discover new active and non-toxic molecules.

1-[4-(5,6-Bis(4-substituted phenyl)-1,2,4-triazin-3-yl)piperazin-1-yl]-2-[benzimidazole/benzoxazole/benzothiazole-2-yl)thio]ethanone (1-9) derivatives were synthesized with a four-step synthetic procedure using toluil, anisil and 4-chlorobenzil as starting materials. The anticancer activity of the compounds was evaluated using the methods MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), BrdU (Bromodeoxyuridine) assays and flow cytometric analysis against lung cancer cell lines. The lipoxygenase enzyme inhibition activity of the compounds were also investigated using the method described by Baylac and Racine.

Compounds was found to have (inhibition concentration) IC_{50} values between 135-500 μg/ml. The early and late apoptotic cell percentage was determined as 6.6 for compound 8 by flow cytometric analysis. The LOX inhibition activity was found 48.35 ± 3.08 for compound 1.

Compound 8 bearing 8-chlorobenzil and benzoxazole moieties was found as the most active compound when we evaluate anticancer potential of all compounds. The LOX enzyme inhibition was indicated for the compound 1 including methyl substituent on phenyl rings. The DNA synthesis inhibition of the compounds has been still studied at the concentrations $IC_{50}/2$, IC_{50} and IC_{50}x2.

P-05.01.1-025
Single amino acid substitutions and deletions modulate the dRP-lyase activity of human DNA polymerase iota

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DNA polymerase iota (Pol ι) is a Y-family DNA polymerase that possesses an unusual combination of properties. Due to the special organization of the active site Pol ι has a very low accuracy of DNA synthesis but possesses an ability to bypass a variety of DNA lesions. In addition to the DNA polymerization activity, human Pol ι also possesses an intrinsic 5′-deoxyribose phosphate (dRP)-lyase activity. Removal of the dRP group is a pivotal step in base excision repair (BER) *in vivo*. Although Pol β plays a key role in the dRP group cleavage and DNA synthesis during BER, Pol ι was shown to complement the *in vitro* single-nucleotide BER deficiency of Pol β null cell extracts and was suggested to be involved in BER under oxidative stress.

The dRP-lyase active site in Pol ι is still not known. To address the mechanism of the dRP-lyase activity of Pol ι we obtained a series of Pol ι mutant variants including point mutations of conserved lysine residues and deletions in different locations. We purified human Pol ι variants from yeast *Saccharomyces cerevisiae* and tested the effect of mutations on the cleavage of an internal 5′-dRP group in oligonucleotide DNA substrates in the presence or absence for Me^{2+} ions.

The experiments revealed several point amino acids substitutions that significantly affected the dRP-lyase activity of Pol ι, thus suggesting a possible location of the dRP-lyase active site. Furthermore, we showed that deletions in the N-terminus of Pol ι and metal ions modulate its dRP-lyase activity, which may play an important role in the regulation of Pol ι activities *in vivo*.

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P-05.01.1-026
Investigation of the rosmarinus officinalis' effect on the chemotherapeutic drug etoposide in glioblastoma (U87 MG) cell culture

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Rosmarinus Officinalis, commonly known as rosemary, is an aromatic plant belongs to Lamiaceae family. From past to now, rosemary have been used as a traditional medicine to cure for various illnesses such as diabetes, rheumatism and cancer. Recent studies have shown that rosemary is effective for various cancer types. In this study we aimed to investigate the effect of rosemary in glioblastoma cells (GBM) by comparison with etoposide and the effect of rosemary by concurrent application with the etoposide.

GBM cells (U87 MG) were seeded into the 24 well plates and cultured with DMEM supplemented with 10% fetal bovine serum. Rosmarinus Officinalis tea was prepared just as traditional usage and filter sterilized. At the second day of the culture rosemary in 1/75 (v/v) dilution ratio was given to first group, 40 μM etoposide was given to second group, 1/75 (v/v) diluted

rosemary and 40 μM etoposide together were given to third group. After one day incubation cell viability was measured by neutral red assay.

It was observed that rosemary reduced the viability of GBM cells by nearly %38, etoposide reduced the viability by nearly % 49 and rosemary with the etoposide reduced the viability by nearly %50. The results showed that rosemary was able to reduce the viability of GBM cells but hadn't got an increasing or inhibiting potential over the etoposide's cytotoxic effect. From our previous studies we know that rosemary increases the proliferation of mouse embryonic fibroblasts. It is considered that rosemary might have a protection potential from DNA damages and when rosemary is used with etoposide during the cancer treatment, it might reduce the side effects on healthy cells. In conclusion Rosemary promises hope for developing new cancer treatment strategies and reducing the side effects of chemotherapeutics. For further studies it is aimed to examine the effects of rosemary with other chemotherapeutics and if rosemary has got a protection potential from the genotoxic stress.

P-05.01.1-027
Novel morpholine dithiocarbamate derivatives induces apoptosis in C6 glioma

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Morpholine moiety has been found to be an excellent pharmacophore in medicinal chemistry and a number of molecules possessing morpholine skeleton are the clinically approved drugs. In this present study, we aimed to investigate the possible underlying apoptotic mechanism for the cytotoxicity of new morpholine dithiocarbamate derivatives bearing 1-(2-aryl-2-oxoethyl)-2-substituted benzimidazole moiety on C6 glioma.

C6 glioma cell lines were used in the studies. The cytotoxic activities of the tested compounds were determined by cell proliferation analysis using standard (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Detection of apoptosis was performed using Annexin V-FITC apoptosis detection kit BD, Pharmingen according to the manufacturer's instruction. All measurements were performed on a FACS-calibur cytometer.

The IC50 values of the compounds were determined for C6 cell line. Compounds 1, 2, 8, 9, and 10, which were including hydrogen, 4-methyl, 3-methoxy, 3-chloro and 3-floro substituents on phenyl acetyl moiety, had significant cytotoxic activity with IC_{50} values lower than 55 μg/ml. Compound 10 showed the highest cytotoxic activity with a IC_{50} value of 28 μg/mL, whereas cisplatin IC_{50} values were 15 μg/mL against C6 cells. Cytotoxic activity of compound 1, 2, 8, 9 and 10 with a IC_{50} value were 42, 55, 50 and 30 μg/mL, respectively. Compound 2, 9 and 10 showed the highest population of early apoptotic cells as 11.5, 10.1, and 10.3% respectively compared to cisplatin (6.2%). Also, compounds caused DNA synthesis inhibition depend on their IC50 values by BRDU assay.

Conclusions: It was concluded that synthesized compounds had considerable anticancer activity against C6 cell lines. However, compound 2, 9 and 10 including 4-methyl, 3-chloro and 3-floro substituents were the most active compounds against the C6 cell line. Also our study results showed that compound 2, 9, 10 induced apoptosis in C6 glioma cells.

P-05.01.1-029
Rutin sensitizes the resistance of prostate cancer cells to TRAIL

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Rutin is a glycosided flavonoid and known to have antioxidant and anti-inflammatory properties.TRAIL induces the apoptosis of tumor cells and has no significant toxic effect on normal cells. Although TRAIL is a promising anticancer agent, TRAIL resistance is a major barrier to effective cancer therapy. This study was conducted to examine the utility of the combined use of rutin and TRAIL in prostate cancer cells.

PC-3 and DU145 prostate cancer cells were treated with rutin (1-1000uM) and/or TRAIL (20 ng/ml), cell viability and migration were examined. Cell viability was determined by trypan blue exclusion and MTT assay. Cell migration was determined by wound healing assay. Furthermore, lactate dehydrogenases (LDH) levels of medium were determined as biochemical markers of cell viability.

PC-3 and DU-145 prostate cancer cells were treated with rutin for 24 and 48 hours incubation and IC_{50} doses for 48 hours incubation were determined 250uM and 1000uM respectively. Treatment with rutin, PC-3 cells is more sensitive than DU145 cells. Rutin and rutin plus TRAIL inhibit prostate cancer cell growth in a dose-dependent manner. Treatment with TRAIL has no effect at inhibiting growth of PC-3 and DU145 prostate cancer cells. The combination of rutin and TRAIL elicit a synergistic antitumor effect on PC-3 and DU145 prostate cancer cells. There is a significant increased in rutin and rutin+TRAIL treatments group of LDH activities with respect to control and TRAIL group.

Conclusion: Present data show that rutin efficiently enhanced TRAIL effects in prostate cancer cells. Combined treatment with rutin and TRAIL is more effective than the individual treatments of TRAIL at inhibiting growth of prostate cancer cells.

P-05.01.1-030
Determination of antigenotoxic, proliferative and cytotoxic properties of ellagic acid

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Since ancient time, people use plant for traditional treatment. Plants or fruits are produced different type of secondary metabolites. Particularly phenolic phytochemicals from plants play an important role in the prevention and treatment of radical damage by inactivating the reactive oxygen compounds due to their antioxidant properties. However, the structure and the activities of many herbal products are not fully elucidated yet and there are several studies about the toxicity of herbal antioxidants and their possible risks to human health. Ellagic acid, phenolic compounds, is an important substance. Ellagic acid is a naturally occurring plant phenol found in numerous fruits, including blackberries, raspberries, strawberries, cranberries, walnuts, pecans, pomegranates and wolfberries. Different researchers give some information about the biological activities of ellagic acid.

In this study, we aimed to determine the cytotoxic, proliferative and antigenotoxic effects of ellagic acid, which is phenolic compounds found in natural products. Cytotoxic effects of ellagic

acid on HUVEC is investigated by lactate dehydrogenase (LDH) and cell proliferation (WST-1) methods; and antigenotoxic effects against CCL_4 on human lymphocytes is investigated by single cell gel electrophoresis (Comet) methods.

The rusults showed that high concentration (100 and 200 μM) of ellagic acid has cytotoxic and mutagenic effects, but showed antiproliferative effects. On the contrary, low concentrations (4, 8, 12.5 μM) of ellagic acid has anticytotoxic and antimutagenic effects.

As a conclusion, low concentrations of ellagic acid might be use treatment of some disease. But high concentrations of ellagic acid constitute a risk factors for people.

Keywords: Cytotoxicity, Antiproliferation, WST-1, LDH, RTCA-SP

P-05.01.1-031
Bortezomib induces DNA damage and apoptosis by NF-kB signaling on myeloid leukemia cell lines

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The constitutive nuclear factor kappa B (NF-kB) activation is widely found in diverse types of hematologic malignancies such as acute myeloid leukemia (AML) and chronic myeloid leukemia (CML) as well as solid tumors. Inhibition of NF-kB signaling via proteasome inhibitors such as bortezomib can induce apoptosis in myeloid leukemia cell lines. However it is not clear whether the cytotoxic effects of bortezomib on myeloid leukemia cell lines is due to direct inhibition of NF-kB or another pathway, such as DNA damage.

In this study, CML cell line K562 and AML cell line HL-60 were treated with bortezomib (Bor), etoposide (Eto) and camptothecin (Cpt) alone or in dual combination with these drugs, following by measuring the effects on cell viability, apoptosis and signal pathways. The effect on cell viability was determined using the MTT assay. The data were used in combination index and isobologram analysis. The expression levels of apoptototic genes (bcl2, bax and caspase 3), the related DNA damage genes (ATM and ATR) and the involved genes in NF-kB signaling (RelA and p50) were determined by real time RT-PCR.

We showed that combinations of Bor with topoisomerase inhibitors (Cpt and Eto) exhibited synergistic cytotoxic effect in K562 cell line but not in HL-60 cell line. The combination treatment increased apoptosis and DNA damage response. DNA-damage-sensing kinases were detected in K562 and HL-60 cells following treatment with Bor as similar as topoisomerase inhibitors. Bor increased the mRNA levels of ATM and ATR dramatically, which indicated active DNA damage in the myeloid cell lines. Furthermore, Bor induced apoptotic cell death by decreasing bcl2 and increasing bax and caspase 3 levels. These effects of Bor were observed to correlated with increasing the p65 expression levels.

This study on the mechanism of action of Bor indicates that this compound affects several pathways involved in the control of cell cycle progression, apoptosis and DNA damage.

P-05.01.1-032
Analysis of molecular cytogenetic alterations in gastric and colon carcinoma by array-based comparative genomic hybridization (Array CGH)

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Introduction: Genomic DNA regions are frequently lost or gained during tumor progression. We aimed to evaluate tumor samples of patients with gastric cancer and colorectal carcinoma to show these genetic alterations by array-based comparative genomic hybridization (Array CGH) method.

Materials and Methods: DNA isolation was performed from the tumor samples obtained from sixteen patients with primary gastric adenocarcinoma and twelve patients with colon adenocarcinoma. Then, agarose gel electrophoresis was performed in those DNA samples. Following electrophoresis of DNA, Array CGH procedure was performed to four patients with gastric adenocarcinoma and three patients with colon adenocarcinoma who had DNA breaks with 100-200 kb.

Results: After Array-CGH study, many common genetic changes in gastric and colon cancer genome were determined. In gastric cancer DNA samples, common losses were detected in chromosome 1p34.1, 1p13.3, 1q22, 3q29, 5p13.2, 6q24.1, 7q11.23, 7q22.1, 8q 12.1, 9p12, 11q13.1, 12 q24.31, 14q32.31, 16q22.1, 17q21.2, 19q13.3, and 20q11.23, and also common gains were detected in chromosome 3p26.1, 6q27, 8q12.1, 15q11.2 and Xq25. In colon cancer DNA samples, common losses were detected in chromosome 1p34.1, 3q29, 7p22.2, 7p11.21, 9q34.11, 12q24.31, 17q21.2, 17q25.1, 19p13.3, 19p13.33, 20q11.21, and 20q11.23, and also common gains were detected.in chromosome 14q32.33, Xp22.33, Xp22.11, Xp13.3, Xp11.1 and Xq25. Both in gastric and colon cancer DNA samples, common losses were detected in chromosome 12q24.31, 17q21.2, and 19p13.3, and common gains were detected in Xq25.

Discussion and Conclusion: We think that these common changes, generally in DNA loss areas harboring tumor suppressor genes and DNA gain areas harboring oncogenes, may important in gastrointestinal tumorigenesis.

P-05.01.1-033
Recruitment of PAR-dependent proteins to sites of laser induced DNA damage

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The DNA of every cell is under a constant attack by various mutagenic factors which damage the DNA and can cause cell cycle arrest and even cell death. Accumulation of DNA damage is the basis for cancer development and one of the reasons for aging of the organisms. In order to preserve the integrity of its DNA cells have evolved an impressive array of DNA repair pathways, which are precisely coordinated with the progression of the cell cycle. One of the first events at the site of DNA

damage is Poly(ADP-ribose) polymerase 1 (PARP1) recruitment which is a sensor for single strand breaks in DNA. PARP1 catalyzes the synthesis of poly(ADP-ribose) or PAR which is needed for the recruitment of many other DNA repair proteins by means of PAR-binding domains.

We used high speed confocal spinning-disk microscopy of living cells to obtain precise kinetics of recruitment of PAR-dependent proteins to the sites of laser induced DNA damage.

Our results show that the investigated PAR-dependent proteins are recruited to DNA damage sites in the matter of seconds, they reach peak intensities for 20 to 30 seconds after damage infliction and start dissociating. The recruitment of the proteins is entirely dependent on PAR because addition of PARP inhibitor abbrogated their recruitment.

The use of spinning-disk microscopy of living cells allowed us to obtain the kinetics of recruitment of the studied proteins to the sites of DNA damage. The results are consistent with the fact that PARP1 and PAR-dependent proteins are quickly recruited to damage sites and generation of PAR is essential for other DNA repair protein recruitment. The precise kinetic curves may serve as a basis for investigating how they will change or if they will change at all when cells are put in different conditions or treated with various chemical substances affecting DNA metabolism and repair.

P-05.01.1-035
A benzamide derivative XT5 and imatinib combination induced apoptosis in İmatinib resistant K562 cell line

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Introduction: Chronic myeloid leukemia (CML) is a myeloproliferative disease associated with reciprocal translocation between chromosomes 9 and 22. BCR-ABL fusion gene which exhibits constitutively active tyrosine kinase activity has a main role in CML. The tyrosine kinase inhibitor imatinib is used as a first line treatment in CML patients, but imatinib resistance leads to failure in therapy. The application of imatinib in combination with other anticancer agents may be a strategy to increase the antileukemic effect of imatinib. In this study, we have investigated the antiproliferative effect two novel agents: a benzamide derivative XT5 and a benzoxazole derivative XT2B in combination with imatinib. These molecules were investigated in imatinib-sensitive (K562s) and imatinib-resistant (K562r) CML cell lines.
Materials and Methods: Antiproliferative and apoptotic effects were assessed by MTT assays and flow-cytometry, respectively. We also evaluated the effects of these compounds on the expression of apoptosis-related genes BAX, BCL-2, BAD, BIM, BCL-XL and MCL1 by real-time quantitative PCR.
Results: Treatment of K562 cells with XT5 increased the expression levels of the pro-apoptotic genes BAX, BAD and BIM in both sensitive and resistant cells. However, XT2B was not found to have similar effects on K562r and K562s cells. Combined application of XT5 increased cell death in the MTT assay. MTT assay demonstrated that IC50 for XT5 treated cells in K562r with imatinib (IC50 = 3.5) is lower than K562r without imatinib (IC50 = 8.5).
Discussion and Conclusion: Our results showed that combining XT5 with imatinib has more antiproliferative and apoptotic effect on a CML cell line. As a result combination of XT5 with imatinib can be an alternative approach to overcome imatinib resistance.

P-05.01.1-036
Effects of MLH1 and MSH2 expression on imatinib resistance in CML

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Introduction: The MMR(Mismatche Repair) system recognizes base–base mismatches and insertion or deletion loops in double-stranded DNA, and it degrades the error-containing region of the newly synthesized strand, allowing the polymerase to correctly resynthesize the second strand according to the template sequence. The human MMR system includes the MLH1 and MSH2. Alteration in expression or a defect in MLH1 or MSH2 can cause resistance to anti-cancer drugs used in chemotherapy. The attempt of the MMR system to detect drug induced DNA damage, triggers the activation of apoptosis, a mechanism which may enhance the cytotoxicity of chemotherapy. Loss of the MMR system would make the neoplastic cell less able to initiate apoptosis. Inability to initiate apoptosis could be a mechanism of resistance to drugs. Chronic myeloid leukemia (CML) is a clonal disease originating from aberrations in hematopoietic stem cell. Imatinib, a tyrosine kinase inhibitor has significantly improved clinical outcome for CML patients. However, patients develop resistance when the disease progresses to the blast phase (BP) and there are several mechanisms involved in imatinib resistance. In this study we investigated the role of MMR system in imatinib resistance.
Materials and Methods: K562s (sensitive) and K562r (resistance) were grown in RPMI-1640. K562r cells were maintained in RPMI-1640 medium supplemented with 5 μM imatinib RNA isolation, cDNA synthesis, RT- PCR was performed respectively.
Results: The results demonstrated that expression of MLH1 in K562r cells is dramatically lower than equal amount of imatinib treated K562s cells, whereas MSH2 expression level did not change in both cell lines.
Conclusion: It can be suggested that alteration and down-regulation of MLH1 genes leads to imatinib resistance.

P-05.01.1-037
Characterization of interaction between Rad51 inhibitor DIDS and human serum albumin

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4′-diisothiocyanostilbene-2,2′-disulfonic acid (DIDS) has been largely used during the last 30 years for its inhibitory effect on anion transporters and channels. More recently, Ishida and colleagues have described a possible mechanism by which DIDS inhibits Rad51-mediated homologous pairing and strand exchange, key processes in DNA repair by Homologous Recombination. Thus, DIDS could act as a potential revertant of radio- and chemo-resistance in cancer cells, which is the major cause of failure during therapeutic protocols. New drugs targeting Rad51 protein have since been developed with potential use for medical applications. In this context, we attempted to determine the behaviour of DIDS towards blood and plasma proteins such as serum albumins. Firstly, we analysed the effects of several environmental factors such as solvent polarity, which may affect the stability of the molecule. Secondly, we analysed the spectroscopic properties of DIDS in the presence of Human or Bovine Serum

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Albumin proteins. UV-visible absorption, Circular Dichroism, Fluorescence Spectroscopy and Isothermal Calorimetry were used. Here we show for the first time that DIDS can interact with both Serum Albumins. We have also determined the characteristics of these interactions. The comparison of several DIDS derivatives led us to identify the essential chemical moiety of this compound involved in the interaction. Moreover, by using site competition approaches we show that the main binding site for this molecule is in subdomain IB of the protein. These findings show that the binding of DIDS to serum albumin proteins may change the equilibrium between the free and bound DIDS forms, thereby affecting its bioavailability and efficiency against the Rad51 recombinase protein.

P-05.01.1-038
Mechanism of TAp73 Beta-MDM2 autoregulation

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p73 is a transcription factor which is the member of a p53 family. It regulates many cellular processes, such as apoptosis, cell cycle, and senescence. In contrast to p53, p73 is rarely mutated in tumors and elevated p73 expression is observed in many types of cancers including hepatocellular carcinoma, neuroblastoma, and lung. Defining regulatory mechanisms which control p73 protein abundance and activity will be crucial for the development of new therapeutic strategies for cancers. MDM2 is known as the key player in regulation stability and activity of p53. In addition, p53 induces MDM2 transcriptional activity, and Caspase-2, 3 activations which cleave MDM2 N-terminal at Asp 367. Cleaved form of MDM2 binds p53 and promotes its stabilization. MDM2 suggested as a candidate to modulate p73 activity and stability too. However, an interaction between p73 and MDM2 has not defined well. In this study, we aimed to analyze the role of MDM2 in p73 stability. To define this relationship, firstly, we overexpressed the TAp73beta isoform using TREx system in Hep3B. TAp73 beta and MDM2 protein levels were determined by western blot. To examine whether MDM2 mediate TAp73 beta protein degradation by the proteasomes, cells were treated with proteasome inhibitor, MG132 for 4 hours prior to analysis. Previous studies showed that p53-induced Caspase-2 and Caspase-3 activation cleaves MDM2. Considering this, we firstly examined Caspase-3 activation by western blot in Hep3B TAp73 Beta cells. Then we analyzed expression of cleaved MDM2 and TAp73 beta levels following caspase inhibitor, Z-VAD-FMK treatment. As a conclusion, TAp73 beta-induced full-length MDM-2 expression. Furthermore, TAp73 beta enhanced cleavage of MDM2 via increased Caspase-3 activation. In addition, inhibition of Caspase-3 activation caused a decrease in cleaved-MDM2 levels in parallel with TAp73 Beta expression repression. Our results suggested positive regulation between MDM2-TAp73 beta.

P-05.01.1-039
TAp73 beta induces tumor inhibition in hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is one of the most common type of liver cancer and third leading cause of cancer related deaths in worldwide. Discovery of new targets is important in survival of HCC patients. p73 is a transcription factor which is the member of p53 family. It has two promoters; while p1 promoter expresses apoptotic TA isoforms, p2 promoter expresses anti-apoptotic DN isoforms. In addition, alternative splicing in C terminal creates many isoforms of TA and DN p73. It has been shown that both TAp73 and DNp73 isoforms are expressed in HCC patient tissue and cell lines. The ratio between TAp73 and DNp73 affects the apoptotic response, drug response and prognosis. Accordingly, identification of the role of p73 and its targets are important in discovery of new treatment strategies in HCC. To understand the role of p73 isoforms in HCC, firstly we performed MTT assays following DNA-damaging drugs and multikinase inhibitor, Sorafenib treatment to categorize HCC cell lines as resistant or sensitive. After that, we analyzed the expression levels of TAp73 isoforms via Western Blot in all HCC cell lines. Then we overexpressed the TAp73beta isoform using TREx system in Hep3B and SNU449 cells. These two clones were analyzed for DNA damaging drug response by MTT, cell cycle and apoptosis by Flow cytometry, and tumor formation by *in vitro* and *in vivo* experiments.

In scope of our study; 1. Only TAp73 alpha isoform is expressed in a few HCC cell lines. 2. There is no correlation between basal expression of p73 isoforms and drug responses in HCC cell lines. 3. There is no change in expression of p73 isoforms after treatment of drugs. 4. We showed that the ectopic expression of TAp73beta in Hep3B arrested the cell cycle in G1/S and decreased the colony formation. Therefore, the capacity of tumor formation of the cells dramatically decreased in SCID mice. As a result, we revealed that TAp73 beta play role in tumor formation, cell cycle arrest, DNA damage responses in HCC.

P-05.01.1-040
Biochemical characterization of exonuclease III-family AP endonuclease point mutants reveals role of conserved amino acid residues in the NIR-specific enzymes

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Oxidative DNA damage caused by reactive oxygen species is believed to be a major type of endogenous cellular damage. Oxidatively damaged DNA bases are substrates for two overlapping repair pathways: DNA glycosylase-initiated base excision (BER) and apurinic/apyrimidinic (AP) endonuclease-initiated nucleotide incision repair (NIR). In the BER pathway, an AP

endonuclease cleaves DNA at AP sites and 3′- blocking moieties generated by DNA glycosylases, whereas in the NIR pathway, the same AP endonuclease incises DNA 5′ to a number of oxidized bases. Majority of characterized AP endonucleases possess classic BER activities and about half of them are able to catalyze NIR activity. At present, the molecular basis of DNA substrate specificities of various AP endonucleases remains unclear. Here, we examined amino-acid sequence requirement of the NIR activity of human major AP endonuclease 1 (APE1). Amino acid sequence alignment of various AP endonucleases including E coli exonuclease III (Xth), human APE1 and archaeal Mth212 revealed conserved amino acid residues in the NIR-specific AP endonucleases APE1, Mth212 and ExoA that are absent in Xth. Based on these data, we constructed four APE1 point mutants Y128H, N174Q, G231S and T268D and examined their DNA substrate specificities. Results obtained from biochemical characterization of APE1 mutants are discussed in the light of the evolutionary conserved DNA repair functions of AP endonucleases and whether these functions can be mutationally separated from.

P-05.01.1-042
Combining NMR (Nuclear Magnetic Resonance) and Raman spectroscopy reveals structural and functional features of a new cisplatin derivative

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Since its discovery some 40 years ago, cisplatin has evolved for its efficacy in one of the most used drugs in treatment of various cancer types. Huge effort was invested in understanding the action of cisplatin and development of more potent drugs. They target mainly neighboring purine bases of nuclear DNA forming covalent intra- or inter-strand cross-links that affect inhibition of replication and transcription, cell cycle arrest, and attempted repair of the damaged nucleotides. If such damage cannot be removed the cell dies.

We have studied the details of the binding site of the short oligonucleotide modified by a platinum compound using complementary solution techniques used in modern structural biology, including Raman spectroscopy with DFT calculations aided interpretation of the obtained vibrational spectra. Moreover, the calculated structure of the DNA duplex was verified using SAXS (Small Angle X-ray Scattering) curve.

In our contribution, we will present an NMR structure of a DNA cross-linked with a cisplatin derivative containing a cyclohexane ring. At this atomic level resolution, structural features probably influencing cytostatic effects are described and compared with previously published structures.

Common structural features of previously determined structures are: a significant roll (25–60°) of the guanine bases involved in the cross-link, bending and unwinding of the double helix at the site of cross-link and orientation towards the major groove. Also, the platinum-guanine plane angle varies between 19 and 54°. Although the experimental structures were often used as the starting models for molecular dynamics (MD) simulations, results of these MD still leave many questions unresolved. The results of

this research have been acquired within CEITEC 2020 (LQ1601) project with financial contribution made by the Ministry of Education, Youths and Sports of the Czech Republic within special support paid from the National Programme for Sustainability II funds.

P-05.01.1-043
ERCC2/XPD polymorphisms and colorectal cancer risk: a case control study in a north eastern Iranian population

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Excision repair cross-complimentary group 2 (ERCC2) is one of the important DNA repair genes.ERCC2 codon 751 and 312 polymorphisms has been shown to modulate cancer risk. We therefore assessed the relationship between the ERCC2 polymorphisms and the susceptibility to colorectal cancer in a case-control study. There were 105 lung cancer cases and matched healthy controls in this study. Information concerning demographic and risk factors was obtained, each person donated 2 ml blood for biomarker testing. ERCC2 genotypes were determined by T-ARMS-PCR method. All of the statistical analyses were performed with SPSS (v 20.0). There was significant difference between the frequencies of ERCC2 polymorphism in cancer cases and controls ($p < 0.05$). The frequencies of ERCC2 751 Gln allele were 6.2% in controls and 13.8% in cancer cases. The individuals with Lys/Gln+Gln/Gln combined genotype were at an increased risk for lung cancer as compared with those carrying the Lys/Lys genotype (adjusted OR=2.80, 95%=CI 1.21−6.48). The above findings indicate that the genetic polymorphism in the ERCC2 codon 751 is associated with the risk of colorectal cancer in an Iranian population (Neyshabur citizenship).

Sunday 4 September 12:30–14:30

New optical methods for studying neuronal structure and function

P-09.01.1-001
Bioengineering fluorescent system to study peptide blockers of potassium voltage-gated Kv1.6 channel

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Peptide pore blockers are potent tools to study structure and function of potassium voltage-gated channels (Kv). KcsA-Kv1.x chimeras, in which a ligand-binding site of eukaryotic Kv-channel is inserted into bacterial KcsA channel, mimic properly the pore domain of Kv-channels. A fluorescence-based approach to study the binding of peptide blockers with KcsA-Kv1.1–KcsA-Kv1.3 chimeras was developed by us. This approach rested on high-level expression of KcsA-Kv1.x chimeras in *E.coli* inner membrane, binding of fluorescently-labeled toxin at the surface of the spheroplast and analysis of competitive binding of studied ligands by laser scanning confocal microscopy (LSCM).

Here we report on a new analytical system for search and study of Kv1.6-channel blockers that combines BL21 (DE3) cells expressing KcsA-Kv1.6 and rhodamine-labelled agitoxin 2 (Rh-

The FEBS Journal **283** (Suppl. 1) (2016) 129–417 DOI: 10.1111/febs.13808

AgTx2) as a fluorescent probe. By tuning cultivation conditions, the high-level of membrane expression of KcsA-Kv1.6 was achieved. It was found that lowering both the growth temperature and the concentration of inducer resulted in significant increase in membrane-embedded KcsA-Kv1.6. For system validation, wellknown Kv1 channel blockers were studied by the method of competitive binding, and equilibrium dissociation constants were estimated for AgTx2, OSK1, and kaliotoxin. A new system was applied to study molecular determinants of peptide-Kv1.6 channel binding using a number of AgTx2 mutants constructed by us, whose affinities to KcsAKv1.6 were measured.

A new bioengineering fluorescent system is a robust and sensitive assay for assessing the binding activity of Kv1.6 channel blockers. It can be used to study interaction interfaces of toxin-channel complexes, to search for novel peptide blockers and to develop new potent and selective Kv1.6-blockers for scientific and medical purposes.

The work was supported by the grant 14-14-00239 from Russian Science Foundation.

Sunday 4 September 12:30–14:30

Miscellaneous

P-MIS-001
Optimizing the anti-inflammatory activity of liposomes of Asparagus racemosus extracts derived from various methods

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Asparagus racemosus root extracts (AR) have been exhibited to show a wide range of pharmacological benefits. In this study, liposomes of AR were developed and assessed their physicochemical properties and anti-inflammatory activity in monocytic leukemia cell line (THP-1). Liposomes containing ratios of AR to lipid and phosphatidylcholine to cholesterol ratio were synthesized by thin-film hydration (TF), reverse-phase evaporation (REV), and polyol dilution (PD). The *in vitro* anti-inflammatory activity was assessed in terms of inhibition of tumor necrosis factor alpha (TNF-α) in lipopolysaccharide activated THP-1 by ELISA. The size of AR liposomes prepared by TF were larger, whereas those prepared by REV and PD were smaller. AR to lipid ratio was shown to have no influence on particle size, whereas zeta potential enhanced with increasing AR to lipid ratio. AR liposomes with lipid ratio of 1:5 achieved the highest value of entrapment efficiency and were at the highest with polyol dilution method. AR was found to have no toxic effects on THP-1 cells. The anti-inflammatory activities of AR and AR liposomes in terms of TNF-α in THP-1 cells were was exhibited to possess the highest values of around 52% at AR concentration of 1 μg/ml and % TNF-α inhibition tended to decline with the increasing amount of AR. This result may be attributed to the increased amount of liposomal particles being uptaken into the cells as a result of the increasing AR concentrations. It can be suggested that AR liposomes could be an alternative choice of topical/transdermal drug delivery for anti-inflammatory activity.

P-MIS-002
Inhibition of IRE1 signaling enzyme increases the expression of tumor suppressor genes and modifies their hypoxic regulation in U87 glioma cells

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Gliomas constitute one of the most aggressive groups of malignant neoplasms with poor survival prognosis and scarce therapeutic options. Plentiful studies have proven the connection between endoplasmic reticulum stress and malignant growth. We have studied the effect of inhibition of IRE1 (inositol requiring enzyme 1), which is a central mediator of endoplasmic reticulum stress and controls cell proliferation and tumor growth, on hypoxic regulation of the expression of different proliferation related genes in U87 glioma cells. It was shown that inhibition of IRE1 leads to up-regulation of the expression of *KRT18, CD24, MEST, CENPU, MYL9, ING1, ING2, MYBL1*, and *MYBL2* genes at the mRNA level in U87 glioma cells, with more profound changes for *MEST, MYBL1,* and *CD24* genes. Hypoxia leads to up-regulation of the expression of *CD24, ING1,* and *ING2* genes and to down-regulation – of *KRT18* gene in glioma cells. At the same time, inhibition of IRE1 modifies the effect of hypoxia on the expression of all studied genes: suppresses effect of hypoxia on *ING1* gene, eliminates hypoxic regulation of *KRT18, CD24*, and ING2 genes in glioma cells. The present study demonstrates that inhibition of IRE1 enhances the expression of all studied genes and modifies the hypoxic regulation of these gene expressions in gene specific manner and thus possibly contributes to slower glioma cell proliferation, but several aspects of this regulation remain to be further clarified.

P-MIS-003
Amplification and clonig of DNA polymerase 1 (pol1) of Thermus scotoductus K1 isolated from an Armenian goethermal spring

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The most important enzyme "mined" from thermophilic microorganisms is DNA polymerase, which widely used in molecular biological studies. Although DNA polymerase produced by *Thermus aquaticus* (*Taq* polymerase) was launched into the market long back, isolation of more processive, reliable and stable DNA polymerases from other species is a demand. The purpose of this work was to amplify and clone the *pol1* gene of *T. scotoductus* strain K1 recently isolated from an Armenian geothermal spring.

The draft genome sequence of strain K1 was deposited under accession number LJJR00000000.1. Genomic DNA was isolated using GenElute Bacterial Genomic DNA Kit. Primers for the *pol1* gene were designed manually. The gene was amplified using *Pfu* polymerase, and amplicons (~2.5 Kb) were ligated into the pET-21b(+) vector (Novagen) and transformed into chemically competent TOP10 *Escherichia coli*. Inserts were sequenced with T7Prom and T7Term primers, which showed that the gene sequence was correct and in the right reading frame and could be expressed in mesophilic *E.coli*.

DNA polymerases patented form different species of *Thermus* are mostly comparable, suggesting that only limited natural variations in *Taq*-like DNA polymerase may be discovered. The pol1 gene from K1 shares 99% and 83% similarity with pol1 of *T. scotoductus* SA-01 (2.0 kb) and *T. aquaticus,* respectively. Although the difference is not huge at sequence level, possible

functional differences (e.g. stability, proofreading activity, resistance to different PCR inhibitors etc.) may occur. Therefore, it is important to express and purify DNA polymerase from strain K1 for further investigations.

P-MIS-004
Peptide ligands of the immunoglobulin G Fc region identified by screening phage libraries and site-directed mutagenesis

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Affinity chromatography based on immunoglobulin (Ig)-binding proteins, such as staphylococcal protein A and streptococcal protein G, typically represents the initial step in therapeutic antibody purification process. However, this approach suffers from high cost, poor ligand stability and the requirement for relatively harsh elution conditions that can negatively impact activity and immunogenicity of antibodies. Compared to protein ligands, peptides represent an interesting alternative due to higher stability and less expensive production. Furthermore, the expected lower affinity for immunoglobulins should allow for elution under milder conditions. The aim of our research was to identify short peptide ligands for the Fc region of human IgGs.

We have screened three commercially available phage display libraries of random cyclic and linear peptides for binding to human Fc region in solution using an optimized biopanning approach.

Five non-homologous linear peptides were shown to specifically interact with the Fc portion of immunoglobulins as verified by a set of phage ELISA assays. Individual phage-displayed peptides were able to recognize specific subclasses of IgG. The highest-affinity peptide (12L-19Fc), which competed for Fc binding with protein A, was subjected to mutagenesis studies. We displayed on phage several variants of 12L-19Fc with individual amino acid residues exchanged for alanine as well fragments of the parent peptide of different lengths and evaluated binding to Fc with phage ELISA to identify the minimal binding motif. Binding characteristics of the minimized peptide were further analyzed using SPR biosensor. The details will be disclosed at the Meeting.

P-MIS-005
Diverse effects of ganoderma lucidum in combination with tamoxifen citrate and doxorubicin in MCF-7 breast cancer cells

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Ganoderma lucidum, an edible medicinal fungus, has been known with its anti-metastatic, anti-carcinogenic bioactivities and widely used in Asian countries in complementary and alternative medicine. However, there is no information regarding its combined usage with tamoxifen and doxorubicin in breast cancer treatment. We investigated the interactions between *Ganoderma lucidum* and tamoxifen or doxorubicin in MCF-7 human estrogen receptor positive breast cancer cell line. Anti-proliferative properties of six extracts were assessed by WST-8 method. The most effective extract in inhibition of MCF-7 cell viability was then evaluated in terms of its anti-metastatic activity by Boyden Chamber Assay. Apoptosis and cell cycle assays were performed by flow cytometry. *Ganoderma lucidum* ether extract (G.Ether) was the most effective extract on inhibition of cell viability among others with $IC_{(50)}$ values of 100 μg/ml and 12.82 μg/ml at 48 h. and 72 h. respectively. We found that G.Ether is capable of inducing apoptosis and changing cell cycle dynamics. However, incubation with G.Ether did not affect MCF-7 cell motility significantly. We then assessed the interactions between G.Ether and tamoxifen or doxorubicin in MCF-7 cells. The interactions between G.Ether and cancer therapeutics were examined by combination index analysis and MacSynergy II software. Interestingly, G.Ether increased the anti-proliferative effect of tamoxifen although exhibited strong antagonism with doxorubicin in MCF-7 cell line.

P-MIS-006
Testing the best matrix/analyte combination for MALDI TOF mass spectrometric detection of steroid hormones, amino acids, vitamins and carbohydrates

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In spite of numerous advantages, there are serious drawbacks of the application of matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI TOF MS) for small-molecule analyses (below 500 Da) and quantification. The main problem is the background interference from commonly used MALDI matrix materials. The aim of this work is to evaluate MALDI TOF mass spectra of physiologically relevant small molecules: steroid hormones, vitamins, amino acids and carbohydrates, acquired with several organic, traditional matrices.

Small volume, 0.5 μL, of each sample solution (testosterone, progesterone, estradiol, L-cysteine, L-alanine, DL-methionine, glutathione, D-(+)-glucose, D-(+)-maltose, vitamin A, vitamin E) was mixed on the sample plate with the same volume of organic matrix solutions (DHB, THAP, CHCA, 9-AA). For each molecule/matrix pair, we determined quantitative and qualitative parameters of MS analysis. To calculate within day and day-to-day variation we used Excel tools (ANOVA tests). In addition, homogeneity of the sample/matrix distribution on the target was also calculated and expressed as the coefficient of variation of a series of measurements.

Our results show selectivity of the detection of individual molecules related with the matrix applied. The statistical analysis of certain molecule/matrix pairs gave within and day-to-day variations less than 15%. Additionally, homogeneity of the sample/matrix mixture distribution on the target plate was with some matrices, also less than 15%.

Some of the used matrices have a great potential for the analysis of small molecules with good analytical parameters, with low variations and high homogeneity of samples on the MALDI target plate.

These results hold potential for quantification of metabolically-significant small molecules and are very promising for future applications of MALDI TOF MS analyses.

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P-MIS-007
Stress causes different expression of mitochondrial biogenesis markers in rat steroid-producing cells of adrenal gland and testes

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Functional mitochondria of steroid producing cells of adrenal cortex and Leydig cells of testes are essential for steroid hormones biosynthesis and regulation.

The aim of this study was to determine transcriptional profile of mitochondrial biogenesis markers in adrenal cortex and Leydig cells by applying *in vivo* and *in vitro* studies.

Immobilization stress (IMO), was performed for 2 hours daily for one (1xIMO), two (2xIMO) or ten (10xIMO) consecutive days. In *in vitro* studies, primary cultures of purified Leydig cells from undisturbed rats were stimulated with stress hormone adrenaline, propranolol (nonselective β-ADRs-blocker) and prazosin (the selective α1-ADRs antagonist).

RQ-PCR results showed that the transcription of the main regulator of mitochondrial biogenesis, *Ppargc1a* and *Ppargc1b,* significantly decreased in adrenal cortex of 10xIMO rats. Oppositely, the significant increase of the same transcript was registered in Leydig cells from the same rats. In parallel, transcription of *Ucp1*, the mediator of regulated proton leak, decreased in adrenal cortex, but increased in Leydig cells of the same group of rats. Incubation of Leydig cells with adrenaline, increased transcription of the main markers of mitochondrial biogenesis (*Ppargc1a*, *Ppargc1b*, *Nrf1* and *Nrf2a*). Nonselective β-ADRs-blocker attenuated this effect. The selective α1-ADRs antagonist did not change adrenaline-induced stimulation of *Ppargc1a, Ppargc1b, Nrf1* and *Nrf2a* transcription in Leydig cells, indicating that the most of the effects are probably mediated by β-adrenergic receptors, not by α1-ADRs of Leydig cells.

In summary, the results suggest that reduction of transcription of mitochondrial biogenesis markers could be a possible mechanism that protects body from excessive glucocorticoid production from adrenal glands in stress conditions, while at the same time stimulation of mitochondrial biogenesis markers transcription in Leydig cells could serve as mechanism to preserve testosterone production.

P-MIS-008
Generation of new mitochondria is possible protection mechanism of basal steroidogenesis in Leydig cells

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Mitochondria are the most important component of stress response in all cells and for steroid-hormones-producing cells they are the starting point for steroid biosynthesis. Here we investigated the parameters of mitochondrial biogenesis in these cells from rats exposed to the psychophysical stress by immobilization (IMO).

IMO stress was applied for 2 hours daily for one (1xIMO), two (2xIMO) or ten (10xIMO) days.Hormone levels were measured employing EIA, ELISA kit or RIA. Mitochondrial membrane potential ($\Delta\psi m$) was measured by TMRE fluorescence, mitochondrial mass was detected by quantitative analysis of MitoTracker-Green fluorescence as well as relative intensity of fluorescence, since number of mitochondria and mitochondrial architecture were defined using transmission electron microscopy. Relative gene expression and proteins analyses were performed by RQ-PCR and Western blot.

There was positive correlation between $\Delta\psi_m$ of Leydig cells and androgens production of Leydig cells. Both of them were reduced in all stressed rats but partially recovered in 10xIMO group. The mitochondrial mass in Leydig cells from 10xIMO group was increased. Transmission electron microscopy analyses showed that acute and two times repeated stress altered architecture of mitochondrial cristae, while 10xIMO increased number of mitochondria and recovered mitochondrial architecture. There was significant increase in the expression of the all markers of mitochondrial biogenesis in Leydig cells from 10xIMO rats compared with other groups. Accordingly, stress-triggered mitochondrial biogenesis represents an adaptive mechanism and does not only correlate with but also is an essential for testosterone production, being both events depend on the same regulators.

Supporting the evidence that stress, a constant factor in life of humans, induces mitochondrial biogenesis in Leydig cells, our results indicate this mechanism probably protects the basal steroid production in stress conditions.

P-MIS-009
Targeting survival pathways in leukemic cells through synergism of metformin and thymoquinone

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Generation of resistance to current treatment options is common problem in the therapy of many hematological malignancies. Combined therapies utilizing compounds with low toxicity that act synergistically, are proposed to overcome this problem. Metformin and thymoquinone (TQ) are two molecules which have proven safety profile and represent potential candidates for treatment of hematological malignancies. There are more than 150 clinical trials, at different stages, exploring metformin anticancer activity. Metformin activates AMP activated protein kinase (AMPK) leading to inhibition of the mammalian target of rapamycin (mTOR) and induction of apoptosis in different cancers. However, human leukemic cells with increased basal Protein kinase B (Akt) phosphorylation were shown to be resistant to metformin-induced apoptosis. It was found that activity of metformin can be enhanced by combination with Akt and/or Nuclear factor 'kappa-lightchain-enhancer' of activated B-cells (NF-κB) inhibitors. TQ is phytochemical compound that has shown inhibitory capacity on both of these targets.

WST-1 assay was used to evaluate the effects of metformin and TQ in DHL4 (B cell lymphoma) and K562 (chronic myelogenous leukemia) cell lines. CompuSyn software was used in order to calculate the combination index (CI). The CI value indicates whether two drugs have synergistic (CI<1), additive (CI=1) or antagonistic effects (CI>1).

We have shown that separately, metformin and TQ, exhibit dose dependent inhibition of DHL4 and K562 cells. In combinatorial study with fixed constant ratio and simultaneous drug exposure, in DHL4 and K562 cell lines, CI values were 0.68 and 0.66, respectively.

To our knowledge, this is the first report showing synergistic effects of metformin and TQ in lymphoma and chronic myelogenous leukemia derived cell lines.

These promising data are currently being investigated in order to obtain the insight into their molecular mechanisms.

P-MIS-010
Classification of *E. coli* promoters with DNA open states dynamic characteristics

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For the last decade many methods of calculating and analysing the physical characteristics of DNA has been developed. These methods allow to estimate distributions of free energy, propensity to bend, stress-induced duplex destabilization (SIDD), electrostatic potential (EP) etc. And most of them have been used for prediction of genomic regulatory site positions. The main idea of such approach is that proteins recognize genome regulatory sites by these physical and chemical properties, so the physical characteristics are used to predict the location of regulatory sites. Most of the characteristics mentioned above describe properties of DNA at equilibrium or steady state, but we propose to use characteristics of internal DNA dynamics.

In this work we used the coarse-grained model of DNA, developed recently, to simulate dynamics of the DNA open states. With this model we were able to calculate trajectories of the open states moving along the molecule and their dynamical characteristics, such as: open state activation energy, size, half decay time and sound velocity in DNA. We use distribution of four dynamical characteristics around transcription start site of experimentally found *E.coli* promoters taken from Regulon DB to organise them in stable clusters. Clusterization was made with Ward method and consensus clustering technique was applied to clusterization results for analysis of its consistency. The same procedure was applied to equilibrium DNA characteristics for comparison. Distribution of GO functions among clusters was also analysed.

Stable promoter clusters obtained with different physical properties share some similarity. It was not surprise that clusters obtained with dynamical characteristics of DNA more similar to SIDD clusters then to EP clusters. The data highlights the possible role of DNA dynamical properties in transcription initiation and its applicability to promoter identification together with other physical and textual properties of DNA.

P-MIS-011
Chromium complex with 5-hydroxyflavone acts on metabolic pathways

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The development of novel therapeutic strategies for obesity treatment are urgently required as obesity is currently the main leading cause in type II diabetes and insulin resistance. Among natural compounds, flavonoids have recently gained interest due to their positive role in maintaining blood glucose levels and insulin secretion. Their association with trace elements, well-known for their capacity in increasing the efficiency of insulin, might potentiate flavonoids biological effects. In this context, the aim of our study was to investigate the *in vitro* changes in energetic metabolism related genes expression profile in the presence of a chromium complex with 5-hydroxyflavone.

DNA microarray technology was used for a large scale screening of differentially expressed genes in human adipose stem cells (hASCs) after 3 weeks of adipogenic induction in the presence of the chromium complex with 5-hydroxyflavone. Moreover, perilipin expression was assessed by flowcytometry.

The chromium complex with primuletin negatively regulates the expression of key genes involved in adipogenesis and also modulates the expression of the genes associated with triglyceride synthesis and subsequent fat storage in mature adipocytes.

Consequently, the chromium complex with 5-hydroxyflavone can be further employed in studies on animal models to investigate the possible improvement of metabolic disorders.

P-MIS-012
Gfh factors stimulate transcriptional pausing and termination by *Deinococcus radiodurans* RNA polymerase

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Deinococcus radiodurans is a highly radioresistant and stress-resistant bacterium. Despite extensive studies, the mechanisms of transcription regulation that contribute to the stress-resistance are still poorly understood. *D. radiodurans* encodes multipe stress-related proteins including three members of the Gre-family of transcription factors: GreA, Gfh1 and Gfh2. While GreA is a universal bacterial factor that stimulates RNA cleavage by RNA polymerase (RNAP), the functions of lineage-specific Gfh proteins remain unknown.

We cloned, expressed and purified *D. radiodurans* RNAP and Gfh factors and their mutant variants and analyzed their properties using various *in vitro* transcription approaches. We tested Gfh effects on RNAP activity in promoter, elongation and termination complexes assembled on natural and synthetic DNA templates under different conditions.

We found that the Gfh factors strongly enhance site-specific pausing and intrinsic transcription termination by *D. radiodurans* RNAP but do not act on active transcription complexes and do not compete with the GreA factor. Uniquely, the pause-stimulatory activity of Gfh is greatly enhanced by manganese ions, which are accumulated in *D. radiodurans* cells under stress conditions, and is modulated by the secondary RNA structure. We revealed functionally important regions in the Gfh factors and the RNAP active site involved in transcriptional pausing.

We propose that Gfh factors inhibit RNA extension in paused complexes through binding within the secondary RNAP channel, coordinating metal ions in the RNAP active site and stabilizing an inactive enzyme conformation. This may serve as a sensitive mechanism to regulate transcription under stress conditions and coordinate it with DNA repair and replication. Our data suggest that Gre and Gfh proteins target different structural states of the transcription elongation complex and reveal functional diversity of the factors that bind within the secondary channel of RNAP.

P-MIS-013
Deciphering the role of a new key player in the transition between motility and biofilm formation

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Bacteria are extremely versatile organisms, which rapidly adjust their gene expression to induce physiological and molecular adaptation to changing environments. When bacterial cells switch

from planktonic to biofilm state of growth, flagella formation is turned off, and the production of fimbriae and extracellular polysaccharides is activated. BolA protein is widespread in nature and has been associated with several cellular processes.

Using High-troughput techniques we showed that BolA protein is a new bacterial transcription factor, which regulates the switch between motile and sessile lifestyle. It negatively modulates flagellar biosynthesis and swimming capacity in *Escherichia coli*. Moreover, BolA overexpression favors biofilm development, involving fimbriae-like adhesins and curli production. Our recent results show that BolA action in these pathways is related with c-di-GMP a relevant intracellular signaling molecule involved in biofilm formation. We demonstrate that BolA contributes to a fine-tuned expression of different diguanylate cyclases and phosphodiesterases and c-di-GMP has a negative influence in the *bolA* mRNA transcription.

Herein we propose that BolA is a key player in motile/adhesive transcriptional switch, contributing to a fine-tuned regulation of these important pathways.

P-MIS-014
Frequency and association of the genetic mutations in patients with deep venous thrombosis and healthy controls

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Background: Deep venous thrombosis (DVT) is an important health problem worldwide. Its pathophysiology is multicausal and involves environmental, genetic and acquired factors. Factor V Leiden (FVL), prothrombin G20210A (PT G20210A), and Methylenetetrahydrofolate reductase (MTHFR) gene mutations are to predispose to venous thrombosis. The aim of this study was to compare the frequency of FVL, PT G20210A and MTHFR polymorphisms between patients with DVT and healthy controls.

Methods: This study was conducted at the Bozok University Hospital. Total 220 participants were included in this study, 126 patients with DVT and 94 healthy blood donors. In order to identify FVL, PT G20210A, MTHFR C677T and MTHFR A1298C, the polymerase chain reaction (PCR) method was utilized combined with the amplification refractory mutation system.

Results: In 126 patients FVL was present in 54 (42.8%) patients while in controls FVL was present in only 18 (19.1%). Frequency of FVL was significantly higher in cases as compared to controls ($p < 0.05$). PT G20210A mutation was present in 13 patients (10.3%) and in 5 healthy participants (5.3%). MTHFR C677T and MTHFR A1298C polymorphisms were almost equally distributed among patients and healthy participants. However, the concomitant presence of FVL and double heterozygous polymorphisms of MTHFR C677T/A1298C was found in 11 patients (8.7%) and in 2 healthy controls (2.1%), showing significant association with deep venous thrombosis.

Conclusion: In this study, the frequencies of FVL and PT G20210A polymorphisms were found significantly higher in patients with DVT than those in healthy participants. Thus, FVL and PT G20210A polymorphisms have a contributory role on the development of DVT In contrast, MTHFR C677T and MTHFR A1298C genotypes were not associated with a predisposition to development of DVT. But, a combination of double heterozygous polymorphisms of MTHFR C677T/A1298C with FVL may be associated with increased risk of DVT.

P-MIS-016
Self-assembling micellar clusters comprising drugs, nanoparticles and fluorescent compounds for bilogical applications

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When designing drug carriers, the drug-carrier ratio is an important consideration, because the use of wrong drug-carriers relation can result in toxicity as a consequence of poor metabolism and elimination of the carriers. Solubility problem of various substances also plays an important role in many aspects of fundamental science and practical field. Specifically, it is an important parameter as well as bioavailability, which determines the required concentration of drug in the body needed to achieve a pharmacological response. Among the variety of solubilization methods micellar solubilization is widely used as an alternative to the dissolution of poorly soluble drugs.

Here, we show a specific approach based on sequential self-assembly of nonionoc detergent micelles (T × 100, TX114) followed by enacpsulation of various nanoparticles (noble metals, magnet etc.), drugs, fluorescent compounds leads to the formation of stable micellar nano- amd microcomplexes.

We propose 3 ways of micellar clusterisation. In the first one micelles are modified by semi-hydrophobic chelator followed by addition of metal ion to make cross-linking. The second way is similar to the first one and suggests application of the metal complex with incresed denticity instead of naked metal ion, and the third one involves micelles clusterisation by semi-hydrophobyc metal complex directly. Therefore, one can stabilize micellar network by means of 'interactions on interface': semi-hydrophobyc metal complexes are embedded inside micelle due to hydrophobyc interactions.

Hydrophobic fluorescent compounds-loaded micellar complexes demonstrates better optical response in aqueous media without crystallization. Such obtaining clusters are also very flexible and can be modified by nanoparticles to obtain various nanocomposites, such as fluoromagnetic clusters.

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P-MIS-017
Lamellipodia and membrane blebs utilize different signalling pathways to induce directional movement of Walker Carcinosarcoma WC 256 cells in a physiological electric field

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Sensing of the electric fields (EF) is important for directional cell migration during wound healing, development and cancer metastasis but the underlying mechanism is poorly understood. We demonstrated that blebbing, similarly to lamellipodia formation, enables directed cell motility in external EFs, however it is not

clear if those reactions are mediated by similar mechanisms. To establish that, we performed proteomic analysis and subsequent investigation of the role of differential signalling pathways in electrotaxis of cells representing various strategies of movement.

Cells were exposed to EF in galvanotaxis apparatus and their reaction was recorded. In some experiments cells were pre-incubated with ERK1/2 or Btk-1 inhibitors. The phosphorylation of ERK1/2 and Btk-1 was determined by Western blot analysis. Proteomic analysis was performed by UltiMate 3000RS LC nanoSystem coupled with a Q-Exactive mass spectrometer.

Both blebbing (BC) and lamellipodial (LC) cells show cathodal migration in a physiological EF (1 V/cm). Comparative analysis of BC and LC cells proteomes revealed about 150 differential proteins. Functional analysis in Ingenuity Analysis Pathway allowed to determine the statistically significant signalling pathways in which these proteins are engaged. Among the most distinctively regulated pathways are Tec Kinase and ERK/MAPK signalling activated in LC but not BC. It was found that Btk-1 is required for directional movement of LC but not for BC cells. Moreover, EF induced stronger and faster Btk-1 phosphorylation in LC than BC cells. In contrast ERK 1/2 activity was not necessary for electrotaxis of LC cells and EF did not induce ERK 1/2 phosphorylation.

Our results reveal that both lamellipodia and membrane blebs can efficiently drive electrotactic migration of WC256 cells but it is mediated by different signalling pathways.

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P-MIS-018
Newborn screening for congenital hypothyroidism in Turkey: a regional evaluation

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Congenital hypothroidism (CH) is the most common congenital endocrine disorder and the most important cause of preventable mental retardation. It is important to begin the treatment within 2 weeks before the development of brain damage. TSH based newborn screening programs are shown to be useful for implementing early treatment of CH. In this study, regional results of CH screening program in Turkey between 2006 and 2015 were assessed retrospectively. We have evaluated the results of Marmara, Central Anatolia, Aegean and Mediterranean regions in which our laboratories are located.

Screening was based on TSH determination in dried blood spot specimens. TSH limits determined to be 10 μu/ml for cut off point and 25μU/ml for clinical decision point. TSH was measured using enzyme immune assay (EIA). Blood spot TSH data for 65857 newborns during this time period were evaluated. Permanent or transient CH was determined according to the results of thyroid function tests. Confirmed CH cases were based on local endocrinologists' report and initiation of thyroxine treatment.

The frequency of neonatal TSH levels were found to be under the cut off level of 10μU/ml in 63513 (96.44%), between 10 and 25μU/ml in 2287 (3.47%) and above the level of 25μU/ml in 57 (0.09%) babies, respectively. Recall rate was 3.5%. CH cases of neonatal TSH levels greater than 25μU/ml were 26. The incidence of CH of this group was 1:2532. There were no significant differences in the number of congenital hypothyroidism between males and females ($p > 0.05$). The preliminary results of our study indicate that the incidence of CH in our region is higher than the worldwide reports as has been proved by preceding studies. Iodine deficiency, dyshormonogenesis, highly consanguineous population, may contribute to the high incidence of CH in Turkey. Newborn screening of CH must be developed for detecting true cases and TSH cut off point must be reviewed for decreasing redundant recall rate.

P-MIS-020
In silico analysis of the first complete genome sequence of *Lactobacillus acidipiscis* species

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Introduction: Lactic acid bacteria (LAB) constitute a significant group of microorganisms for the food industry, as they play a key role in food fermentation and consequently in human health. *Lactobacillus acidipiscis* ACA-DC 1533 is a Gram-positive, motile, rod-shaped LAB isolated from traditional Greek Kopanisti cheese. Here we present the *in silico* analysis of the first complete genome sequence of *L. acidipiscis* in order to explore the biology of the species.

Materials and Methods: Sequencing of *L. acidipiscis* genome was performed using the HiSeq 2000 and PacBio RSII sequencing platform technologies and the genome assembly was validated against an *Nhe*I optical map of the *L. acidipiscis* genome. Protein-coding sequences were predicted by Glimmer, rRNA genes by RNAmmer and tRNA genes by the tRNAscan-SE server. Potential genomic islands were detected using the IslandViewer software tool, prophage regions by PHAST and the subsystem-based annotation by RAST server. Finally, the circular representation of *L. acidipiscis* genome keyed to the COG groups was constructed by CGView server.

Results: The sequencing analysis resulted in one continuous genomic scaffold of 2,678,726 bp with a G+C content of 39.75%. The genome contains 2,525 protein-coding genes on the chromosome covering up to 82.09% of the genome sequence, 63 tRNA and 18 rRNA. According to the subsystem-based annotation, 1,562 protein-coding genes were assigned to 310 metabolic subsystems. The most abundant of the subsystems are related to carbohydrates (n = 287, 18.37% of total protein-coding genes) and protein metabolism (n = 173, 11.08% of total protein-coding genes). Furthermore, three prophage regions were detected; one intact (43.5Kb), one incomplete (14.7 Kb) and one questionable (29.3 Kb).

Discussion and Conclusion: The whole genome analysis of *L. acidipiscis* ACA-DC 1533 provided interesting information about a not well-studied species.

P-MIS-021
Investigation of serum irisin levels of patients with metformin taking new onset type 2 diabetes mellitus

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Irisin, a recently defined hormone, is a myokine which is released into blood from the skeletal muscle by proteolytic cleavage of fibronectin type III domain containing protein 5 (FNDC5). Irisin

increases glucose tolerance and energy expenditure and improves carbohydrate homeostasis. Metformin is a biguanidine class antidiabetic drug which inhibits liver gluconeogenesis and decreases insulin resistance and is frequently recommended in treatment of new onset type 2 diabetes mellitus (T2DM). Irisin has a role in the regulation of energy metabolism pathways and its level in blood of persons with T2DM has been reported to decrease.

Regarding this relationship, it was aimed to reveal the effect of metformin on serum irisin levels. 20 patients with impaired oral glucose tolerance test were included to this investigation. They were recommended to take metformin and to change their life style, such as exercise and diet. Their blood were taken at the beginning and after 1 month. Also, a healthy control group (n = 20) was formed from persons with similar age and sexual distribution as the patient group. Irisin levels of their sera were measured by Enzyme-Linked ImmunoSorbent Assay (ELISA) method.

Statistical evaluation of the measurements showed no significant difference ($p = 0.780$) between the irisin levels of the patients at the beginning and after 1 month treatment. A similar result was found between the control and the treated groups ($p = 0.170$), while a significant difference ($p = 0.002$) was observed between the control and untreated patients groups.

The results obtained from this study do not show a clear and significant change in the blood irisin levels of the patients with new onset T2DM taking metformin together with life style change. A longer period of treatment and a higher number of patients may be needed for more reliable results.

P-MIS-022
Thermodynamics of DNA ligands binding at specific sites of telomeric G-quadruplex

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DNA G-quadruplexes are a perspective target for anticancer therapy. For example stabilization of the telomeric G-quadruplex DNA formed by single-stranded ends of the chromosomes leads to inhibition of telomerase, which is active in 90% of cancer cells. Similarly, small molecules targeted to a specific G-quadruplex would inhibit various cellular processes. Stoichiometry and affinity of interaction of these compounds to DNA is determined by specific structural motifs within a G-quadruplex. Rational design of novel chemical compounds requires an in depth knowledge of interactions between known ligands and G-quadruplex structures.

Experimental methods that are used for determination of thermodynamic binding parameters, such as isothermal titration calorimetry, differential scanning calorimetry, ultraviolet absorption and circular dichroism spectroscopy provide a collective characteristic for all of the ligand molecules bound to DNA, while the information on ligand affinity to individual DNA binding sites is lost.

We propose a complimentary method for detailed analysis of thermodynamic parameters of ligand binding based on the introduction of fluorescent probes in the structure of G-quadruplex. Monitoring fluorescence quenching of the fluorescent labels allows to derive binding constants of the DNA-ligand interaction at a specific binding site. Temperature dependence of the fluorescence quenching determines the thermodynamics of the DNA-ligand complex formation. Since only a proximal ligand is able to quench the fluorescence, this method allows characterization of the ligand binding to a particular site the G-quadruplex structure. The study was supported by project no. 16-14-10396 of the Russian Science Foundation.

P-MIS-023
The correlation between biochemical and dynamic surface tension parameters of calves blood serum

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During the animal ontogenesis, as well as by various pathologies or poor diet, the imbalance of protein, mineral, lipid components is observed (the changes in all parameters of biological liquids are accompanied of these metabolism peculiarities). The dynamic surface tension (DST) of serum essentially depends on these factors and (in combination with the biochemical parameters) can provide the valuable information for evaluation of the physiological and biochemical status of the organism (can be used as an express test for animal diagnostics in future).

The aim of the work was to study DST and biochemical parameters of calve serum, as well as their correlations, as the main indicators of the animals. The major DST (average equilibrium value 46.92 ± 2.54 mN/m and curve tilt 0.31 ± 0.39 $mN*m^{-1}s^{/2}$) and biochemical parameters (total protein 79.51 ± 5.55 g/l, albumin 25.86 ± 1.40 g/l, cholesterol 2.61 ± 0.40 mM, urea 2.74 ± 0.64 mM, billirubin 10.16 ± 3.34 mM, calcium 2.87 ± 0.18 mM, magnesium 0.82 ± 0.35 mM, phosphorus 2.49 ± 0.65 mM, etc.) of calve serum were in the range of the normal values for healthy animals and can be considered as reference data for animal science and practice. The obtained results enable us to establish correlations between the DST and biochemical parameters of calves serum. This work was supported by the Russian Scientific Foundation (grant 14-16-00046). The middle strong correlations of DST values of calves serum with the level of total protein, albumin, billirubin, some enzymes and cholesterol, whereas only weak correlations with the other biochemical parameters (urea, calcium, magnesium, phosphorus, etc.) were found. In the veterinary science and practice such correlations are important for the estimation of the organism physiologicaland biochemical status, for general inspections of cattle before vaccination (immunization) or slaughter, for "quick separation" of healthy and ill animals in the case of infection, etc.

P-MIS-024
Role of protein kinase C in the regulation of astrocytic glutamine transporter SN1 in ammonia-exposed mouse cortical astrocytes

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It has been established that SN1, the system N astroglial L-glutamine (Gln) transporter, shows dynamic membrane trafficking that appears to be negatively regulated by PKC. The question arose whether the specific role of PKC in the SN1 regulation holds for primary cortical astrocytes treated with ammonia, a condition mimicking hyperammonemia.

Cortical astrocytes from newborn mice were treated with 5 mM NH_4Cl (ammonia) for 24 h and/or co-treated with PKC activator – phorbol 12-myristate 13-acetate (PMA; 200 nM) in the presence or absence of PKC inhibitor bisindolylmaleimide I

(BisI; 1 μM). Total PKC activity was analyzed by a direct PKC assay and phosphoserine detection by Western Blot (WB) analysis. Protein level of SN1 and SN2, second astrocytic Gln transporter belonging to system N, in a membrane fraction was also analyzed. The total uptake and system N-mediated (L-Ala and L-Leu-inhibitable) Gln uptake was tested.

Treatment of astrocytes with ammonia resulted in a decrease of PKC activity, whereas PMA treatment increased PKC activity in ammonia-independent way. BisI treatment reversed fully, and ammonia partially, the PMA-induced PKC activity. PMA treatment resulted in only a slight decrease in SN1 protein level in both control and ammonia-treated astrocytes, while a decrease of total and system N-mediated Gln uptake were noted in control astrocytes, an effect not exacerbated by ammonia. In turn, co-treatment with PMA and BisI reversed the decrease of total Gln uptake and showed tendency towards increase in system N-mediated Gln transport.

The results suggest that: a) Ammonia changes the dominating direction of system N transport from release to uptake, which may be related to decreased phosphorylation or to alterations in relative phosphorylation by different PKC isoforms. This inference remains to be verified in further studies; b) Changes in system N transporter function induced by ammonia appear to involve mechanisms other than changes in transporter expression.

P-MIS-025
Evidence for human ghrelin GHS-R1a and orexin OX1 heteroreceptor complex formation in a heterologous system

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Ghrelin and Orexin are two peptides implicated in the regulation of energy balance and modulation of food-related motivation at the level of the midbrain dopamine reward system. Their function in the hypothalamic arcuate nucleus and the ventral tegmental area (VTA) has already been described, but the modulation at the level of receptors remains unclear. The action of these peptides is mediated by G-protein-coupled receptors (GPCRs): ghrelin 1a and 1b (GHS-$R1_a$, GHS-$R1_b$) for ghrelin, and orexin 1 and 2 (OX_1, OX_2) for orexin.

Traditional approaches to know the mechanism of neurotransmission of dopaminergic neurons in the mesolimbic system have focused on targeting neuronal receptors as single entities. From the discovery that GPCRs for neuromodulators may form heteroreceptor complexes, our hypothesis is that Ghrelin and Orexin receptors may interact and form novel functional units that may specifically participate in the central regulation of food intake and energy balance. As a proof of concept we have investigated the potential of human GHS-$R1_a$ and OX_1 receptors to form heterocomplexes.

Formation of GHS-$R1_a$ -OX_1 receptor heteromers in transfected HEK293T cells was detected by Bioluminescence Resonance Energy Transfer (BRET) and Proximity Ligation (PLA) assays. Furthermore, a negative crosstalk was identified in cells co-expressing both receptors by assessing mitogen-activated protein kinase (MAPK) and adenylyl cyclase (cAMP) pathways, and by a label-free dynamic mass redistribution assay.

Experiments in sources endogenously expressing GHS-$R1_a$ and OX_1 receptors are needed to know the functional relevance of the heteromer. From the negative crosstalk here identified, it is tempting to speculate that GHS-$R1_a$-OX_1 receptor heteromers are important players in mediating the response to the combination of different orexigenic signals.

P-MIS-026
Glycocerebrosidase enzyme activity in Dried Blood Spot (DBS) sample is closely related to the number of leucocytes

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Lysosomal storage diseases which are related to deficiency of specific lysosomal hydrolases resulted to clinical aspects due to accumulation of substrates in different tissues. Since Dried Blood Spot (DBS) is non-invasive, low-cost, easy transportable, acceptable enzyme stability compared to leucocyte and/or fibroblast culture, it's recommended as a first screening test. However the false positive rate with DBS sample is higher compared to other samples. We aimed to investigate any possible effect of leucocyte number on enzyme activity in dried blood samples in a retrospective study.

We re-evaluated the lysosomal enzyme activity results in regard to leucocyte number among data within last 1 year. Enzyme activities had measured by using fluorometric and LC MSMS method. We determined the correlations between the lysosomal enzyme activities of alpha glycosidase, glycocerebrosidase, alpha galactosidase, sphingomyelinase, galactocerebrosidase and alpha-L-iduronidase in healthy population (n = 220).

While glycocerebrosidase and galactocerebrosidase positively correlated with the number of neutrophils, alpha galactosidase, sphingomyelinase and alpha-L-iduronidase positively correlated with the number of lymphocytes. Alpha glycosidase activity showed a correlation both lymphocytes and neutrophils. The patients having the glycocerebrosidase enzyme activity which was lower than 0.6 nmol/ml/hour (which is accepted as the cut off value to recall the patients) existed significantly lower number of leukocyte, lymphocyte and neutrophil compared to those of patients having higher enzyme activity than 0.6.

Our data indicated that the enzyme activity in dried blood samples including low leucocyte number might be found lower than reference intervals resulting in false positive diagnosis. Therefore we suggest that the laboratory scientists should evaluate the number of leucocyte levels while they were interpreting data.

P-MIS-027
Using DNA-markers for estimation of genetical variability of two Kazakh sheep breeds

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To compare the frequencies of different microsatellite loci in sheep breeds subpopulations genomic structure of Edilbay and Kazakh Archaromerinos was investigated. Different methods for homogeneity testing of two breeds were elaborated. Inter Simple Sequence Repeats (ISSR) PCR analysis of the breeds studied displayed species and breed specific fragments with different frequencies (population frequency more than 0.4) There were found rarely met fragments (frequency lower than 0.4). The combinations of these fragments present the specific ISSR-spectra which arrange genofond profiles of breeds. Using panels of microsatellits (recommended by ISAG) 2 breeds (5 populations) were characterized. Informative value and resolving capacity of the sum of 32 STR-loci were estimated. Wide polymorphism of alleles length

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was demonstrated both when the breeds were compared and within the breeds. 10 informative markers were chosen for both two breeds, 7 markers being used for both breeds, while other 3 markers were informative for one of the breed only. When the animals of one breed were compared unique alleles which were met only within one of populations were of much interest. For example the allele 174 of BM1824 was met in Birlik population of Edilbay breed as often as in 59% of animals while in two other populations there were no this allele. In Kumtekey population one can meet 70% animals having particular locus (DYMS1), while in the other population (CF Ablay) this locus was not met at all. Basing on genetical distances obtained using fragment analysis phylogenetic relationships between populations were estimated. So for example Edilbay population of CF Ajar has the larger distance from two other populations (Birlik and Bayserke-Agro) than each of them from one another. Two subpopulations of Kazakh Arkharomerinos breed (CF Kumtekei and CF Ablay) also have the genetical difference.

P-MIS-028
How preeclampsia affects oxidant status and antiinflammatory potential of breast milk?

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Preeclampsia is a pregnancy syndrome associated with hypertension, proteinuria and edema, leading to maternal morbidity/mortality and preterm delivery. In this study we aimed to investigate if the breast milk of preeclamptic mothers is effected in oxidative status and anti-inflammatory activity in comparison to the breast milk of mothers with healthy pregnancies.

For the aim of the study, hyaluronidase and myeloperoxidase activities (MPO), total oxidant status (TOS), total antioxidant status (TAS), oxidative stress index (OSI) and TBARS levels were measured in breast milks of 20 preeclamptic mothers and 20 mothers with healthy pregnancies as control group. When the control group and preeclamptic group were compared, hyaluronidase activity, TAS, TOS and OSI levels showed statistically significant differences in the preeclamptic group.

Hyaluronidase activity was significantly higher in the preeclamptic mothers' breast milk (734 vs 594 U/ml, $p = 0.046$). While TOS levels were significantly higher in the preeclampsia group (7.48 vs 4.51 μmol/l, $p = 0.001$), the TAS levels were significantly higher in the control breast milks (0.557 vs 0.813 mmol/l, $p = 0.011$). As expected OSI levels (TOS/TAS ratio) were significantly higher in the preeclampsia group. Even though the mean levels were higher in preeclamptic group, the difference in MPO activities and TBARS levels did not show statistic significance.

Oxidant status parameters also suggest that preeclampsia effects in both ways by increasing oxidant status and also decreasing antioxidant capacity shifting the balance to the increased oxidant stress side. As the results showed that the preeclampsia group had higher hyaluronidase activity, this can be interpreted as preeclamptic mothers' milk have higher inflammatory potential as this enzyme enhances inflammation by catalyzing the depolymerization of certain acidic glycosaminoglcans.

P-MIS-030
Investigation of relationship between postprandial lipemia and erythrocyte membrane cholesterol level

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Postprandial lipemia is a metabolic condition related to an increase in plasma triglycerides. Remnant-like lipoprotein particles are predominant in postprandial phase and they play an important role in development of atherosclerosis. Cholesterol is a prominent component of erythrocyte membranes and regulates the membrane functions such as viscosity and permeability. Free cholesterol derived from erythrocytes is thought to participate in the atherosclerotic plaque formation.

In the current study, it was aimed to investigate the relationship between postprandial lipemia and erythrocyte membrane cholesterol level in healthy subjects. Study group included 87 subjects (39 female and 48 male with age range of 18–55 years). Then these individuals were divided into three groups according to the values of area under curve (AUC) calculated by using triglyceride levels at the fasting state and at 2nd, 4th and 6th hours after the high fat diet (OTTT).

Lipid and erythrocyte membrane cholesterol (EMC) values were compared between groups with low and high OTTT response. While TC, TG, LDL-C and EMC were significantly higher, HDL-C was significantly lower in high OTTT response group than low OTTT response group. It was not observed any statistically significant difference when compared EMC values between women and men study groups. On the other part, it was seen positive correlation between EMC and AUC ($r = 0.33$, $p = 0.002$), TG ($r = 0.26$, $p = 0.016$), TC ($r = 0.30$, $p = 0.005$), LDL-C ($r = 0.29$, $p = 0.007$) in the total study group.

It was concluded that, postprandial lipemia may show atherosclerotic tendency not only with atherogenic lipid profile but also with increasing EMC.

P-MIS-031
EU-OPENSCREEN: the European infrastructure for chemical biology

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Small molecules that can be applied as chemical 'tool' compounds (or 'probes') have become indispensable in basic research for the elucidation of fundamental biological mechanisms. They act directly with the protein-of-interest and often allow for the interrogation of biological processes that cannot be properly studied with traditional genetic or RNA interference approaches.

EU-OPENSCREEN (www.eu-openscreen.eu) is the largest emerging academic chemical biology research infrastructure initiative in Europe and will provide access for molecular and cell biologists to screening infrastructure, well-characterized high-quality chemical libraries, and facilities for medicinal chemistry services for compound optimization.

Molecular biologists who have a robust and suitable biological assay and are interested in collaboratively developing chemical tool compounds to validate *their* targets-of-interest are welcome to work

with EU-OPENSCREEN. Selected assays are screened against a collection of more than 100,000 compounds, incl. confirmatory and counter screening, IC/EC50 determination, SAR (structure-activity relationships) and QC of confirmed hit compounds.

EU-OPENSCREEN will start operations in 2017, but it can already look back on a growing number of transnational activities: joint screening projects, exchange of local compound libraries, development of new design principles for its compound collection; exchange of experimental data through its pilot database etc.

P-MIS-032
Steps towards an Arthrobacter nicotinovorans based biotechnology for production of 6-hidroxy-nicotine

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As the archetypal agonist of nAchR, nicotine stands up as a powerful scaffold for developing new Alzheimer disease therapeutic agents in form of nicotine derivatives. In this context, *Arthrobacter nicotinovorans* pAO1 and its wide range of nicotine-derivatives produced when grown on nicotine have a huge biotechnological potential. Indeed, the metabolic intermediate 6-Hydroxy-Nicotine (6HNic) produced by *A. nicotinovorans* pAO1 was shown to bind to the nAChRs, and by modulating their function, to sustain spatial memory formation in a rat model of AD.

The current work presents the first attempts to produce and isolate 6HNic by using a genetically engineered *A. nicotinovorans* strain. The growth and the 6HNic accumulation were compared for two strains: 1. *A. nicotinovorans* pAO1 wild type strain and 2. a genetically engineered *A. nicotinovorans* pAO1 strain (pART2NDH) containing the nicotine-dehydrogenase (NDH) genes cloned in the nicotine inducible pART2 vector. The growth curves were followed spectrophotometrically. The consumption of nicotine and accumulation of 6HNic were monitored by HPLC using a MN Nucleodur 100-3 C18ec column and 0.1 M sulfuric acid at a flow rate of 1 ml/minute.

The growth curve of the pART2NDH strain shows that the bacteria grow slower when compared with the *wt*. As a result, in the wt strain, the nicotine is quickly depleted from the medium and only low amounts of 6HNic are observed. Although the SDS-PAGE analysis of the total protein extracts from the pART2NDH strain did not show clear signs of NDH overexpression, the enzyme is produced and is active, allowing a 5 fold accumulation of 6HNic in the growth medium. The first attempts to purify NDH from the pART2NDH strain using IMAC were nevertheless unsuccessful.

In conclusion, using the pART2NDH strain for 6HNic production is feasible. Further improvements of the growth condition and strain are envisioned (i.e. knocking the NDH downstream genes; adding inhibitors for the downstream enzymes).

P-MIS-033
Design, synthesis and kinetic evaluation of cinchonines and cinchonidines as selective human butyrylcholinesterase inhibitors

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Studies on the impact of butyrylcholinesterase (BChE) on the symptoms and progression of cognitive impairments like Alzheimer's disease (AD) or other neurodegenerative disruptions speak in favour of selective BChE inhibitors as a new approach in future AD pharmacotherapy. Some derivatives of quinine and quinidine, present in the Cinchona species bark, have already been identified as selective BChE inhibitors with respect to acetylcholinesterase (AChE); therefore, further investigation of these compounds might result in promising leads for enhanced anti-AD drugs.

We synthesised ten quaternary derivatives of cinchonines and their corresponding pseudo-enantiomeric cinchonidines. Quaternization of quinuclidine moiety was carried out with groups diverse in size: methyl and differently *meta* and *para* substituted benzyl groups. All of the compounds were prepared in good yields, characterized by standard analytical spectroscopy methods, and were tested for their BChE and AChE inhibition potency. The inhibition potency of the compounds was defined by the dissociation constants of the enzyme–inhibitor complex (Ki).

All of the tested compounds reversibly inhibited both human BChE and AChE. The compounds inhibited BChE with Ki constants in the range of 0.04–30 μM, and AChE in the range 2.5–70 μM. Five cinchonidines displayed a 95–510 times higher inhibition selectivity to BChE over AChE, and four of them were potent BChE inhibitors with Ki constants up to 100 nM.

BChE affinity toward the studied compounds depended on the size of the substituent on the nitrogen of the quinuclidinium part of the molecule and on the resonance stabilization of the substituent at the quaternized nitrogen.

Based on the presented results, cinchonidine CD-(pBr) can be pointed out as a potent and selective BChE inhibitor that could be considered for further research in Alzheimer disease pharmacotherapy.

P-MIS-034
Long-term exposure of cultured mouse astrocytes to NMDA modulates expression of mRNAs coding for critical astrocytic proteins

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NMDA receptors are present in rodent astrocytes and their activation elicits a rapid calcium signal. We set out to analyze the as yet poorly known response of NMDA receptors present in astrocytes to long-term exposure to excess glutamate (Glu) or NMDA, conditions mimicking different brain pathologies.

Here, expression of mRNAs coding for: the inward rectifying potassium channel Kir4.1, the water channel aquaporin-4 (AQP4) and glutamine synthetase (GS) and for the NMDA receptor subunits was analyzed using real-time PCR in primary cultures of mouse cortical astrocytes exposed for 8–72 h to NMDA, Glu and the inflammatory cytokine TNFα.

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Exposure to NMDA (100 μM) for 72 h increased the expression of Kir4.1 mRNA and decreased that AQP4- and GS mRNA. The expression of Kir4.1 was decreased by 72 h exposure to Glu (2 mM) and TNFα (50 ng/ml). At 8 h incubation, NMDA induced a decrease of Kir4.1 expression in the presence but not in the absence of calcium in the medium. NMDA did not alter the expression of NMDA receptor subunits. TNFα increased the expression of the NR1 subunit, and decreased that of NR2B mRNA. Glu decreased the expression of 3 out of 7 subunits.

The study demonstrates, to our knowledge for the first time, that prolonged exposure of astrocytes to NMDA alters the expression of mRNA coding for critical astrocytic proteins. The dependence of the decrease of Kir4.1 mRNA expression on extracellular calcium suggests the ionotropic nature of NMDA receptor stimulation. The effects of NMDA receptor stimulation occurred by a mechanism bypassing changes in subunit composition of the NMDA receptor. Experiments are under way to establish whether the TNFα-induced changes in the expression of NMDA receptor subunits contribute to modulation of NMDA receptor stimulation by inflammation.

P-MIS-035
The importance of education in reducing preanalytical errors

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The preanalytical phase includes the request of test, the preparation of patient, the obtaining of sample from the patient, the transport of the sample to the laboratory, and the pretreatment of sample. The preparation of patient and the obtaining of sample are considered as the most common error sources. In order to reduce preanalytical errors, we aimed to provide training for phlebotomists and to also determine their knowledge level about the preanalytical phase before and after these training.

It was given the training related with preanalytic phases to 130 pediatric nurses and 350 adult nurses, other phlebotomists in March. The surveys which are made before and after the training were consisted of 20 questions that are related with demographyic features and preanalytic phases. In order to determine the effects of training to the preanalytic phase, the preanalytic error rates before (in February) and after (in April) traning was calculated with the formula of: (the number of rejected samples/the number of total samples)x100.

The average age of participants was 35 ± 9 years. It was not found significant difference between their correct answers rate before the training and the education degree of the participants. The correct answer rate before the training was 59% and after the training it was 92%, which showed an increase of 55%. The preanalytic error rates which were 0.61% in February were decreased to 0.40% in April.

In our study, the positive results were obtained through the training aimed to reduce the preanalytical errors. By providing regular training to the phlebotomists and also providing pretraining to the beginners, the updating of their information about preanalytic phase can be achieved. In this way, the loss of labor and economic related to preanalytical errors can be avoided and the accurate results can be obtained in short time.

P-MIS-036
Serum vitamin D levels in patients with hypothyroidism

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Aim: Vitamin D deficiency is a global health problem. Over a billion people worldwide are vitamin D deficient or insufficient. Both vitamin D and thyroid hormone bind to similar receptors called steroid hormone receptors. Our aim was to examine the serum vitamin D levels in patients with hypothyroidism.
Material and Methods: This is a retrospective observational study; evaluating the vitamin D values of 47 healthy controls aged 37.5 ± 13.1 years and 1104 patients with hypothyroidism aged 36.3 ± 16.5 years. Mass spectrometric analyses were performed using an Shimadzu LC-20-AD (Kyoto, Japan) coupled with a ABSCIEX API 3200 triple quadrupole mass spectrometer (USA) equipped with an atmospheric pressure chemical ionisation (APCI) operating in positive mode for determination of vitamin D. Statistical analysis was performed with SPSS v16.
Results: The mean of vitamin D values in patients with hypothyroidism (14.67 ± 7.28) were significantly lower compared to control group (21.18 ± 12.89) ($p < 0.001$).
Conclusions: Vitamin D has various effects on body's metabolic pathways. Besides bone mineral homeostasis, Vitamin D deficiency has been associated with a wide range of non-skeletal effects. According to this study's results, decreased vitamin D levels may be related to thyroid function disorders.

P-MIS-037
Co-encapsulation of curcumin and piperin in zein-chitosane nanocapsules by electrospray

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Curcumin, the active compound of turmeric (Curcuma longa) has antiinflammatory, antioxidative and antitumour effects. Unfortunately, curcumin has a poor absorption and low stability. Both can be solved by encapsulation of curcumin using a proper technique like electrospray. It was reported that piperin, the active compound of black pepper, enhances the intestinal absorption of curcumin and thus its bioavailability.

Due to these facts it was aimed in this study to nanoencapsulate turmeric extract in order to enhance its absorption and stability. For that purpose, it was encapsulated with the maize protein zein, chitosane and black pepper extract by varying the voltage and flow rate of electrospray and the concentration of the compounds. The nanocapsules were characterised by measuring their particle size and with help of SEM photographs. The particle size of the final nanocapsule formulation was 550 nm and had a sufficient stability over a period of 6 months, visually determined.

By encapsulating turmeric extract into double layer nanocapsules with help of black pepper extract, zein and chitosane, the turmeric extract could be protected from degradation, which was observed for the pure turmeric extract in form of clearing its yellow colour.

P-MIS-038
Oxidation of oncogene promoter G-quadruplexes with cationic porphyrins

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Analysis of the human genome reveals that potential G-quadruplex sequences are enriched in promoters of the oncogenes. Growing body of evidence suggests that G-quadruplexes (G4) may play putative roles in various biological processes, such as the regulation of gene expression. Consequently, targeting the oncogenic G-quadruplexes using small molecules is an alternative strategy for the potential treatment of cancers. Porphyrin derivatives are promising class of drug in this respect, being nucleic acids binders and generators of reactive oxygen species under visual light irradiation. Interaction between porphyrin derivatives and G4 DNA from oncogene promoter region has been studied *in vitro*. We applied chemical probing, circular dichroism spectroscopy and UV melting techniques in order to map the oxidized bases, monitor structural rearrangements and evaluate stability of the resulting DNA structures. Specifically, we observed that G4 DNA is considerably more susceptible to light-induced modification than duplex DNA; 5′-terminal tetrads of the G4 DNA are preferably oxidized; structural changes induced by oxidation result in decrease of the thermodynamic stability of the G4 DNA. Irreversibility of these effects on DNA make porphyrin derivatives perspective lead compounds for rational design of ligands targeting human oncogenes. The study was financially supported by project no. 16-14-10396 from the Russian Science Foundation.

P-MIS-039
Resistin levels in denervated obese rats

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The sympathetic nervous system is an important factor affecting the metabolic and secretory function of the white adipose tissue. Resistin is mainly expressed by mononuclear cells, also it is expressed by adipocytes, pancreatic cells, and muscle. Resistin induces insulin resistance and glucose intolerance in mice. Resistin plasma levels depend on fat depots size and sex. Resistin levels decrease in short- term fasting in mice, then it increase refeeding. Also, it increase as a response to fed with the high fat diet. In our study we aimed to determination of the effect of high-fat diet and denervation on serum resistin levels in rats.

In this study 4 experimental groups were formed each consisted of 8 rats. During 10 weeks, first two groups are fed with high-fat diet and other two groups are fed with standart diet which they purchased from *Research Diets* company. At the beginning of the feeding periods, retroperitoneal fat tissiues of animals assigned to the first and the third groups were denervated. Second and fourth groups were not denervated. At the end of the 10 week feeding periods, blood collected from rats and blood resistin concentration was determinated by ELISA.

In denervated and fed with high fat diet groups serum resistin levels higher than control groups ($p < 0.05$).

According to our literature research, there are no studies demonstrating the relationship between resistin and the sympathetic nervous system. Also, denervation may lead to increase in serum resistin levels. The amount of resistin is possibly reduced by β -adrenergic activation. In conclusion, it was concluded that there is differences on serum resistin levels depending on diet in bilateral denervation of retroperitoneal fat tissues of rats.

P-MIS-040
Stress activated protein kinases regulates the ribosomal frameshift rate in EST3 gene, encoding subunit of telomerase

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EST3 gene (Ever Shorter Telomere) of *S. cerevisiae* encodes one of the essential subunits of telomerase enzyme. Expression of *EST3* gene is regulated at the translation level by +1 Programmed Ribosomal Frameshift (PRF). It is known that the physiological stresses affect telomere length. In this study, we have investigated the effects of stress activated protein kinases Snf1p (AMPK) and Gcn2p (eIF2 kinase) on the PRF rate in *EST3* gene.

PRF rate of *EST3* gene was quantified in plasmid based expression system. Expression vectors were transformed in to the wild type and mutant yeast strains that deleted for *SNF1*, or *GCN2* genes. Yeast cells were grown in normal conditions or subjected to acid stress, osmotic stress, or glucose limitations to activate protein kinases Gcn2p, and Snf1p, respectively.

PRF rate of *EST3* gene was measured as 13% in the normal growth conditions in the wild type cells. But, the PRF rate of the wild type strain grown in glucose limited conditions decreased more than 10-fold, giving less than 1% PRF rate. Contrary to glucose limitation, osmotic or acid stress activated frameshift rate by 2-fold in the wild type cells and PRF rate increased to 25%. When the PRF rate was analyzed in gcn2 and snf1 mutants, frame shift rate of EST3 was 4–5% in normal growth conditions. When these mutants were subjected to acidic or osmotic stress, PRF rate activated slightly. We have also shown that Gcn1p and Gcn20p, positive regulator of Gcn2p, is also essential for the regulation of PRF in *EST3* in response to stress conditions.

It is clear that the basal level expression of *EST3* is highly dependent on the Gcn2p kinase complex. Gcn2p is also associates with ribosomes, indicating that Gcn2p might have a significant function in connecting the stress signals to biosynthesis of the full length Est3 peptide. This regulation might also link the biosynthesis of functional telomerase and telomere replications to cell physiology through protein kinases such as Snf1p and Gcn2p.

P-MIS-041
Inflammation might have a role in erosive esophagitis but not in non-erosive reflux disease

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The relationship between inflammatory activation mechanisms and acid-peptic injured esophageal tissue is not clear. We evaluated whether there are differences between inflammation and tight junctional proteins such as e-cadherine among subtypes of gastroesophageal reflux disease. The aim of this study was to investigate any possible role of inflammation in pathologic mechanism of reflux disease by determining the inflammatory markers in injured esophageal tissue as well as serum of patients.

Three groups (erosive-EE, n = 18; nonerosive-NERD, n = 12; healthy controls-HC, n = 13) were evaluated with upper gastrointestinal endoscopy. The esophageal biopsies and blood samples were collected. Serum e-cadherine levels, NFkB, chitotirosidase (CHIT), myeloperoxidase (MPO) activities in serum and homogenized tissues were determined.

NKFB levels in tissue was significantly higher in subjects with EE (4.9 ± 2.53 ng/mg.prt) versus HC (2.95 ± 1.51 ng/mg.prt, p = 0.018). MPO tissue activities in EE group were significantly lower (0.07 ± 0.06 u/mg.prt) than HC (0.23 ± 0.22 u/mg.prt, p = 0.025) while MPO serum levels were higher in EE (1.15 ± 1.63 uL) versus HC (0.56 ± 0.69 uL, p = 0.045). Tissue CHIT levels were three fold increased in EE versus HC (p = 0.071). None of these measurements showed any differences in NERD group. NFKB and MPO levels had a negative correlation (r=−0.408, p = 0.005) in tissue. NFKB and ECAD levels had a positive correlation in serum (r = 0.642, p < 0.0001).

Inflammatory process might play a pivotal role in injured mechanism only in erosive esophagitis but not in NERD. Non-inflammatory mechanisms might be responsible such as hypersensitivity in patients with non-erosive reflux disease.

P-MIS-042
A linear regression model for estimating D-dimer levels from hsCRP, WBC, neutrophil and procalcitonin: a data mining study

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D-dimer (a fibrin degradation product) test is used to aid in the diagnosis of intravascular coagulation. The aim of this study is to investigate the correlation between D-dimer levels and other inflammatory markers including procalcitonin.

Anonymized data on D-dimer, fibrinogen, hsCRP, WBC, Neutrophil% (NEUT%) and procalcitonin levels from 50,107 patients (mean age ± SD, 53.37 ± 20.6) were used for the correlation (Excel Analyze-it v4.60.4) and linear regression (PASW Statistics 18 v18.0) analysis between the measured parameters.

There was a significant (p < 0.05) age-dependent increase in D-dimer levels between different age groups. Patients with the highest D-dimer levels were also found to have an increased frequency of hsCRP levels. D-dimer levels showed a significant correlation with hsCRP, WBC and NEUT%. A model describing the positive association between these parameters were built. The resulting equation is as follows: D-Dimer = (hsCRP*0.054) + (0.011*Age) + (0.006*WBC) + (0.04*NEUT%)−0.929. Correlation analysis between procalcitonin and D-dimer levels gave Pearson's correlation coefficient of 0.159.

Our results suggest that the age-dependent variations should be taken into account while interpreting D-dimer test results. In addition, NEUT% ratio was found to be the most important parameter for estimating D-dimer levels. Our equation can be used when the D-dimer test is not available or for control purposes only.

P-MIS-043
Fullerene C60 as a nanocarrier of doxorubicin for cancer treatment

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In the field of cancer research great hope lies in finding more powerful and selective way for the direct elimination of cancer cells. This task can be solved by means of nanobiotechnology. Recent progress in this field has arisen interest in a carbon nanostructure – fullerene C_{60}. Fullerene exhibits not only unique physico-chemical properties and biological activity but also a significant potential to serve as a nanocarrier for selective drug delivery into cancer cells.

The aim of this study is to analyze a unique tool for cancer therapy. The main idea is realized by the non-covalent conjugation of C_{60} with the well-known anticancer drug – Doxorubicin (Dox). Two types of conjugate with different C_{60}-Dox ratio (1:1 and 2:1) were studied. Conjugates absorbance and fluorescence, size distribution as well as a mass data were recorded utilizing optical and analytical equipment (Microplate Reader, Zetasizer, LC-MS/MS and MALDI-TOF). *In vitro* studies were performed including evaluation of C_{60}-Dox conjugate effects on human leukemic cells (Jurkat, CCRF-CEM, THP1 ad Molt-16) viability. Conjugates accumulation and distribution within cancer cells was monitored using fluorescent microscopy accompanied with fluorescence-activated cell sorting.

It was evidently proven that both C_{60}-Dox conjugates were stable and could be used as reliable candidates for biological application. Cellular accumulation and distribution studies showed that conjugation of Dox with fullerene promoted its entry into leukemic cells. Accumulation of Dox in the form of conjugates within cancer cells was intensified compared to the free drug. The results show that conjugated Dox is more cytotoxic and the value of its IC50 are lower compared with the free Dox. Obtained results confirm nanocarrier function of fullerene C_{60} and the perspective of its application for optimization of Doxorubicin efficiency against leukemic cells.

P-MIS-044
Comperative investigation of protective effects of tea and tea-related wastes on reducing potaential of H2O2-induced erythrocytes

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Tea processing waste (TPW) formed during the tea production process in tea factories is up to 50,000 tones/year in Turkey. TPW is one of the abundant available phenolic biomass among plantal wastes. In this study, black and green teas and their wastes were used. The aim of the study is to determinate the phenolic content and the radical scavenging activities of the samples, and to measure their effects on hydrogen peroxide-induced erythrocyte damage due to analyzing the reducing potential of erythrocyte involving glutathione reductase (GR), glutathione peroxidase (GPx) activities and reduced glutathione (GSH) content. Total polyphenol content of samples was determined as mg catechine per dry mass by using Folin-Ciocalteau reactive and DPPH radical scavenging activity was estimated by Cuendet method as equivalent catechine standard. In erythrocyte, GSH level was measured by method of Sedlak and Lindsay while GR and GPx activities were assayed by the methods of Bergmeyer

and Beutler, respectively. The highest phenolic content was observed in green tea and its wastes ($p < 0.01$) whereas black fiber waste had the lowest phenolic content. Therefore, the highest radical scavenging activity and GSH level were detected in green tea and its wastes ($p < 0.01$). Erythrocyte with the extracts of the teas and their wastes had the similar enzyme activities for both GPx and GR. In sum, the teas and wastes have antioxidant activity but, green tea and its leaf waste hade higher antioxidant activity than other samples. The tea wastes might be evaluated as many of protective health products, particularly in cosmetic fields thus, these by-products no application for any area is expected to become an economical value.

P-MIS-045
Formulation, characterisation and Salmonella/Microsome mutagenicity assay of a novel 5 fluorouracil derivative

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Fluorouracil (5-FU) is a chemotherapeutic drug classified as an "antimetabolite". It works through irreversible inhibition of thymidylate synthase. Chemical derivatization of 5-FU with carbohydrtates is being investigated widely in order to enhance its bioavailability, therapeutic efficiency and to reduce its toxicity. However, water solubility of the newly derived compounds is usually very low. So, in order to obtain a pharmaceutically relevant formulation they need to be formulated appropriately.

In this study, we prepared micellar delivery system for the new tetra-*O*-acetylglycose derivative of 5-FU synthesized via "Click Reaction", namely F1-[{1′-(2″,3″,4″,6″-tetra-*O*-acetyl-*β*-D-glycopyronosyl)-1′H-1′,2′,3′-triazole-4′-yl}methyl]5-fluorouracil. Since the water solubility of this compound is very limited, we tested its solubility in several pharmaceutically relevant solvents by visual estimation after stiring increasing amount of the compound in 1 ml of solvent for 48 h. To estimate the carcinogenic potential of this compound, *Salmonella*/Microsome Mutagenicity Assay (Ames test) was performed in four histidine-requiring strains of *S. typhimurium*, tester strains TA98, TA 1537 (for the detection of frameshift mutations) TA100 and TA 1535 (for detection of base pair substitutions) according to the OECD Guideline 471.

The drug was solubilized (500 μg/ml) with no precipitation in Lutrol®-F68/ethanol/water (2.25:2.25:5.5, wt/wt) micelles (7.3 ± 1.0 nm). The results of Ames test were negative so the compound neither produced frame shift mutations nor base pair mutations in *S. typhimurium* strains.

The results imply that the new compound can be dissolved in aqueous micellar delivery system in order to be used for further studies, and that it was not mutagenic in the tested *S. typhimurium* strains.

In conclusion, the formulation of the newly synthesized compound is not carcinogenic, and can be evaluated for anticancer activity *in vitro* and *in vivo*.

P-MIS-046
Integral metabolism parameters of dairy goats during reproductive cycle periods

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Study of the goat metabolism at different periods of the reproductive cycle allows to correct feeding ration, to increase the age of the productive use of animals and to receive high-quality products. The aim of the work was to determine the metabolic parameters of blood serum of goats, expressed in terms of biochemical parameters and interfacial tensiometry and study their relationship to metabolic processes in the body goats depending on the age and the period of the reproductive cycle. The 90 healthy goats were divided into 5 groups. The dynamic surface tension (DST) parameters were obtained from dependences of a surface tension (σ) vs. time (t): at $t \to 0$ (σ_0), at t = 0.02 s (σ_1), t = 1 s (σ_2) and $t \to \infty$ (σ_3). This work was supported by the Russian Scientific Foundation (14-16-00046). All animals had 4–5% fat content. The contents of total protein (5.4%), albumin (8.4%) and urea (8.8%) are higher for the lactating animals as compared to the normal goat values. The levels of total cholesterol (18.0%) and creatinine (6.2%) are higher for the lactating animals. In lactating animals have the highest level of, which along with high phosphorus level talks about the intensification of energy processes during lactation. The correlations were found between the biochemical and DST parameters of the goat blood: lipids or cholesterol levels with σ_0 ($r = -0.40$), σ_1 ($r = -0.72$), σ_2 ($r = -0.89$); total protein or albumin levels with σ_1 ($r = -0.42$), σ_2 ($r = -0.56$), σ_3 ($r = -0.90$); aminotransferase activity with σ_2 ($r = -0.47$), σ_3 ($r = -0.63$). The correlations were found between the total protein and albumin levels with λ_0 ($r = 0.54$), λ_1 ($r = 0.44$); glucose levels and σ_1 ($r = 0.47$), σ_2 ($r = 0.43$). Thus, the DST and biochemical parameters of goats have strong correlation relationships that are important for biomedical and veterinary applications.

P-MIS-047
The relation of the severity of atherosclerotic disease with oxidative stress in patients with stable coronary artery disease

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Introduction: Because, to the best of our knowledge, the relationship of total oxidant status (TOS) and total antioxidant status (TAS) with the severity of stable Coronary artery disease (CAD) has not been investigated in the literature so far, the present study was conducted to address this issue.
Materials and Methods: This study consisted of 349 consecutive patients and controls who underwent coronary angiography. For each patient, the total Gensiniscore (GS) was calculated andthose with a GS of >20 were classified as the high GS group (HGG), and those with a GS less than 20 were defined as the low GS Group (LGG). The total oxidant status (TOS) and total antioxidant status (TAS) levels were measured using the Erel-method. The OSI, which is an indicator of the oxidative balance, was calculated as the percentage ratio of TOS to TAS.
Results: The TAS was lower in the HGG than LGG. The TOS and OSI were higher in the HGG than LGG. The correlation analysis showed that GS was negatively associated with the TAS and positively with the TOS and the OSI. The multivariate analysis showed that age, TOS, and HDL-C were independent variables for a high GS. The cut-off level of 10.9 μmol H_2O_2 equiv./L for serum TOS levels predicted high GS with a sensitivity of 70% and a specificity of 56%.

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Discussion: Information on the severity of atherosclerosis is requiredtopredicttheprognosis of an individualpatientandtodetermine the proper treatment modality. The GS system has beenproventodemonstratethe severity of atherosclerotic disease. Inthepresentstudy, thepatientswith a high GS had increasedlevels of oxidants. Inaddition, TOS was an independentindicator of theseverity of atherosclerosis. The optimal cut-offvaluefor TOS topredict high-gens score was 10.9 (sensitivity 70% and specificity 56%).
Conclusions: The results suggest that the severity of atherosclerosis in stable CAD is associated with increased oxidative status.

P-MIS-048
Evaluation of roemerine as a multidrug resistance pump inhibitor

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Efflux by multidrug resistance (MDR) pumps is a common defense mechanism used against antimicrobials. By pumping the drugs out, these pumps significantly reduce the efficacy of drugs. One approach to overcome this limitation is offered by the combinatorial therapies where drugs are co-administered with together with pump inhibitors. By simply preventing the efflux of the drug, the presence of inhibitors enhance drug efficacy. (-)-Roemerine is an aporphine type alkaloid with significant antibacterial (against *Bacillus cereus*, *Escherichia coli*) and antifungal (against *Candida* strains) activities. Interestingly, (-)-roemerine was also found to enhance the cytotoxic response mediated by vinblastine in multidrug-resistant KB-V1 cells. In the same study, this finding was linked to its possible interaction with P-glycoprotein, a eukaryotic MDR pump. Taking this finding as the starting point, the current study investigates the potential of roemerine as an inhibitor of the P-glycoprotein homologue pump, BmrA, in *Bacillus subtilis* 168. The antimicrobial agent berberine was used as the model agent since its efficacy is reduced by efflux through MDR pumps. To this end, *Bacillus subtilis* 168 cells were subjected to 100 μg/mL berberine, a value well below the MIC. This concentration only slightly retarded growth for 2 hours but then cells resumed their regular growth. Upon addition of 25 μg/mL (-)-roemerine to the *Bacillus subtilis* 168 cells treated with berberine, growth pattern changed, indicating possible interaction with BmrA. Further investigation for the change in the expression of BmrA was achieved with real time PCR analysis.

P-MIS-049
Synthesis of single glucose oxidase nanoparticles and their potential use in cancer therapy

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Glucose oxidase is an enzyme that catalyzes the oxidation of glucose to D-glucono-1,5-lactone and hydrogen peroxide. We hypothesized that enzyme would cause a double negative effect on cancer cells, by reducing the presence of glucose in cancer microenvironment and producing reactive oxygen species. To increase enzyme stability and enhance cellular uptake we encapsulated the enzyme with a thin acrylamide layer. The purpose of this work was to optimize the synthesis of these glucose oxidase nanoparticles and investigate their effect on cancer cells.

Nanoparticles containing single glucose oxidase were synthesized in two steps; first by introducing the vinyl groups onto the surface of enzyme by acyloylation followed by polymerization step with acrylamide monomers. Encapsulated enzymes are approximately 150 nm in size and retain most of their activity. After the optimization of nanoparticles, the anticancer potency of these nanoparticles was *in vitro* tested in MCF-7 breast cancer cell line.

According to results, both nanoparticles and free enzyme are capable of inhibiting viability of cancer cells in a similar manner at very low concentrations. Currently we are investigating mechanisms involved in this viability inhibition. Initial results demonstrated that glucose supplement does not rescue cells from death induced by the activity of glucose oxidase, suggesting an oxidative stress related cause of inhibition. Further studies are required to elucidate the exact mechanism.

Until now there is no determined advantage of glucose oxidase encapsulation against proteolysis. However, encapsulation may induce the accumulation of enzyme in cancer microenvironment. Furthermore results suggest that glucose oxidase has a high effect on the viability of MCF-7 breast cancer cells indicating that this enzyme may have a potential use in cancer treatment.

P-MIS-050
Studies on the interaction of human phospholipid scramblase 1 with C-terminal domain of topoisomerase IIα

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Human phospholipid scramblase 1 (hPLSCR1) is a multifunctional protein that plays key roles in several cellular processes including apoptosis, tumorigenesis, anti-viral defense, cell signalling and several protein-protein interactions. It has been shown that hPLSCR1 interacts with the C-terminal domain of topoisomerase IIα (topo IIα) and enhances its decatenation activity *in vitro*. The interacting region in topo IIα was identified but till date, no reports exist on the binding region in hPLSCR1. This study aims to identify the region of hPLSCR1 that interacts with topo IIα.

To identify the topo IIα interacting sites in hPLSCR1, N-terminal deletion constructs of hPLSCR1 viz Δ25-hPLSCR1, Δ50-hPLSCR1, Δ75-hPLSCR1, Δ100-hPLSCR1 and Δ160-hPLSCR1 were generated by PCR, cloned, overexpressed and purified to homogeneity using Ni^{2+}-NTA purification. The C-terminal domain (CTD) of topo IIα was cloned in pGEX6P-1 and was expressed as a GST fusion protein. GST pull down assays will be performed with the deletion constructs of hPLSCR1 and the GST-CTD-topo IIα. The binding region in hPLSCR1 will be confirmed by peptide competition assays.

Our initial results show that the decatenation activity of topo IIα was enhanced when the topo II was pretreated with hPLSCR1. Δ100-hPLSCR1 did not show any enhancement of the decatenation activity compared to full length hPLSCR1. Hence, the binding region could be in the 1-100 region of hPLSCR1. Further deletions were done in the 1-100 region of hPLSCR1 as described earlier. GST-pull down assays and decatenation assays will be performed for the deletion constructs to narrow down the region of hPLSCR1 that binds to topo IIα.

We conclude that hPLSCR1 interacts with and enhances the activity of topo IIα and the 1-100 region of hPLSCR1 is critical for enhancement of decatenation activity. Further work is under progress to identify the exact topo IIα binding region of

hPLSCR1 and the physiological relevance of this interaction in the cell.

P-MIS-051
Mutations of analysis of Beta-Thalassemia in antalya

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Beta-Thalassemia is a common autosomal recessive disorder resulting from over 200 different mutations of the beta- globin genes. Our aim was to creat a mutation map of Beta thalassemia in province of Antalya, Turkey. In this study, mutation analysis of a total 150 of Beta-thalassemia patients followed up at the Thalassemia Center of the Antalya Education and Research Hospital, Antalya, Turkey, were included. According to our results, the IVS 1.110 is the most frequent mutation type in our province same as other geographical regions of Turkey. The most frequent mutations in heterozygous or homozygous patients are IVS 1.110, IVS 1.6, IVS 2.1 and IVS 1.1. Our results indicate the importance of micromaping and epidemiology studies of thalassemia, which will assist in establishing the national prevention and control program in Turkey.

Keywords: Beta-Thalassemia, Beta-Globin Gene, Mutation

P-MIS-052
Investigation of the *in vitro* effects of some antibiotics on the purified Beta-glucosidases from the rat liver

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Beta-glucosidases catalyzes the hydrolysis of the glycosidic bonds to terminal non-reducing residues in β-D-glucosides and oligosaccharides.β-glucosidases are widely distributed in the living world.β-glucosidases which in mammals, primarily found in the liver and kidneys;lysosomal β-glucosidase (GBA1),non-lysosomal β-glucosidase (GBA2), cytosolic β-glucosidase (GBA3),intestinal lactase-phloriz the hydrolase (LPH).

Liver tissues of Wistar-Albino rats were homogenized with homogenizer in the extraction buffer and crude extract was obtained after centrifugation.Ammoniumsulfate precipitation range designated crude extract was purified by sepharose 4B-L-tyrosine-1-naphthylamine hydrophobic gel.Commercially availabled antibiotics were prepared with substrate buffer.It was investigated inhbition effects of cefuroximesodium, ampicillin-sulbactam, amoxicillin trihydrate/potassium clavulanate, cefazolin sodium, gentamicin sulfate and ceftriaxone disodium antibiotics onto GBA2.Inhibition types and K_i values of related antibiotics were determined with p-NPG substrate.Lineweaver-Burk plot was used for that purpose.

Rat liver GBA2 was purified at 30.2-fold with 43.4% yield.GBA2 was illustrated 58 and 110 kDa at SDS-PAGE.IC_{50} value of ampicillin/sulbactam antibiotic for GBA2 was found 62.97 mg/ml with competitive type inhibition and other antibiotics didn't inhibit.

Purfication methods are being used in the literature for the purified β-glucosidase from different sources.Purified GBA2 was illustrated 58 and 110 kDa at SDS-PAGE.About molecular weight of β- glucosidases is presented different information in the literatüre. This has been reported because of acid beta glucosidases are abnormal migration at the acrylamide or agarose gels.It was investigated inhbition effects of various antibiotics onto purified GBA2.Ampicillin/sulbactam antibiotic inhibited to purfied GBA2 at the competitive type.Similiar antibiotics studies have been made in the literature for different enzymes.

P-MIS-053
Effect of glutamine on insulin resistance and endoplasmic reticulum stress

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Obesity and diseases are one of the most important public health problems of the world.Excess fat storage in adipocytes leads to the release of increased amounts of non-esterified fatty acids, glycerol, hormones, cytokines, which are factors involved in the development of insulin resistance that cause type 2 diabetes. One of the major differences between obese and lean individuals is the amino acid concentration in the circulation. Although there are many studies about the amino acid metabolism associated with insulin resistance in obese individuals, the effect of glutamine metabolism in insulin resistance mechanisms are not well understood yet. Glutamine can be used as fuel and its levels in tissues and circulation can regulate cell responsiveness to insulin and cellular metabolism. Therefore, glutamine is a potentially important factor that might help us better understand insulin resistance and type 2 diabetes.

To determine whether glutamine effect on insulin resistance and endoplasmic reticulum stress, 3T3-L1 cell is treated with different concentration of glutamine and analyzed by Western Blot for ER stress markers.

Our results indicated that glutamine reduced endoplasmic reticulum stress and related with that attenuated insulin resistance. In case of transport of amino acids, insulin resistance, how it is affected when we have the information about the important tips on energy requirements and metabolism reach insulin resistance and type-2 diabetes treatment is likely to reveal a possible new targets.

P-MIS-054
How does different lead levels affect TSH, FT3, FT4, vitamin B12 and folate?

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Exposure to heavy metals is increasing with the industrialization of society. One of the most intense exposure to heavy metals is Pb on this issue. This study was aimed to determine the relationship between different blood Pb levels and serum thyroid hormones (TH), Vit B12, folate.

The cases were 20–65 years old, male individuals who admitted to our hospital between April 2012- March 2016 for periodic inspections because of occupational exposure to Pb. The parameters of the cases were retrospectively retrieved. According to their Pb levels, exposed workers (n: 9400) were divided into four subgroups; group (G) 1: 0–9.99 μg/dL, G 2: 10–19.99, G 3: 20–39.99, G 4: ≥40. From these, the number of cases whose TH levels were measured (n:3894) given respectively; G 1:3062, G 2:178, G 3:334, G 4:275 cases. Also the number of cases whose Vit B12 and folate levels were measured (n:2807) given respectively; G 1:2139, G 2:143, G 3:276,G 4:249 cases. Levels of Pb were determined by ICP-MS. TH, vit B12, folate were determined by CMIA.

Between the groups formed according to Pb levels, there was no significant difference in terms of average T3, TSH and vitamin B12 ($p > 0.05$). On the other hand there was statistically significant difference between T4 and group 1,3,4 ($p < 0.05$) but there was no difference between group 2 ($p > 0.05$). The average folate belongs to the first group was about 10% higher than the other 3 groups, and found that the difference was statistically significant ($p < 0.05$).

There are many publications which have various results between Pb levels and T3,T4, TSH. But this study is important to compare the effect of different levels of Pb. Up to day there was no publication about the relation between different Pb levels and Vit B12, folate.

It was seen that there was no significant clinical relation between different Pb levels and thyroid parameters, vit B12. But the low levels of folate in the high Pb levels groups shows us that we need further studies about this relationship.

P-MIS-056
Fluorescent study of *in meso* crystallization of membrane proteins

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With the introduction of membrane protein *in meso* crystallization 30 years ago by Landau and Rosenbusch, a new era of membrane protein structural research has emerged (1). Since that time this method became associated with a number of major breakthroughs in the field (2) including exceptional successes in structural studies of microbial rhodopsins and G-protein coupled receptors (3). Here we used fluorescence microscopy to study *in meso* crystallization process of bacteriorhodopsin. Several observations provide new insights into the *in meso* crystallization process. The crystallization starts with formation of microcrystals, followed by growth of a dominating crystal at the expense of smaller ones and formation of a depletion zone around it. These observations demonstrate an Ostwald ripening mechanism of the *in meso* crystal growth. The depletion zone formed around the growing crystal is consistent with the previously proposed analogy relating *in meso* crystallization with the crystallization in a microgravity convection-free environment. This work is supported by RSF 14-14-00995.

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P-MIS-058
STAMP1 is critical for both AR and mTOR signaling in prostate cancer cells

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Androgen receptor (AR) signaling plays a central role in the initiation and progression of prostate cancer (PCa), including when the disease progresses to castration-resistant PCa (CRPC). The second central signaling pathway in PCa, similar to various other cancers, is the PI3K-AKT-mTOR signaling. Importantly, these two oncogenic pathways cross-regulate each other in PCa cells by reciprocal feedback, thereby maintaining tumor cell survival even when one is suppressed. We have previously identified that the six transmembrane protein of prostate 1 (STAMP1) promotes PCa cell proliferation as well as inhibits apoptosis through, at least in part, regulating the ERK MAPK signaling.

Human PCa cell lines LNCaP and VCaP were used in the study. Colony formation, soft-agar growth, prostatosphere formation assays were performed. For *in vivo* xenograft experiment, the cells were implanted subcutaneously into the flanks of nude mice.

Here, we show that STAMP1 knockdown caused defects in colony formation, anchorage-independent growth and prostatosphere formation in LNCaP and VCaP cells both *in vitro*, as well as tumor formation and growth *in vivo*. This may be due to the impaired AR and mTOR signaling in these cells upon STAMP1 knockdown. Interestingly, in the CRPC cell line 22Rv1, where-STAMP1 knockdown did not affect mTOR signaling, there was a remarkable repression of tumor take rate and growth.

These results clearly indicate that STAMP1 is essential for both AR and mTOR signaling, and is crucial for PCa growth *in vitro* and *in vivo*. However, the detailed molecular mechanism requires further investigation.

Taken together, these data unveil a critical role for STAMP1 in coordinating the AR and mTOR signaling pathways in PCa cells, solidifying the basis for its pro-survival effects in PCa, including in advanced disease.

P-MIS-060
Quantification of 2D thin layer chromatograms using 2D gel analysis software and gel documentation system

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Introduction: Thin layer chromatography (TLC) is an important chromatographic technique that is widely used as a cost-effective method for rapid-sensitive analysis of compounds in plants, animals, and humans. However, one dimentional (1D) TLC is not sufficient for the separation of complex compounds. Therefore, two-dimentional (2D) TLC was developed. The quantitative evaluation of plates are performed with TLC scanners or documentation systems. However, these systems specific for 1D plates, and cannot be adapted to quantitative evaluations of 2D plates. In this study, the applicability of the gel documentation systems and 2D analysis software for the analysis of 2D TLC plates were examined.

Material and method: 2D TLC of Lipids: 1st dimension: methyl acetate/n-propanol/chloroform/methanol/0.25% KCl (25/25/28/10/7 v/v); 2nd dimension: chloroform/methanol/acetic acid/water (90/40/12/2 v/v); Detection: Charring.

2D TLC of Aminoacids: 1st dimension: 1.5% (v/v) formic acid; 2nd dimension: toluene/glacial acetic acid (10:1 v/v); Detection: UV.

Phospholipid and aminoacid standards, each include 6 different classes were developed by 2D TLC. Plates visualized with BioRad GelDoc XR, and band volumes on plates were calculated with BioRad PDQuest 2D gel analysis software.

For the method validationa) 5 plates containing same 6 lipid classes were developed in the same day, and results were used for the calculation of intra-assay CV;CV% = average of each sample standard deviation/mean of sample × 100

b) 6 plates containing same 6 lipid classes were developed in 6 different days, and results were used for the calculation of inter-assay CV;

CV% = standard deviation of each sample average/mean of the plates × 100

Results: Volume of each phospholipid and aminoacid had less than 10% intra and inter-assay CV.

Conclusion: Gel documentation system with 2D gel analysis software can be used for the quantitative analysis of the 2D TL plates both at UV and visible light.

P-MIS-061
The role of Na^+K^+ ATPase activity in the vasodilatory effect of N-acetylcysteine

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Introduction: Spasm occurred at the stage of and after the preparation of arterial grafts used in coronary artery bypass surgery (CABG) is effective on morbidity and mortality in the first 24 hours of postoperative patients. N-acetyl cysteine (NAC) that vasodilatory effect is known,may be considered as a suppressor agent for vasospasm developing during CABG. However, for the prevention of complications that may arise during or after CABG mechanism of these vasodilatory effects should be described. This study was aimed to investigate the role of ATPase enzyme on the vasodilatory effect of NAC.

Materials and Methods: In this study, 28 adult male Wistar albino rats were used. Rats were separated into four groups as control rats (G1), 2 mM NAC (G2), 5 mM NAC (G3) and 10 mM NAC (G4). A portion of the thoracic aorta isolated from rats was used for the relaxation response recording, and the other portion was used for measurement of NaKATPase activity. Isolated smooth muscle rings are suspended in the 20 ml organ bath containing Krebs solution for relaxation responses. In all groups, level of smooth muscle contraction were allowed to reach a plateau by adding 60 mM KCl to the organ bath. Then, in the first 10 minutes of application relaxation responses which created by adding NAC to the medium were recorded and the maximum relaxation responses were measured. NaK ATPase activity was determined using the Mazzanti method. Groups means were compared by one-way analysis of variance (ANOVA). The threshold for statistical significance was set at .05.

Results: The contraction force decreased in all NAC dose groups compared to control group and this reduction was statistically significant ($p < 0.05$). Similarly, NaK ATPase activity is also decreased in a dose dependent manner ($p < 0.05$).

Discussion and Conclusion: The findings obtained in this study suggest that vasodilator effect of NAC formed in thoracic aortic smooth muscle was associated with the activity of the enzyme Na K ATPase.

P-MIS-062
Isolation and characterization of feather degrading keratinase from Bacillus sp. UK69

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In the presented study, we isolated and characterized a novel feather-degrading bacterium that shows keratinolytic activity. A Bacillus UK69, which was isolated from the soil samples taken from farmland on Kahramanmaras Sutcu Imam University campus, showed high keratinolytic activity when cultured on feather meal medium. The enzyme activity was studied in the pH range of 5.0–12.0. The optimum temperature for keratinase activity was investigated by varying the incubation temperature between 20°C and 80°C. Optimum keratinolytic activity was observed at 60°C and pH 10.5. The enzyme was stable at 60°C. The activity was investigated in the presence of some chemicals, including SDS, Tween 80, DMSO, Triton X- 100, EDTA, NaCl, ZnCl2, CaCl2, glucose. The keratinolytic activity was inhibited by all chemicals tested to some degree. The molecular weight of keratinase was determined by polyacrylamide gel (10%) using standard molecular weight marker and estimated about 37 kDa by SDS-PAGE. The keratinase isolated from Bacillus UK69 could be used in biotechnological processes i.e. feather degradation, wastewater treatment and in industrial processes, such as detergent, food and leather industries.

P-MIS-064
Alpha/Beta globin mRNA ratio informs the gene function for personalized mutation data in molecular screening of thalasemia carriers

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Introduction: Hemoglobinopathies, including thalassemia, abnormal hemoglobins, constitutes a major group of inherited disorders of hemoglobin synthesis. The reduced or absent of the beta (β) or alpha (α) globin chains of the adult human hemoglobin molecule results beta or alpha thalassemias, leading to imbalanced α-globin/non α-globin chains. The aim of this study was to give a quik desicion with α/β-globin mRNA ratio for sequencing of α or β gene, when the anemia is not detectable.

Materials and Methods: mRNA and cDNA extraction of 25 β-thalassemia and 15 α- (including two of 3.7 Kb Del./HbS) thalassemia subjects and normal controls were accomplished using the High Pure RNA Isolation Kit and Transcriptor First Strand cDNA Synthesis kit, respectively, following the manufacturer's instructions. We used cDNA as a template in the real-time PCR amplification using primers specific for α, β globin genes. Amplification was performed in a LightCycler® 480 instrument. The α/β-globin mRNA ratio of each sample was calculated based on the $2^{-\Delta\Delta CT}$ method.

Results: α/β-globin mRNA ratios calculated in α-thalassemia subjects relative to normal control as a result of numbers of defective α-globin genes. The α/β-globin mRNA ratio was found higher in β-thalassemia subjects. Coinheritance of α-thalassemia in Hb S subjects concluded a stabile α/β-globin mRNA ratio as per α-thalassemia or β-thalassemia subjects.

Discussion and Conclusion: Instability in α/β-globin chains is a significant factor of thalassemia disease severity and can be

used before deciding type of gene sequencing when the anemia is not detectable. This study indicates that imbalance in globin gene expression could be demonstrated by measuring α/β-globin mRNA ratio, which was conveniently and accurately determined by qRT-PCR and give an information about globin gene function which gene should be correct to investigate an individual for globin gene mutation.

P-MIS-067
Self-assembling peptides mimic supramolecular biochirality

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Supramolecular chirality is rooted in asymmetric spatial arrangement of structural elements (e.g. molecules or units with higher hierarchy). Self-assembled systems giving rise to this kind of chirality are of great importance because they closely resemble natural biological systems and potentially can lead to new advanced functional materials. In the process of self-assembly, both molecularly chiral and achiral structural units can organize into chiral nanostructures.

Chiral arrangement of chromophore molecules in space is known to result in emergence of chiroptical properties of a chromophore. Organization of pigment-protein complexes into macrodomains in green plants gives rise to biochirality emanating from long-range chiral order of complexes. Owing to this order, macrodomains start to absorb circularly polarized light intensively and thus exhibit huge circular dichroism (CD) signal. In our study, a simple approach which was aimed at mimicking the biochirality phenomenon makes use of self-assembling peptide amphiphiles and their interactions with pyrene chromophore. Designed peptide amphiphiles are capable of self-assembly into nanofibers with chiral interior, which in principle gives an opportunity to achieve long-range chiral order. Two modes of interaction – covalent and noncovalent – were utilized in order to induce supramolecular chirality. Covalent interaction mode included direct covalent attachment of pyrene to peptide sequence. Upon self-assembly of peptide amphiphile into nanofibers intense circular dichroism phenomenon was observed. Noncovalent interaction mode envisioned encapsulation of pyrene molecules in the hydrophobic core of nanofibers of another peptide amphiphile. Co-assembly of peptide amphiphile and pyrene molecules led to chiral order and intense CD signal. In addition, it was possible to control the sign of CD signals by using either of peptide isomers, L or D.

P-MIS-068
PON1 activity in HDL subgroups of obese, overweight and normal weight subjects

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Objective: The aims of this study were isolation of HDL-C subgroups by using precipitation method, determination of PON-1 activity in both total and HDL3 subgroups, and evaluation of performance characteristics of PON-1 activity measurement method in newly diagnosed obese, overweight and normal subjects.

Material and Methods: The study population consists of newly diagnosed 71 obese, 40 overweight and 30 normal subjects. Fasting morning blood samples were taken from all study groups. HDL3 subgroup was obtained by heparin-Mn-dextran sulphate precipitation method and cholesterol was measured with direct (homegenous) HDL-C method. HDL2-C concentrations were calculated with the subtraction of HDL3-C from total HDL-C. HDL3-C and total PON-1 activity were determined by using Eckerson method. Non-HDL3 PON-1 activitiy was calculated with subtraction of HDL3 PON-1 activity from total PON-1 activity.

Results: Total HDL-C, HDL2-C and HDL3-C concentrations and the activity of total PON-1 and HDL3 PON-1 were found lower in obesity according to overweight and normal subjects ($p < 0.001$). Negative correlations were found between body mass index and HDL3-C, total PON-1 and HDL3 PON- 1 ($r = -0.244$, $p < 0.005$; $r = -0.247$, $p < 0.005$; $r = -0.199$, $p < 0.05$, respectively).

Conclusion: Our findings indicated that HDL-C metabolism and lipoprotein associated antioxidant defense mechanisms were adversely affected with obesity. In conclusion we think that precipitation method using for separating HDL3 subgroup, is simple and cost effective for routine applications in clinical laboratories. Besides HDL3-C measurements, PON1 activity, measurement of total and HDL3-C subgroup might be helpful to evaluate the atherosclerotic process in obese subjects.

Keywords: Obesity, Body Mass Index, Paraoxonase, HDL subgroup, Cholesterol

P-MIS-069
Hepatitis E virus antibody prevelance among persons who work with animals in North Cyprus

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Introductions: Hepatitis E infection is a major cause of viral hepatitis in many developing countries. The objectives of the present study was to determine the seroprevelance of HEV infection in peoples who work with animals in Northern Cyprus.

Materials and Methods: Prevelance of HEV infection were determined in study 4 group population: persons without occupational exposure to animals; persons who work with animals; veterinarian and butcher. A total of 400 blood samples were collected. All serum samples were tested ELISA using a commercially available kit according to the manufacturer's instructions. Ti-test were used for istatistical analyses. $p > 0.05$ was accepted as significant value.

Results: In a study of 400 blood donors (334 male, 66 female), the overall prevelance of anti-HEV IgG antibodies were 3.0%. The blood samples were collected 5 different areas. The prevelance of anti-HEV IgM antibodies was 0.25% and he was 44 years and acting a butcher during 20 years. The prevelance of anti-HEV IgG of women were approximately two fold higher than men. No significant difference in anti-HEV prevelance was observed between the age of the blood donors. According to the anti-HEV IgG prevelance, the without occupation expose to animal animal were 1%, the animal husbandry were 7% and the veterians and the butcher were 2% were found.

Discussion: The prevelance of anti-HEV in the North Cyprus (3%) was found low such as the prevelance of the Turkey (5%). The prevelance of anti-HEV IgG in animal husbandry were higher that the other groups because of they may be more spend of time and contact with animals. The prevelance of IgM results suggested that the possibility of outbreaks may be low in North Cyprus.

Conclusion: This study was the first seroprevelance analysis of North Cyprus according to the population number.The further studies could be included the seroprevelance of anti-HEV from the animals.

P-MIS-070
Preanalytic errors in our laboratory

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Most errors in the clinical laboratory occur in the preanalytical phase The aim of this study was to investigate the causes and rates of rejected samples, regarding to certain test groups in our laboratory.

This study was designed on the rejected samples between January 2015 and January 2016. Clinical chemistry, coagulation, hormone, cardiac markers, total urine evaluation and other (ethanol level, HbA1c, Hb electrophoresis, neonatal bilirubin, drug level, blood gas, fecal occult blood) test groups were included. The total number of specimen and rejected samples was obtained from the Hospital Information System retrospectively. Types of inappropriateness were evaluated as follows: erroneous coding, clotted specimen, hemolysis, insufficient volume, incorrect patient, incorrect tube and *inappropriate specimen*.

It was determined that 873343 blood samples were sent to our laboratory in one-year period. 0.37% of them were rejected because of preanalytical errors. Erroneous coding was found as the most common rejection cause (33%). Rejection rates of clotted specimen, hemolysis, insufficient volume, incorrect patient, incorrect tube and *inappropriate specimen* were found to be 11%, 15%, 19%, 2%, 4% and 16% respectively.

In our study, erroneous coding was the most common cause of preanalytical errors. Education of medical secretaries is relevant and important as can be seen in the decrease of sample errors and the resulting quality improvement.

P-MIS-071
Diagnostic efficiency of glycosylated hemoglobin test in comparison to oral glucose tolerance test: a data mining study

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Glycosylated hemoglobin test (HbA1c) is important for screening, diagnosing, and monitoring diabetes and prediabetes. However, HbA1c levels may dependent on patient ethnicity suggesting that the diagnostic cut-offs should be evaluated for specific populations. Therefore, our aim in this study was to evaluate the efficiency of HbA1c for predicting diabetes in comparison to oral glucose tolerance test (OGTT) results for Turkish population.

The study included anonymous lab results (Acibadem LABMED laboratories in Turkey) of 6270 patients (3913 female, 2351 male) aging 40.4 ± 11.6 years (15–86) who had an initial diagnosis of diabetes. Glucose and insulin levels during OGTT were measured after the initial administration of 75 g sugar (0-hour), 1-hour and 2-hour. These parameters were statistically analyzed in comparison to simultaneous HbA1c results.

Glucose measurements at 1 hour had better distinction power ($p < 0.05$) between these individual groups than initial and 2-hour glucose measurements. The average HbA1c (%) levels for healthy, pre-diabetic and diabetic individuals were 5.4 ± 0.4, 5.7 ± 0.4 and 6.2 ± 0.7, respectively. ROC curve analysis showed 23.4% sensitivity and 98.2% specificity for the clinically accepted HbA1c cut-off value of 6.5%. HbA1c cut-off value of 5.9% had a higher sensitivity of 67.8% and comparable specificity of 85.7%.

The highest discrimination power between healthy, pre-diabetic and diabetic individuals was observed at glucose concentration at 1-hour after sugar administration in OGTT test as opposed 2-hours generally used for diagnosis. Low sensitivity was observed for the clinically adapted 6.5% cut-off value of HbA1c. The cut-off value of 5.9% for HbA1c was found to be more sensitive with comparable specificity than the 6.5% cut-off values for diabetes screening in our population. Our results suggest that 5.9% for HbA1c should be considered for diabetes cut-off value for Turkish population.

P-MIS-074
Induction of the glutathione-dependent detoxification capacity is involved in the hepatoprotective effect of silymarin against acetaminophen-induced hepatotoxicity

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Recent findings in this laboratory showed that silymarin was capable of promoting hepatic glutathione (GSH) synthesis via a modification of the transsulfuration reactions in the liver. To investigate its pharmacological significance, we examined the hepatoprotective effect of silymarin against liver injury induced by acetaminophen (APAP).

Adult male mice were treated with silymarin (200 mg/kg, po) every 12 hours for a total of 3 doses prior to an APAP challenge (500 mg/kg, ip). The APAP-induced liver injury was assessed by histopathological examination and measurement of changes in plasma enzyme activities, lipid peroxidation and formation of nitrotyrosine protein adducts in the liver. Plasma levels of APAP and its major metabolites were monitored for 24 hours to estimate the metabolic transformation of APAP. Also protein and activity of the major Cyp subtypes involved in the metabolic activation of APAP into a toxic metabolite were determined in liver of the mice treated with silymarin only.

Silymarin pretreatment attenuated the APAP-induced liver injury significantly when determined 24 hours later. Plasma concentrations of APAP, APAP-glucuronide or APAP-sulfate in plasma were not changed, but thiol conjugates of APAP, such as APAP-glutathione, APAP-cysteine and APAP-*N*-acetylcysteine, were elevated significantly in the mice pretreated with silymarin. However, silymarin treatment did not affect protein expression of Cyp2e1, Cyp1a2, or Cyp3a11 in the liver. Also hepatic microsomal enzyme activities measured using *p*-nitrophenol, ethoxyresorufin and erythromycin as substrates, were not increased by silymarin, indicating that the elevation of APAP-thiol conjugates should be attributed to an augmentation of the GSH conjugation capacity.

It is suggested that silymarin may protect the liver against an electrophilic substance-induced toxicity by increasing GSH availability which would enhance the detoxifying capacity of liver cells.

P-MIS-075
Intracellular trafficking of STAMP1 in prostate cancer cells

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Prostate cancer (PCa) is the second leading cause of death among men in western countries. We have previously found that the six transmembrane protein of prostate 1 (STAMP1) promotes PCa cell proliferation as well as inhibits apoptosis through, at least in part, regulating the ERK/MAPK signaling. We also found that STAMP1 is highly mobile in PCa cells and shuttles between the plasma membrane and the Golgi, often found in vesiculotubular structures in the cytosol.

Using advanced imaging techniques, we have now characterized the trafficking of STAMP1 from the plasma membrane to early endosomes in LNCAP cells, by analysing its dynamic targeting to the three main endocytosis pathways: clathrin-mediated endocytosis, caveolæ/lipid rafts, and the ARF6-dependent pathway.

We found that STAMP1 fused to Cyan Fluorescent Protein (CFP-STAMP1) is present at the plasma membrane where it accumulates in punctate structures. Live cell confocal imaging showed that these puncta were dynamic over time indicating that STAMP1 may be constitutively delivered to the plasma membrane and removed from it by endocytosis.

Co-expression of CFP-STAMP1 with various fluorescent protein markers revealed that CFP-STAMP1 puncta corresponded to lipid rafts that were labelled with caveolin-1-RFP or antibodies against flotillin. Live cell imaging showed that CFP-STAMP1 and Caveolin-1-RFP disappeared at the same time from the same region of the plasma membrane suggesting that lipid rafts are likely to be responsible for STAMP1 internalization. Notably, STAMP1 was absent from other endocytosis structures such as clathrin-coated pits/vesicles.

Further work is needed to determine whether STAMP1 internalization is required for its function, such as its link to ERK signaling, and whether interference with lipid rafts influences STAMP1 effects on PCa cell proliferation and survival.

P-MIS-076
Antithrombin-III, MPV and plasma total homocysteine levels in Behcet's disease

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Introduction: Behcet's disease is a multi-systemic and chronic inflammatory vasculitis of unknown etiology characterized by recurrent oral and genital ulcers, uveitis, arthritis, arterial aneurysms, venous thrombosis and skin lesions. Platelet indices such as mean platelet volume (MPV) is a standart indicator of platelet function in disease pathophysiology. Antithrombin, a glycoprotein synthesized in the liver, is the major plasma inhibitor of thrombin thus modulating blood coagulation. Antithrombi-III (AT-III) is a enzyme even moderate deficiency significantly increases the risk of thrombosis. Homocysteine (Hcy), that is formed during the metabolism of methionine. Several clinical studies have clarified that elevated blood Hcy levels are related to atherosclerotic disease. In our study, we investigated Anthrombin-III levels, MPV values and Homocysteine values in Behcet Diease.

Material and Method: We investigated 46 patients with Behcet Disease. MPV levels calculated with Abbott Cell Dyne Hematology analyzer, AT-III levels calculated with Sysmex CA-1500 analyzer, Homocysteine levels calculated with SHIMADZU-HPLC. Statistical analysis was performed with SPSS v16.

Results: The Homocysteine values was 14.16 ± 8.24 μmol/L. MPV values was 9.16 ± 1.6. AT-III vasues was 100.08 ± 16.04. Our study has showed us AT-III and MPV values has a negative correlation ($p = 0.016$). When MPV values increase in patients with Behcet Disease, AT-III levels were decreasing. Homocysteine levels and AT-III levels is not correlated in Behcet Disease (p:0.24).

Conclusions: Our analyses showed that; homocysteine levels and AT-III levels is not correlated in Behcet Disease, and increase in MPV values with patients Behcet Disease, AT-III levels decrease and this finding might be useful and valuable in the etiopathogenesis of Behcet Disease.

P-MIS-077
Effect of Tryptophan 104 modification on anti-papain and anti-legumain activities of ovocystatin

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Ovocystatin is one of the best characterized members of cystatin superfamily of protease inhibitors, and it has been frequently used for pathophysiological studies as the model protein, representative for this superfamily. Its application has been supported by high structural similarity to human cystatin C as well as several common biological activities. As regard to biological activity, cystatins, including ovocystatin, are best characterized as inhibitors of cysteine proteases of papain family (C1), such as cathepsins B, H, L and S. These inhibitors participate in intra- and extracellular control of proteolytic events, both in physiological and pathological states. In the recent decade also new activities of cystatins, not assigned to inhibition of papain-like cysteine cathepsins, were found. These activities are associated with an alternative active center for legumain-type proteases in the molecule. Here we report a chemical modification of ovocystatin that disables the anti-papain activity of the inhibitor but does not affect its anti-legumain activity.

The chemical knockout has been obtained by reaction with 2-Hydroxy-5-nitrobenzyl bromide (HNBB) that covalently modifies the Trp104 residue in the molecule. The reaction has been monitored by UV-VIS and fluorescence spectroscopy. The anti-papain activity of the inhibitor has been measured colorimetrically against BANA as a substrate. The anti-legumain activity was assessed fluorometrically using Z-Ala-Ala-Asn-AMC.

The reacted inhibitor exhibited an additional, characteristic for HNBB, band at 410 nm in UV-VIS scan. Accordingly, an ablation of Trp fluorescence was also observed. The molecule fully retained the anti-legumain activity, while only residual anti-papain activity (10%) was observed.

The modified ovocystatin can be a useful molecular tool for studying the physiological and pathological processes specifically associated with legumain activity.

P-MIS-078
Study of PFKFB2 isoforms in pancreatic duct cells transformed with mutant K-Ras

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6-phosphofructo-2-kinase/fructose-2,6-bisphospatase (PFKFB) family of enzymes are responsible for the conversion of fructose-6-phosphate (F6P) to fructose-2,6-bisphosphate (F2,6BP) and *vice versa*, and F2,6BP is an allosteric activator of phosphofructokinase-1 (PFK1), a rate-limiting enzyme of glycolysis. Among the four identified PFKFB isozymes (PFKFB1-4), PFKFB2 is the least studied isozyme in human cancers. There exists two different splice variants of PFKFB2, variant-1 and variant-2, coding two different isoforms, isoform a and b, respectively.

In this study, we first analyzed the effect of K-Ras(G12D)-induced oncogenic transformation on PFKFB2 expression in pancreatic duct cells. We found that oncogenic K-Ras induction in immortalized pancreatic duct cells (iPDE) was associated with decreases in total PFKFB2 mRNA and protein expressions (mRNA; iPDE: 1 ± 0.15; iPDE+KRas: 0.78 ± 0.24 and protein; iPDE: 1 iPDE+KRas: 0.55). We then, checked individual expressions of splice variants and observed that while PFKFB2 splice variant-1 (P2-v1) expression was reduced by K-Ras induction (iPDE:100; iPDE+KRas:81.50), PFKFB2 splice variant-2 (P2-v2) expression was increased (iPDE:100; iPDE+KRas:125.70). Then, we checked effects of P2-v1 and P2-v2 on glycolytic phenotype of iPDE and iPDE+KRas cells. Over-expression of PFKFB2 variants increased F2,6BP concentration (P2-v1: 1.96; P2-v2: 1.72 fold; compared to empty vec), glucose uptake (P2-v1: 16%; P2-v2: 30%) and glycolysis (P2-v1: 20%; P2-v2: 30%) in iPDE+K-Ras cells.

We next analyzed the subcellular localizations of PFKFB2 isoforms and observed that both PFKFB2 isozymes localize to the nucleus, with more prominent nuclear localization of P2-v1 compared to P2-v2. Also, nuclear localization ratio of P2-v2 increases after oncogenic transformation with mutant K-Ras.

Taken together, these results suggest that PFKFB2 may have a role in the glycolytic phenotype of pancreatic cancers characterized with hyperactive K-Ras signaling.

P-MIS-079
Effects of p38 MAP kinase inhibitors on MDA-MB-231 cell line

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Introduction: p38 MAPK phosphorylates serine and/or threonine residues of the target proteins. The activation of p38 MAPK leads to cell growth, differentiation, survival or apoptosis. In this study, we tested the effect p38 MAPK SB203580 and SB202190 on MDA-MB-231 cells to further elucidate the controversial role of p38 MAPK on cell proliferation or cell migration.

Materials and Methods: MDA-MB-231 cancer line was cultured in RPMI-1640 supplemented with 10% FBS. The cytotoxic and cell migration effects of SB203580 and SB202190 inhibitors were tested by MTT assay and wound assay, respectively. The effects of both inhibitors on proliferation and adhesion of MD-MB-231 cells were determined by iCELLigence system.

Results: It was found that SB202190 p38 MAP kinase inhibitor was more effective than SB203580. However, no significant effects of low doses of 1 μM and 5 μM of both inhibitors were seen on cell proliferation as compared to the DMSO-treated control cells for up to 96 hours as determined by iCELLigence system. On the other hand, both SB203580 and SB202190 significantly prevented cell proliferation at a concentration of 50 μM. Both SB203580 and SB202190 significantly reduced cell migration in a time-dependent manner at a concentration of 50 μM. Then, we tested whether each p38 MAPK inhibitors have any effect on cell adhesion during a treatment period of 3 hours using iCELLigence system. Only 50 μM concentration of SB202190 reduced cell adhesion for about 1.5 hour ($p < 0.001$).

Conclusion: p38 MAPK inhibitors SB203580 and SB202190 differentially affect cell proliferation, survival and migration.

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P-MIS-080
Mutagenicity of a series efficacious benzoxazine derivatives – a new approach to evaluate ames test data

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Testing safety of drug candidates is as crucial as evaluating their efficacy in early drug development. We previously synthesized a series of 1,4-benzoxazine-3-one derivatives showing significant antimicrobial, *in vitro* anticancer, topoisomerase I inhibitory activities and studied their several mechanisms of action. In this present study, we have evaluated mutagenic activities of these compounds and their potential metabolites. Moreover, we aimed to develop a new statistical algorithm available for structure-activity relationship analysis to identify the regions responsible for the activity.

To evaluate mutagenicity of the compounds, Ames *Salmonella*/microsome test was used. *Salmonella typhimurium* TA98 and TA100 strains were used to detect for frameshift and base-pair substitution mutagens, respectively. Additionally, mutagenicity of potential metabolites of them were evaluated by adding metabolic activation system (S9) which was prepared from a pool of male *Sprague Dawley* rats. Results were evaluated with Student's-T test. Following regression model estimation analysis, we detected minimum mutagenic doses of all tested compounds for generating a 3D-common features pharmacophore model with HipHop method.

According to the results, only BS12, BS13, BS16 and BS17 exhibited strong mutagenic effects on both strains in the presence and absence of S9. Additionally BS10, BS7, BS1 and BS15 (in the absence of the S9), BS18, BS4 and BS7 (in the presence of the S9) showed weak mutagenic effects on TA98. HipHop analysis results revealed that mutagenicity was increased in the presence of aromatic desactivating groups which might form hydrogen bonds at the position of R3 and hydrophobic groups at the position of R2 of the benzene ring in the structure of benzoxazine.

The new statistical approach developed in this study can be useful for assessing the Ames test data available for structure activity relationship analyses.

P-MIS-081
Expanded newborn screening results by tandem mass spectrometry

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Background: Recently more than thirty different diseases can screen simultaneously with Expanded Newborn Screening (NBS) programs by tandem MS.Expanded NBS with tandem MS is performed routinely at Akdeniz University Hospital Central Laboratory since 2008.The aim of this study was to evaluate our NBS results with some second-tier and confirmatory tests.
Materials and Methods: NBS results (n = 1863) were evaluated in dried blood samples which sent to our laboratory for the study between August 2014 and August 2015. Electrospray Ionisation (ESI)triple quadrupole mass spectrometer (Shimadzu LC-MS/MS 8030,Japan) was used for NBS analysis,acylcarnitine and amino acid profile were screened with MRM (multiple reaction monitoring) spectrum within 2 minutes.Second-tier tests were performed as urine organic acid analysis by gas chromatography-mass spectrometry (GC-MS),plasma and urine quantitative amino acid analysis by high pressure liquid chromatography (HPLC).Pathological NBS results were assessed in three separate groups as amino acid metabolism disorders, fatty acid oxidation defects and organic acidemias.
Results: Metabolic diseases were found in 69 (3.70%) patients by the second-tier tests performed.There were detected amino acid metabolism disorders in 13,organic acidemia in 42,fatty acid oxidation defects in 14 patients.
Conclusions: The reason of high positive results in our laboratory could explain that our study includes both screening and monitoring of previously diagnosed metabolic patients.NBS is performed in only a few centers in Turkey although there were the national screening programs included NBS in many foreign countries.More expanded NBS programmes in our country is required to start treatment of patients before irreversible damage is not occured.

P-MIS-082
Specific role of calpain-1 associated to N-methyl-D-aspartate receptor in lipid rafts microdomains

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Although many reports indicate the involvement of calpain in several human pathologies, it is not yet clarified how the protease can recognize the substrates to digest and how can escape to its natural inhibitor calpastatin. Answers to these questions have been obtained by identifying specific intracellular localizations of calpain and its substrates and analyzing the interactions of the protease with calpastatin.

These studies were carried out using human SKNBE neuroblastoma cells. Protein-protein interactions and intracellular localization of calpain and the related proteins were determined by immunoprecipitation and isolation of membrane microdomains.

We have observed that small amounts of calpain-1 are localized in lipid rafts microdomains together with N-methyl-D-aspartate receptor (NMDAR) containing NR1/NR2B subunits. Immunoprecipitation experiments have demonstrated that NMDAR containing NR1/NR2B subunits, calpain-1, HSP90 and neuronal nitric oxide synthase (nNOS) but not calpastatin and calpain-2 are present in specific protein complexes. Thus, in this localization calpain activity is regulated by HSP90 that reduces the affinity for Ca^{2+} of the protease. Cell stimulation with NMDAR agonists induces calpain activation that specifically cleaves the subunits NR2B of the receptor promoting changes in lipid rafts organization and internalization of NMDAR without affecting cell viability. Moreover, in these conditions, also nNOS is digested and converted in the active form by calpain-1.

Our data suggest a physiological role of calpain-1 at specific cell sites. The protease inserted in lipid rafts microdomains is in strict contact with its targets and escapes to calpastatin which is not inserted in these structures. Following an increase in Ca^{2+} influx, the activated protease regulated by HSP90, promotes the removal of NMDAR from the plasma membranes, decreasing Ca^{2+} entrance through this receptor-channel and protecting cells from Ca^{2+} overloading.

P-MIS-083
Role of tissue transglutaminase transaminase and GTP-binding functions on renal cancer cell migration

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Tissue transglutaminase 2 (TG2) is a multifunctional protein complex that can act as a crosslinking enzyme, GTPase/ATPase, protein kinase and protein disulfide isomerase. At the cell surface, TG2 was shown to be involved in adhesion, migration, invasion, growth, epithelial mesenchymal transition and hence implied in the metastatic development of many different tumor types. Renal cell cancer (RCC) is one of the most common type of cancer in adult males that generally grows as a single tumor within a kidney. Our previous findings indicate that the increased expression of TG2 in RCC results in tumor metastasis with a significant decrease in disease- and cancer-specific survival outcome. Herewith, the role of TG2 in cell migration of RCC was investigated in this study by transducing the model RCC mouse cell line RenCa with a series of TG2 mutant constructs.

RenCa cells were transduced by lentiviral particles encoding wtTG2, transaminase-defective TG2-C277S form with low GTP-binding affinity, GTP-binding deficient form TG2-R580A, and transaminase-inactive TG2-W241A. In order to investigate the role of TG2 transamidating and GTPase activity in cell migration, scattering assay was used where 7 colonies for each mutant clone was followed for a time interval of 24 hours.

Our results showed that non-transduced control and TG2-C277S mutant Renca cells demonstrated a similar migration pattern with a 20% of scatter activity. On the other hand, 52% colonies formed by Renca cells overexpressing wtTG2 and TG2-W241A mutant scattered away from each other. A small insignificant increase in scattering was seen in 28% of the total number of 10 colonies for RenCa cells overexpressing TG-R580A construct.

Data from this study supports that GTP-binding activity of TG2 is the drive force in migration driven scattering of Renca cells, suggesting that inhibitors targeting the GTP-binding activity of TG2 may serve as a new therapeutic approach in the treatment of RCC.

P-MIS-084
Altered 25-hydroxy (OH) vitamin D levels in subclinic thyroid disorders

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Background: In this study, we aimed to investigate the relationship between level of vitamin D with subclinical hypothyroidism and subclinical hyperthyroidism.
Material and metod: Study groups planned as three groups such as euthyroid (N = 11966), subclinical hypothyroid (N = 1040), subclinical hyperthyroid (N = 795). Serum TSH, free T4 (fT4) and free T3 (fT3) levels were determined by chemiluminescence immunoassay and serum 25-hydroxy (OH) vitamin D 25 (OH) D level were determined by liquid chromatography-tandem mass spectrometry. Euthyroidism was defined as a normal level of TSH (range, 0.4 to 4.2 mIU/L), fT4 (range, 0.8 to 2.3 ng/dL) and fT3 (range, 2.3 to 4.2 ng/dL). Subclinical hypothyroidism is defined as an elevated serum TSH level associated with normal total or free T4 and T3 levels. Subclinical hyperthyroidism is defined as low serum TSH levels associated with normal free T4 and free T3 levels.
Results: Subclinical hyperthyroid group had significantly higher 25 (OH) vitamin D levels compared to the euthyroid and subclinical hypothyroid groups ($p < 0.05$). 25 (OH) vitamin D levels in subclinical hypothyroid group was not statistically significant when compared with the euthyroid group.
Conclusion: Complex metabolic pathways play crucial roles in subclinical hyperthyroid patients in regulating 25 (OH) vitamin D levels.
Keywords: TSH, fT4, fT3, 25 (OH) vitamin D.

P-MIS-085
New approaches in bioethanol production

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Food processing wastes provide carbon sources in high amounts for fermentative microorganisms to produce energy. Converting carbon-rich biomass into bioethanol through fermentation by microorganisms both provides energy requirement for humankind and also decrease pollutant gases like CO_2, NO_x and SO_x (Ghorbani et al., 2011). Fermentation processes for bio-ethanol production could be achieved by *Saccharomyces cerevisiae*, *Zymomonas mobilis*, and *Escherichia coli*.

Bacterial hemoglobin (*Vitreoscilla* hemoglobin, VHb) is the first and best characterized prokaryotic hemoglobin molecule. The function of VHb is supporting the cellular respiration through binding to oxygen at microaerobic environment, transferring it to the terminal respiration oxidases (Geckil et al., 2004) and thus improving growth and productivity of the microorganisms.

In this study, *E.coli* strains FBR5, TS3 and TS4 were used as ethanologenic microorganisms. Expression of VHb in TS3 is lower than in TS4 strain. For the efficient ethanol production effect of different inoculum sizes, sugar species and sugar concentrations in the growth medium were investigated. VHb expression increased effectively the viability of TS3 strain by up to $1.8x10^9$ cfu per ml of fructose (3%, w/v) supplemented LB medium starting with small inoculum for fermentation. This indicates that *vgb* expression should be at the certain level to maintain sufficient the cell growth for ethanol production.

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Ghorbani, F., Younesi, H., Sari, A. E., Najafpour, G. (2011). Cane molasses fermentation for continuous ethanol production in an immobilized cells reactor by *Saccharomyces cerevisiae*. Renewable Energy: Volume 36, Issue 2, February 2011, Pages 503–509. doi:10.1016/j.renene.2010.07.016.

P-MIS-086
Ethanol production from dairy industry by product using bacterial hemoglobin

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Bioethanol production from biomass has a great potential to reduce greenhouse gases emissions. Ethanol has several applications in industries (chemical, medical, pharmaceutical, food etc.) in the form of raw material, solvent and fuel. One of the most abundant liquid wastes is cheese whey generated from dairy industries. Whey powder is concentrated form of whey and contains lactose and also protein, lipid, minerals and vitamins. *Vitreoscilla* hemoglobin (VHb) is the first bacterial hemoglobin. The main function of this molecule is to improve oxygen transfer to cellular oxidases and thus supporting cellular growth and productivity at low oxygen levels (Kallio et al. 1994).

In this work, *E. coli* strains FBR5, TS3 (low level VHb expressing) and TS4 (high level VHb expressing) were used as ethanol producing microorganisms. Fermentation medium containing whey powder supplemented with LB material was inoculated with these strains and incubated for 48 hours at 37 °C and 180 rpm in a 1000 ml Erlenmayer Flask. The ethanol production was improved over 300% by using lower VHb expressing strain. The ethanol levels (v/v, %) were determined as 1.08, 4.99 and 1.51 for FBR5, TS3 and TS4 strains respectively. It is shown that the certain levels of VHb could be useful tool to increase the growth and productivity of ethanol from dairy industry wastes.

Kallio P.T., Kim D.J., Tsai P.S. and Bailey J.E. (1994). "Intracellular expression of *Vitreoscilla* hemoglobin alters *Escherichia coli* energy metabolism under oxygen-limited conditions", European Journal of Biochemistry, 219 (1–2): 201–208.

P-MIS-087
As a useful tool for inhibitors derived from lignocellulosic hydrolysates in ethanol production: *Vitreoscilla* hemoglobin (VHb)

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Bioethanol is usually produced from cellulose, hemicellulose and lignin. The lignocellulosic wastes should be hydrolysed into fermentable sugars by using enzymes or dilute acids before microbial fermentation. Acidic hydrolysis methodology is cheaper than enzymatic hydrolysis but it can cause production of some inhibitors like aliphatic acids, which affect the growth of microorganisms. *Vitreoscilla* hemoglobin (VHb) is the first described prokaryotic hemoglobin. The recombinant strains carrying *vgb* gene *(E. coli, P. aureginosa)* which encodes VHb showed increased bacterial growth, productivity of metabolites compared to untransformant counterparts under low oxygen concentrations [Nasr et al., 2001; Geckil et al., 2004]. In this study, ethanologic *E. coli* strains FBR5, its derivative strains TS3 (*vgb*+) and TS4

(*vgb*+) were used. TS4 was constructed in such that it could express more VHb than TS3. Bioethanol production by these strains in presence of lignocellulosic hydrolysates derived inhibitors was investigated. Different acetic acid concentrations (2.5–10 mM) were used as inhibitors from lignocellulose hydrolysate. 10.0 mM acetic acid was used as an inhibitor. The growth of VHb expressing TS3 and TS4 strains was inhibited about 31% after 48 hours fermentation time. Strain FBR5 was inhibited as high as 76% by using the same inhibitor including growth medium. It was shown that the expression of VHb could improve growth and productivity in presence of lignocellulosic inhibitors.

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Geckil H., Barak Z., Chipman D. M., Erenler S. O., Webster D. A., Stark B. C., (2004), "Enhanced production of acetoin and butanediol in recombinant *Enterobacter aerogenes* carrying *Vitreoscilla* hemoglobin gene", Bioprocess and Biosystems Engineering, 26, 325–330.

P-MIS-088
Inhibition of adipogenesis by AZD1208, an inhibitor of the family of Pim kinases through down-regulation of C/EBP-a, PPAR-r, FAS, Perilipin A, and STAT-3

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Differentiation of preadipocyte, also called adipogenesis, leads to the phenotype of mature adipocyte. However, excessive adipogenesis is closely linked to the development of obesity. Thus, any drug or chemical that can inhibit adipogenesis may have preventive and/or therapeutic potential against obesity and related diseases. AZD1208, an inhibitor of the family of Pim kinases, is known for anti-cancer activity. Here we investigated the effect of AZD1208 on adipogenesis in 3T3-L1 preadipocytes. Notably, AZD1208 treatment led to a concentration-dependent inhibition of both lipid accumulation and triglyceride (TG) synthesis during the differentiation of 3T3-L1 preadipocytes into adipocytes with no cytotoxicity. On mechanistic levels, AZD1208 strongly reduced not only the expression levels of CCAAT/enhancer-binding protein-α (C/EBP-α), peroxisome proliferator-activated receptor-γ (PPAR-γ), fatty acid synthase (FAS), and perilipin A but also the phosphorylation levels of signal transducer and activator of transcription-3 (STAT-3) during adipocyte differentiation. Furthermore, AZD1208 largely decreased leptin, but not adiponectin, mRNA expressions during adipocyte differentiation. Collectively, these results demonstrate that AZD1208 inhibits adipogenesis in 3T3-L1 preadipocytes and the inhibition is largely attributable to the reduced expression and/or phosphorylation levels of C/EBP-α, PPAR-γ, FAS, perilipin A, and STAT-3.

P-MIS-089
Effect of intrauterin exposure to artificial food colourings on DNA damage in rats

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In many research genotoxic potential of food additives has been investigated. However there are few findings about the effect of artificial food colourings (AFC) on DNA. In this experimental study, we aimed to analyze whether *in utero* exposed artificial food colourings would have effect on DNA and cause damage.Thirteen female rats were included to the study which were equally divided into two groups as control (CG, n = 15) and food colouring (FCG, n = 15) groups. A mixture of nine food colours were given daily to FCG by oral gavage from preconception to birth. No adverse effect level (NOAEL) of artificial food colourings for each colouring was administered to FCG. Three months after the birth, 6 offspring from each group were selected randomly as control (CG) and experiment (EG) groups. Then they were sacrified under anesthesia. For performing the Alkaline comet comet assay leukocytes were seperated from whole blood samples. The alkaline comet assay was performed. The extent of DNA damage was assessed from the length of DNA migration derived by subtracting the diameter of the nucleus from the total length of the image and graded into 5 categories and these grades were converted into arbitrary unit (AU). Differences between the means of data were compared by Independent Samples T test. The results were given as the mean±SD, p values of less than 0.05 were considered as statistically significant. Although the extent of DNA damage was higher in EG, the comparison of experiment (13.50 ± 1.76) and control (11.66 ± 1.36) groups showed no statistical difference ($p = 0.072$).

P-MIS-091
Relationship between glucocorticoid receptor gene polymorphisms and recurrent depression

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Objective: Sensitivity to glucocorticoids varies between individuals and these differences have been implicated in the etiology of psychiatric diseases such as depression. Recent studies have found relationship between common glucocorticoid receptor (GR) gene (NR3C1) polymorphisms and unipolar or bipolar depression. The NR3C1 gene is a candidate gene affecting depressive disorder risk and management. The aim of the present study was to evaluate the relative distribution of specific polymorphisms of NR3C1 (Bcl1 and rs33388) in recurrent depressive disorder (rDD) patients.
Methods: Our study included 100 volunteers with recurrent depressive disorder and 100 healthy individuals without any mental illness. Depression was assessed by Hamilton and MADRS depression scale. NR3C1 gene polymorphisms were detected by Real-Time PCR, with hybridization probe method. Allele and

genotype frequencies at two loci (BclI and rs33388) were investigated in rDD patients and controls.

Results: Genotype distribution among RDD patients and the control group for Bcl-1 (G/C) were as follows: CC 3% and 5%, GC 27% and 34%, GG 70% and 61%, respectively. There was not a significant difference when the frequency of the allele ($p = 0.204$) and genotype frequency ($p = 0.382$) were compared between the patients and the control. Genotype distribution in the rs33388region (A/T) of the patients and controls were TT 29% and 25%, TA 49% and 54%, AA 22% and 21%, respectively. Allele frequency ($p = 0.841$) and the genotype frequencies ($p = 0.754$) were not significantly different among the groups.

Conclusion: Numerous NR3C1 gene polymorphisms were previously reported in association with modification of depressive disorders. The results of our study showed no association between GR genotype and recurrent depressive disorder. NR3C1 polymorphism does not play a role in the development of recurrent depressive disorder.

P-MIS-092
Determining the effects of Thymoquinone on apoptosis, cell cycle, invasion, migration and colony formation in SH-SY5Y neuroblastoma cell line

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Thymoquinone (TQ) has been shown to supress the proliferation of various tumor cells, while it is minimally toxic to normal cells. The aim of this study is to investigate the potential therapeutic effects of TQ on cell proliferation, apoptosis, invasion, migration, colony formation and wound-healing in SH-SY5Y human neuroblastoma cell line.

SH-SY5Y cell line treated with 5–400 μM TQ by solving medium for 24, 48 and 72 h considering a time- and dose-dependent manner. The cytotoxic effect of TQ was determined by MTT method. Total RNA was isolated by TRIzol reagent. cDNA synthesis was performed by using commercial kit. *MDM2, p53, p21, AKT, PTEN, CDK4, CYCLIN D1, CASPASE-3, -8, -9, -10, BCL-2, BAX, PARP, BCL-XL, BID, DR4, DR5, PUMA, NOXA, MMP-2, -9, TIMP-1, -2* and *GAPDH* gene expression profiles were analysed by real-time PCR method. Effects of TQ in SH-SY5Y cells on invasion, colony formation and cell migration were detected by matrigel-chamber, colony formation assay and wound-healing assay, respectively. Statistical analysis were performed with RT^2Profiles Array Data Analysis by using Student's t test.

IC_{50} value of TQ in SH-SY5Y cells was detected as 15 μM at 48^{th} hours. By RT-PCR results, it was determined that TQ caused a decrease in the expression of *MDM2, AKT, CDK4, CYCLIN D1, BCL-2* and *MMP-2*. It is also observed that TQ caused a significant increase in the expression of *p53, PTEN, CASPASE-3, -10, BID, DR4, PUMA, NOXA* and *TIMP-1*. It was also found that TQ in SH-SY5Y cells suppressed invasion, migration and colony formation by using matrigel invasion chamber, wound healing and colony formation assay, respectively.

In conclusion, we demonstrate that TQ significantly effect cell cycle, apoptosis, invasion, migration and colony formation of SH-SY5Y cells. TQ may be a potential candidate as chemotherapeutic agent for the treatment of neuroblastoma. More studies have to be performed to profile the mechanisms and genome wide effects of TQ to prove its therapeutic potential.

P-MIS-093
Efficient technique for the selection of DNA aptamers to soluble recombinant proteins

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DNA aptamers can achieve a very high affinity to the target due to the potential of developing broad target-binding interface. However, classic strategy selection of aptamer binders is a challenging task requiring multiple rounds of panning and post-selection optimization. We have developed fast and convenient technique for the selection of DNA aptamers based on the off-rate selection and tandem affinity purification (TAP).

We constructed and produced in E.coli recombinant chimeric protein, comprising two affinity tags (His6 and GST) separated from each other and from the target protein (anthrax protective antigen domain IV, PAdIV) by SUMO protease recognition polypeptide and synthetic cleavage site for the anthrax lethal factor (LF). The protein bound to aptamer library is first captured by IMAC resin, cleaved by SUMO protease, captured by GST resin and eluted by LF following the lines of the TAP method. The GST-captured aptamer-target complexes were subjected to the off-rate selection using soluble PAdIV as the competitor.

Multiple selection rounds are cumbersome and can result in carryover. High abundance of moderate affinity aptamers in the resulting pools obtained by classic selection approaches suggests that the procedure to counter-select them at the beginning of panning is needed. Reduction of the contact duration between the aptamer library and the target was crucial for efficient selection of high-affinity binders. On the other hand, TAP prevents contamination, and bundled with the off-rate selection, allows for clean isolation of high-affinity binders with affinity in the low nanomolar range.

The developed technique is applicable for efficient selection of high affinity DNA binders to soluble recombinant proteins and their fragments. DNA aptamers obtained will be further used for the development of diagnostic and therapeutic tools for the detection and treatment of anthrax.

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P-MIS-094
The role of MacAB efflux pump in protection of Serratia marcescens against antibiotics and oxidative stress

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The emergence of bacterial multi-drug resistance is a growing problem of public health worldwide. Bacterial drug efflux pumps are membrane protein complexes that function to expulse drugs from the cell. They play a crucial role in the rising rates of antibiotic therapy failures. The homolog of macrolide-specific pump MacAB was identified in opportunistic pathogen *Serratia marcescens* and was used in this study to characterize its role in protection against antimicrobials and other processes beyond the active efflux of antibiotics.

Here we used method of serial dilutions to determine minimum inhibitory concentration (MIC) for *S. marcescens* SM6 wild type (WT) and its isogenic Δ*macAB* mutant strains. We also used

H_2O_2 survival assay to evaluate the ability of WT and the mutant strain to withstand an oxidative stress. Finally, we used β-galactosidase assay to evaluate *macAB* promotor activation in the reporter strain and followed MacAB expression by Western blotting analysis using MacAB-6xHis strain.

We show that in contrary to its *E. coli* homolog, MacAB pump in *S. marcescens* is not involved in the protection against macrolides but instead it is required for protection against aminoglycosides. We further show that similar to its *Salmonella* Typhimurium homolog, *S. marcescens* MacAB is essential for protection of bacteria against H_2O_2. Transcriptional analyses demonstrate that while low level of *macAB* promotor activity can be detected after 2 hours of growth in LB-broth there is at least 5-fold increase in expression in response to the presence of H_2O_2. On the protein level MacAB can be detected starting from 3 hours of growth in LB-broth and it reaches maximum expression on 16 hour of growth.

Our data suggest that MacAB pump in *S. marcescens* is involved in protection of bacteria against aminoglycoside antibiotics and is crucial for protection against reactive oxygen species. We are currently working on identification of MacAB substrate with anti-H_2O_2 properties.

P-MIS-095
Antiproliferative and apoptotic effects of noscapine on MCF-7 and MDA-MB-231 human breast cancer cell lines

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Approximately 10–17% of breast cancers are negative for estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2. These are most aggressive tumor and a clinical problem because of lack of targeted therapies. Noscapine is an alkaloid from opium. Noscapine is a microtubule-interfering agent. It causes mitotic arrest, induces apoptosis. In this study, we aimed to investigate the effects of Noscapine in MCF-7 and MDA-MB-231 human breast cancer cell lines.

The cytotoxic effects of Docetaxel, Tamoxifen, and Noscapine on the MCF-7 and MDA-MB-231 cell lines were analyzed by Roche xCELLigence system. The cells were cultured in 10% fetal bovine serum containing Dulbecco's Modified Eagle Medium at 37°C in a humidified atmosphere containing 5% CO_2. 24 h after seeding, the cells were treated with 4 different doses of Docetaxel (12.5 to 100 nM), Tamoxifen (12.5 to 100 μM), and Noscapine (12.5 to 100 μM). Cultured cells were harvested, fixed with 10% formalin, and centrifuged. Pellet was blocked, fixed, and embedded in paraffin. Paraffin-embedded cells blocks were sectioned at 4 μm thickness and stained with H&E, Ki-67, Bcl-2, Cyclin-D1, and BAX. Sides were assessed under a light microscope. Quantification of the analyzed proteins were evaluated by the percentage of positive cells.

All drugs showed cytotoxic effects on both cell lines. All drugs inhibited the proliferation of breast cancer cells, but effects were dependent on time and dose. All drugs were especially more effective on MCF-7 cells. Immunohistochemical examinations revealed that Tamoxifen was more effective on MCF-7 cells, hovewer Docetaxel and Noscapine were more effective on MDA-MB-231 cells. Tamoxifen has more apoptotic and antiproliferative effects on MCF-7 cells. Docetaxel and Noscapine showed more apoptotic and antiproliferative effects on MDA-MB-231 cells.

Noscapine may be an effective anticancer agent due to antiproliferative and apoptotic effects on breast cancer cells.

P-MIS-096
Negative selection of DNA aptamers to reduce non-specific binding in solid-phase-based selection procedures

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Carryover by binders specific to the components of the selection system can be a serious issue in hampering the aptamer selection campaign. Solution- or "mass"-based techniques still cannot substitute classic phase-separation strategies.

One approach to prevent selection of "passenger" phase-specific (plastic, beads) or blocking agent specific aptamer species is their depletion from the initial library pool. Our aim was to develop the universal technique for removal of such aptamers exemplified by BSA- and casein-specific binders, while preserving the initial library complexity.

The DNA aptamer library was subjected to three rounds of depletion using magnetic beads with covalently attached casein and BSA. To ensure high depletion efficiency, beads were pelleted in a 15-ml centrifuge tube by a neodymium magnet through a 10-cm cushion of 20% sucrose, thus preventing weakly bound aptamers from re-populating the library. High complexity of the input library helped to avoid PCR amplification after depletion rounds preventing the library bias introduced by DNA amplification. The depletion effciency was confirmed by real-time PCR.

Resulting oligonucleotide sub-library was analyzed for binding to the targets using solid-phase real-time PCR assay.

We have shown that three rounds of panning under the conditions employed provided full depletion of the initial DNA pool from nucleic acid structures capable of binding to protein competitors and hampering the process of aptamer selection. We compared selection efficiency of aptamers specific to type A botulinum neurotoxin light chain in depleted vs undepleted library. The yield of the target-specific aptamers was 10-fold higher in the library subjected to the depletion procedure.

Removal of undesired binders from aptamer libraries appears an important step of solid-phase SELEX procedure. It can become a useful approach in optimizing solid-phase SELEX.

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P-MIS-097
SIP1 induced epithelial mesenchymal transition promotes metastasis and alters chemokine (C-C motif) ligand 5 expression by modulating tumour microenvironment in colorectal cancer

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Epithelial mesenchymal transition (EMT) is a critical trans-differentiation program driving cancer metastasis. Patients showing signs of EMT or presence of distant metastasis have poor prognosis. Another well-known feature of decreased cancer-associated survival is the lack of anti-cancer immune responses. Thus we hypothesized that the EMT and anti-tumor response should be linked via altered secretion of soluble factors by metastatic cells.

All cell lines were grown in DMEM. EMT status of CRC cell lines were assessed by investigating canonical markers of EMT. Cytokine/chemokine expression of CRC cells was performed using R&D systems antibody arrays and validated using CCL5 sandwich ELISA and RT-PCR. The mechanism of action of ZEB1/2 on *CCL5* promoter has been studied by luciferace assay and ChIP. CCL5 coding region was cloned into pCDNA3.1 and stably transfected into DLD-1 cells. CCL5 deficient CT26 cells were generated using lentivirual shRNA transduction. Cells over-expressing or knock/down CCL5 were injected orthotopically into mice. T lymphocyte (TIL) infiltration in respect to CCL5 and SIP1 expression was studied using IHC or flow cytometry.

EMT status catagorised 13 CRC cell lines into epithelial, intermediate epithelial, intermediate mesechymal and mesenchymal. Cytokine/chemokine antibody arrays showed a significant increase in CCL5 in induced DLD-SIP1 cells. ELISA, Multiplex assays and RT-PCR confirmed a significant increase of secreted CCL5 in the induced DLD-SIP1 cells as well as mesenchymal CRC cells as compared to epithelial ones ($p = 0.027$). Promoter studies showed that ZEB1/2 bind to *CCL5* promoter and and activate *CCL5* gene expression. No metastasis was observed for DLD-1 cells overexpressing CCL5 but significant alterations of tumour associated lymphocytes were identified in syngeneic orthotopic CRC models.

Our data shows that CCL5 is up-regulated by EMT inducing transcription factor SIP1, and mesenchymal (metastatic) CRC cells secrete significantly more CCL5 compared to epithelial (non-metastatic) ones. CCL5 did not induce EMT *per se* but abundant secretion of CCL5 by metastatic CRC cells was a crucial regulator of immune infiltrate in CRC. Inhibiting CCL5 in metastatic CRC may have a therapeutic potential.

P-MIS-098
Vitamin E (α-tocopherol) contents and antimutagenicity potentials Talbina (*Hordeum vulgare* L.)

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Barley (Hordeum vulgare L.) belongs to the grass family, Poaceae (Gramineae). It is the fourth most important cereal crop after wheat, maize and rice and is among the top ten crop plants in the world. Talbina was used to be recommended for the sick and for one who is grieving over a dead person. Talbina is made by adding one or two tablespoon of barley flour (must be 100 percent wholegrain barley flour) to one-and-a-half cups of water and placed on low heat for 10–15 minutes (optional: add milk or yoghurt and sweeten with honey). The main objectives of this investigation were determine the α-tocopherol contents and antimutagenicity activity of Talbina (Hordeum vulgare L.). Our results showed that the total tocopherol content was in the range of 0.25 to 1.03 μmol/g FW. Talbina extract was shown to have greater antimutagenic activity observed in the 2500 μg/plate concentration S. typhimurium TA98. At all the doses antimutagenic response was significant at ($p < 0.01$) against both the strains with a percent mutagenicity decrease from 40 to 25 for TA98 followed by TA100 with percent antimutagenicity from 30 to 11. The results of the study concluded that Talbina is a better antimutagenic agent than vitamin E and combination of vitamins did not produce any synergistic activity.

P-MIS-099
Biological properties of some novel thiadiazole compounds

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The compounds containing thiadiazoles have diverse applications as antifungals, anticancer agents, antibacterial, antiinflammatory drugs, antidepressants and carbonic anhydrase inhibitors according to literature. In this study some novel thiadiazole compounds [(1,4,10,13)-tetrathia[4.4](2,5)-1,3,4-thiadiazolophane; (4,16)-dioxo-1,7,13,19)-tetrathia[7.7](2,5)-1,3,4-thiadiazolophane; (4,7,19,22)-tetraoxo-(1,10,16,25)-tetrathia[10.10](2,5)-1,3,4-thiadiazolophane and (4,7,10,22,25,28)-hexaoxo-(1,13,19,31)-tetrathia [13.13] (2,5)-1,3,4- thiadiazolophane] were used to evaluate the cytotoxicity on healthy human lymphocytes and the antibacterial activities. Cytotoxicity tests were perfomed using MTS Assay and the trypan blue test. Cells were incubated with the compounds for 72 hours. At the end of the each 24 hour, cell vitality was assessed by measuring the absorbance (490 nm) of each well using a microplate reader for MTS assay. In addition, viability percents of the cells were determined after trypan blue test. As a result, the compounds showed cytotoxicity in a dose dependent manner. For the concentrations of 1:1000 of 0.5 mg/mL, the cytotoxic effect was eliminated. Also, antioxidant capacity was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent. Moreover, the antibacterial activities of the compounds were analyzed using a microdilution test against *E. coli* and *S.aureus*. Compounds having various concentrations showed different antibacterial effects against these two bacteria.

P-MIS-101
Arabidopsis thaliana ecotypes vary in their ability to utilize organic P substrates

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Insufficient quantity of inorganic phosphorus in soil is an ever-growing problem that affects many fields of agriculture. Unlike inorganic phosphates, organic phosphorus compounds are very common in many soil types, but plants are often unable to efficiently utilize them. To better characterize the extent of natural variation in the ability of the model plant *Arabidopsis thaliana* to grow on organic phosphorus compounds, we grew 19 Arabidopsis ecotypes on several organic and inorganic sources of phosphorus. Plants were grown in liquid or solid media containing Na-phosphate, phytate and ATP as the sole supply of phosphorus or in absence of any phosphorus source. After several weeks of growth, plants were assayed for changes in their morphological and physiological characteristics.

Phytate was shown to be the least preferred source of phosphorus compared to inorganic phosphate and ATP. The rate of biomass accumulation in all ecotypes decreased in the following order from inorganic phosphate to ATP to phytate. Lateral root formation was markedly reduced in the absence of any phosphorus source or in the presence of phytate. We also showed that phosphomonoesterase activity in intact roots increased when plants were grown on ATP and phytate. Overall phosphorus content in leaves and roots was similar when plants were grown on ATP or inorganic phosphate, but it was markedly reduced on

phytate. Substantial differences between ecotypes were also observed in root length, P content in ash and phosphomonoesterase activity in intact roots. Our analysis of the ability of Arabidopsis ecotypes to grow on several different phosphorus sources provides a unique opportunity to investigate the degree of natural variation in this plant's ability to adapt to different nutritional environments. Analysis of many important morphological and physiological changes observed in these plants can further extend our understanding of the full range of plant responses to phosphorus availability.

P-MIS-102
An example for investigation of unnecessary laboratory testing: free PSA test

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Laboratory tests are important in terms of confirmation of diagnosis given by clinics and implementation of appropriate treatment protocols for patients. Laboratory tests used by the clinics have been increased in parallel with time.There are many reasons for increased use of the test such as increase of elderly population, increase in standard of care, lack of information and shortening of turn around time. Unnecessary laboratory testing also constitute one of the reasons for increased use of laboratory tests. In our study we aimed to investigate the unnecessary laboratory testing for fPSA test.

fPSA tests which are ordered with total psa tests that values of less than 4 ng/ml or greater than 10 ng/ml were accepted as inappropriate initial testing.

9759 fPSA tests were evaluated as unnecessary laboratory testing. The clinic which ordered the maximum unnecessary laboratory testing with 3315 was urology within all the clinics. Although to the restrictions about the ordering of total PSA and fPSA tests there were no decrease in the number of unnecessary laboratory testing. Unnecessary usage of laboratory testing may cause increase of false positive results, increase in the use of invasive testing, unnecessary drug consumption and increase of healtcare costs. Some precautions may be effective in reducing unnecessary tests such as to inform clinicians about the cost of laboratory tests, to increase the clinician education programs and to develop usage of disease specific diagnostic algorithms about test ordering.

P-MIS-103
Local clinical validation of blood collection tubes

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Although the tubes with gel and clot activator are widely used due to the advantages, there are ongoing discussions about the effects of the blood collection tube on clinical outcomes in the analysis of biochemical parameters. Therefore, we aimed to prove the local clinical validation of the new produced blood collection tubes with low-volume.

The blood samples of 40 patients who referred to the hospital phlebotomy unit were collected using holder into the 3 different tubes. First tube was 5 ml glass tube and with no additive, second was 5 ml tube with gel separator, third was 1 ml tube with gel separator. Serum was separated and immediadiately analysed for 25 biochemical parameters. The difference between the analyte amounts in the different tubes was evaluated using paired t-test. The clinical significance was evaluated using significant change limit. Bias (%) between the other tubes with the reference tube was also evaluated according to the "allowable total error".

When we compared the other test tubes to a glass tube which was assumed reference tube, total protein, albumin, amylase, calcium, triglyceride, cholesterol, HDL-cholesterol, total and direct bilirubin, iron, gamma glutamyl transferase, magnesium, phosphorus results were statistically significant. But the results of all the analytes were within the significant changes limit and the allowable total error was not significant.

While a biochemical parameters have analysed, it may be absorbed into the gel and this may caused from factors such as the chemical structure of the gel, analyte itself, the residence time in the gel, storage temperature and volume of the sample e.g. As well as the leaking of gel material to the sample was reported to be another factor for affecting the analysis. Despite these factors, we observed that neither gel-clot activator tube with low nor high volume affect the clinical results.

P-MIS-104
The research of the frequency of interference in thyroid function tests

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Interference is defined as the effect of substance in the sample which changes the correct value of laboratory results. The frequency of interference in immune techniques is varied. The frequency of interference depends on population of the study, technique for detecting the reaction and researcher's method. Unexpected or inconsistent results with clinical findings should suggest the possibility of interference. In this study It is aimed to investigate the frequency of interference in thyroid function tests (TSH, fT3, fT4) which are the most common requested laboratory tests.

Thyroid function tests of 47915 patients are analyzed in Ankara Numune Education and Research Hospital in October 2014- May 2015. Five samples which had the incompatible results with clinical findings are re-evaluated just because of the suspicion of interference. The detection of interference included; repetition of test via different immune techniques, serial dilution, polyethylene glycol (PEG) precipitation and incubation with heterophilic blocking tubes (HBT).

The results of two different immune techniques and before/after incubation with HBT showed no significant difference. Linear curves had observed in serial dilution. After PEG precipitation; below 40% of recovery had obtained in one sample, therefore it is interpreted as macro-TSH. The frequency of interference in thyroid function tests for 8-month study period was 0.01%.

No information is found about the best test for defining the cross reaction. It is also aforethought that interference should not be excluded by using any single procedure.

P-MIS-105
Development of polyclonal and monoclonal antibodies against fatty acid binding protein 4 (FABP4/AP2)

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Recombinant proteins and antibodies can be use for therapeutic or diagnostic purposes which produced in many different host organisms.

The technique for the production of immortal cell making single antibody, fusing target antibody-forming B lymphocyte precursor with a suitable myeloma cells. The fused hybrid cells (called hybridomas), as a cancer cell will reproduce rapidly and will produce large amounts of the desired antibodies.

Fatty acid binding protein (FABP4) is a well characterized intracellular lipid transport protein and plays a key role in the intracellular fatty acid transport and adipose tissue metabolism. FABP4 as a adipokine that regulates glucose homeostasis and has various features for metabolic syndrome associated with obesity.

In this study, production of monoclonal antibodies against immunogenic FABP4 protein made by recombinant DNA technology. Recombinant His-FABP4 was expressed in e.coli and purified. Balb/C mice used for immunization and serum anti-FABP4 antibodies determined by enzyme-linked immunosorbent assay (ELISA). Hybridoma cells created by fusion of splenocytes and myeloma partner cells. After selection of antibody producing cell clones, injecting hybridomas into the peritoneal cavity in Balb/C mice ascites fluids was obtained.

We have selected fifteen hybridoma clones that produced antibodies specific for FABP4, as shown by western blotting and immunocytochemistry. As a result we produced MAbs that will be useful for the scientific community working on fatty acid binding proteins and lipid metabolism. In near future, therapeutic approach for this antibody maybe a possibility in metabolic syndrome.

P-MIS-106
Thioridazine, an anti-psychotic drug, inhibits migration, invasion and epithelial mesenchymal transition in breast cancer cell lines

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Thioridazine (THZ), an antipsychotic drug, exhibits anti-angiogenic effects on breast cancer cell lines. However the mechanistic insight in exerting antiangiogenic effect is not clearly understood. The objective was to investigate the role of THZ in epithelial-mesenchymal transition (EMT) by using cell migration assay, scratch assay, Western blot (WB) and immunocytochemistry.

THZ treatment reduced cell viability on MDA-MB-231, MCF-7 and CD44 + /CD24- cells and IC50 values of THZ were found to be 9 μM, 16.4 μM and 18 μM respectively, at 24 hours. Invasion potency of MCF-7, CD44 + /CD24- and MDA-MB-231 cells were determined as 29%, 24%, 16.5% when compared to relevant treatment controls. Migration potency of MCF-7, CD44 + /CD24- and MDA-MB-231 cells was determined as 28.5%, 63.2%, 61% respectively. Among the three cell lines MDA-MB-231 cells display enhanced invasive and migration ability when compared to other cell lines. Western blotting results demonstrate that THZ significantly increases E-cadherin, Cytokeratin-18, β-Catenin, while inhibiting N-cadherin, Vimentin, Fibronectin. Immunocytochemistry studies revealed decrease in E cadherin and a concomitant increase in Vimentin level for all three cell lines upon treatment with THZ. Moreover THZ significantly inhibited the cell migration, invasion and EMT in MDA-MB-231, MCF-7 and CD44 + /CD24 cell lines by suppressing mesenchymal markers.

In conclusion, these data suggest that THZ might be a novel anti-proliferative and anti-metastatic agent for treatment of breast cancer.

P-MIS-107
Effect of seasonal temperature and humidity on urine density in children

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Environmental heat and humidity are important factors affecting hydration status in childhood. Hereby, we aimed to investigate the effects of seasonal climate changes on urine density of children living in Mediterranean climate, Cyprus. 1700 healthy 0–18 year children's (850 girls, 850 boys) age, sex and urine density results were collected retrospectively for three consecutive years. The correlation of urine density with each seasonal and 12 months' average temperature and humidity has been analysed. The urine density results had a positive correlation with temperature ($r = 0.083$, $p = 0.001$) and a negative correlation with humidity ($r = -0.072$, $p = 0.003$). Mean urine density in spring was higher than that of autumn ($p = 0.02$) and winter ($p = 0.00$). Mean value of summer was higher than autumn ($p = 0.03$) and winter ($p = 0.00$). 0–24 months age group had lower urine density. Evaluation of urine density based on gender and puberty revealed no statistically significant difference. Seasonal Mediterranean climate changes have an impact on urine density in children which may affect hydration status especially in infants < 2 yrs of age. During high temperature seasons ensuring adequate water intake is essential in this age group in Mediterranean climate.

P-MIS-108
Implementation related to the use of antibiotics and data sources by community pharmacists in North Cyprus

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As the resistance to antibiotics is gaining importance in today's world;the solution to this problem is possible through a common consciousness of the doctor who prescribes antibiotics,the pharmacist who sells and the patient who consumes antibiotics. Irrational use of drugs is an economic and medical problem in many developed and developing countries around the world.The aim of this study is to determine the sales ratio of non-prescription antibiotics in pharmacies which is the biggest category of the antibiotic group sold as well as the indications that lead to its' prescription.

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Eighty-four pharmacies out of 168 pharmacies located in North Cyprus were involved in the study with 50%stratified systematic sampling, questionnaires were filled and a consent form was signed by the participating pharmacists.

The pharmacists involved in the study stated that non-prescribed antibiotics were demanded from the pharmacists and all except two (97.6%),responded positively to this demand. It has also been identified in the study that 41.5% of the daily sale of antibiotics in the first half of the year 2014 was non-prescribed. The most purchased antibiotics either with or without prescription was found to be the penicillin and its derivatives with 76.2% and upper respiratory tract with 86.9%. When the level of self-awareness of the pharmacists was examined,

The rate is found in North Cyprus to be (41.5%),compared with the studies conducted in Greece,Italy,Malta and Spain 47% and Egypt 50.4%that designated the non prescribed antibiotics purchased from the public pharmacies.

The rate of sale of non-prescribed antibiotics in North Cyprus has been found to be at a higher level compared to the rates in many developed and developing countries. Furthermore, the upper respiratory tract infections are amongst the most common viral causes which lead to a high consumption of both prescribed and non-prescribed antibiotics.

This study was supported by Turkish Viral Hepatitis Prevention Society.

P-MIS-109
Acrylamide has cytotoxic, antiproliferative and apoptotic effects on human lung adeno carcinoma cell line A549

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Acrylamide (AA), a widespread substance in many fields, forms in foods during high temperature processing such as baking, roasting, frying. AA is a potent neurotoxic, genotoxic and clastogenic agent being a strong electrophile and forming adduct with biological molecules or potent nucleophiles. Up to now, several studies confirmed the toxicity of acrylamide to several organs. On the other hand, AA is reported to have inhibition effects both on proliferation and differentiation of different cancer cells in a time and dose- dependent manner. In addition, natural and synthetic acrylamide derivatives are also used as potent anti-cancer agents. Moreover, inhibition concentration (IC50) values of AA against these cancer cells have not been investigated in detail yet. Thus, the goal of this study is to investigate the cytotoxicity of AA on A549 cells including with ultrastructural and morphological effects.

IC50 value of AA on A549 cells for 24 h was detected with MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) colorimetric assay. We evaluated morphological changes under confocal microscopy and ultrastructural changes under transmission electron microscopy (TEM).

Our results demonstrate that AA inhibits the proliferation of A549 cells in dose-dependent manner and IC50 on A549 cells was found to be 4.6 mM for 24 hours. Confocal microscopy evaluations showed that AA caused nuclear condensations, fragmentations, cytoskeleton lacerations and membrane blebbing. TEM results revealed membrane blebbing, chromatin condensations and cell shrinkage.

Although AA is a probable carcinogen substance, it drastically inhibited cell viability in dose-dependent manner. From microscopic assessments, AA is suggested to induce apoptosis in A549 cells.

In conclusion, the present study confirms the high potential of AA for cytotoxic, antiproliferative and apoptotic activity on A549 cells. However, appropriate AA dose is critical to prevent its possible adverse effects.

P-MIS-110
Effect of hemolysis and lipemia on some immunochemical tests in Beckman Coulter Unicell DXI 800 immunoassay analyzer

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The aim of the study was to investigate the effects of *in vitro* hemolysis and lipemia on 25 immunoassays studied by the Beckman Coulter Unicell DXI 800 immunoassay analyzer.

We prepared a serum pool without hemolysis, lipemia and icterus. Baseline serum pool concentrations of 25 tests were measured by the Beckman Coulter Unicell DXI 800. Dİfferent serum pools, six for hemolysis and five for lipemia, were spiked with increasing concentrations of hemoglobin (0.6, 1.2, 2.4, 4.5, 6.6 and 8.6 g/l hemoglobin) and Intralipid (0.625, 1.25, 2.5, 5 and 10 g/l intralipid). The hemolysate was prepared by osmotic shock method. Intralipid (20%, Baxter, Deerfield, IL) was used to mimic the effect of lipemia. The hemolysis (H), lipemia (L) and icterus (I) indices were measured on Beckman Coulter AU 5800. After spiking the pools, the 25 tests were measured again in duplicate on Beckman-Coulter DXI 800 analyzer. A change of 10% from baseline results was taken as evidence of interference and the interfered tests were also evaluated according to total analytical error based on analytical imprecision and intraindividual biological variation.

We observed a positive interference due to hemolysis for folat, vitamin B12, testosterone and by lipemia for cortisol. There was a negative interference of hemolysis for CA 19.9, CA 125, CA 15.3, insulin, PTH and E2, and of lipemia for progesterone, CA 19.9, vitamin B12 and PTH.

We found clinically significant effect (>total analytical error) of hemolysis on folate and insulin, and lipemia on cortisol.

P-MIS-111
Investigation of the effect of two different p38MAPK inhibitors in rats subjected to isoproterenol-induced acute myocardial injury: an experimental study

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Objective: Acute myocardial infarction is a serious acute condition. In the current study, we aimed to investigate the possible effect of two different mitogen-activated protein kinase (p38MAPK) inhibitors in rats subjected to isoproterenol (ISO)-induced myocardial injury.

Materials and Methods: A total of 32 male Wistar-Albino rats were equally and randomly seperated into four groups as follows: Control, ISO, ISO plus SB203580 andISO plus TAK-715. Treatment agents were orally administered and myocardial injury was induced by subcutaneous injection of ISO. Serum cardiac troponin-I (cTnI), ischemia modified albumin (IMA), heart fatty acid binding protein (HFABP) levels and paraoxonase-1 (PON-1) activity, tissue TOS (total oxidant status), TAS (total antioxidant status), TT (total thiol), tumor necrosis factor-α (TNF-α) levels,

superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activity levels were measured. Tissue mRNA levels of NF-κB, p38 MAPK and nuclear factor erythroid 2-related factor 2 (Nrf2) were analyzed. Heart tissues were also immunohistochemically and histopathologically evaluated.

Results: Both compounds have led to a decrement in myocardial damage, apoptosis, cTnI, IMA, HFABP, TOS, and TNF-α levels, NF-κB, p38 MAPK, phosphorylated c-Jun N-terminal protein kinase (pJNK 1/2) expressions. On the other hand, the applied treatment increased SOD, GSH-Px, TAS and TT levels, as well as phosphorylated extracellular signal-regulated kinase (pERK 1/2) and Nrf2 expressions.

Conclusion: Data established from the current study suggest that administered agents have protective effect against cardiac injury induced by ISO, which was more prominent in rats received SB203580 treatment. p38MAPK inhibitors may constitute a useful choice as cardioprotective agents due to their anti-inflammatory, antioxidant and anti-apoptotic effects.

Keywords: İsoproterenol, myocardial infarction, myocardial ischemia, p38 mitogen-activated protein kinases, SB203580, TAK-715.

P-MIS-112
Comparison of serum adenosine deaminase activity and spirometric values in patients with silicosis

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Silicosis composes the vast majority of occupational lung diseases. Silicosis, caused by inhalation of crystalline silica, is a chronic lung disease characterized by parenchymal nodules and pulmonary fibrosis. The susceptibility of patients with silicosis to infection is thought to be due to toxic effects of silica on pulmonary macrophages. ADA activity is considered as a nonspecific marker of T cell activation and cellular immunity. This study aimed to compare the serum ADA activity in silicosis patients with spirometric values.

In this study there were 35 males in each groups which contained patients with silicosis (group 1), individuals having similar symptoms with silicosis from same occupational area (group 2) and healthy subjects (group 3). Routine hematological and biochemical parameters were also measured. The serum ADA activity and spirometric values (FEV1, FEV1%, FEV1/FVC, FEV1/FVC%, FEF25-75 and FEF25-75%) were compared.

The average age of group1, 2 and 3 are 38.5 ± 10.6, 39.5 ± 2 and 51 ± 10.5 years, respectively. There was a significant difference between group 1 and 3 in terms of the ADA level ($p < 0.05$). There was a negative correlation between ADA activity and FEV1, FEV1%, FEV1/FVC, FEV1/FVC%, FEF25-75 values.

Elevated serum ADA activity has been shown in many diseases with induced cellular immunity. Despite initially toxic effects were lead to a little immunological reaction in patients with silicosis, continuation of this immunological response is important in some chronic manifestations of silicosis. The release of chemotactic factors and inflammatory mediators cause the migration of polymorphonuclear leukocytes, T lymphocytes and macrophages. In this study, the ADA activity was significantly higher in patients with silicosis than others. Increased immunity in patients with silicosis is being considered, increasing ADA activity might be help of earlier recognition of these patients and to take better quality of life.

P-MIS-113
Intracellular proteolysis as a regulator of *Salmo salar* L. parr phenotypic speciation

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Atlantic salmon (*Salmo salar* L.) is an important model system in evolutionary and conservation biology that provides fundamental knowledge into population persistence, adaptive response and the effects of anthropogenic change. The role of behavioral and body size variation in environmental adaptation of Atlantic salmon is well known, by contrast, the underlying biochemical mechanisms are largely unknown. Intracellular proteases, such as cathepsins B and D in lysosomes and calpains and proteasome in cytosol, due to their metabolic and regulatory role may contribute to phenotyping speciation of salmon young. We examined the activity of intracellular proteolytic enzymes in skeletal muscles of Atlantic salmon parr from two local habitats of the Varzuga river (the main channel and small tributaries) differing in hydrological and feeding parameters.

Calpain and proteasome activities were determined by casein or Suc-LLVY-AMC hydrolysis in the skeletal muscles of *S. salar* from Varzuga river (Kola peninsula, Russia).

It is known that salmon parr originated from a common hatch became phenotypically divergent during the settle in the biotopes. Reliable difference in studied enzyme activities in the salmon parr from two local habitats was found; furthermore, calpain and cathepsin B proteolytic activities were found to negatively correlate with parr body size.

Muscle proteolytic activity data support an idea on protease contribution to environmentally-driven adaptation and speciation process in fish. The work was supported by the Russian Scientific Foundation, project no. 14-24-00102.

P-MIS-114
The phylogenetic analyses of anthriscus (Apiacea) species from Turkey based on Non-Coding "trn" regions of chloroplast genome

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Anthriscus Pers. (Apiaceae) species belongs to Apiaceae family and is represented by 16 genus on the world and by 8 genus in Turkey. Anhriscus species are used extensively for treatment various disease such as asthma, alzheimer and show anti-tumoral, anti-microbial, antioxidant features. For determining exact species which treat disease it is necessary sorting species correctly with molecular markers to support morphological features.

Anthriscus species were defined by examining insufficient quantity of samples in Turkey flora. Besides, no detailed study was found in our country after flora study. For this reason a revision study was made with the aim of solving some systematical problems in 2013 by Tekin. The result of the study provided important contribution to the systematic of the species in Turkey. However a molecular study was also required for building the obtained results on a more solid ground.

In this study, the aim to reveal systematic and phylogenetic relationship among species of *Anthriscus* in Turkey, by using

*trn*L-F region in chloroplast genome. DNA was isolated by CTAB method and amplified in PCR by using e-f primaries. The obtained data was evaluated by Mega 7.0 program and phylogenetic tree was prepared by using Maximum Likelihood method.

According to the phylogenetic tree that we prepared by using the sequence line up of *trn*L-F section, it was observed that *A. cerefolium, A. caucalis* and *A. tenerrima* species completed their speciation and an isolation with other species in terms of speciation was provided. It was also observed that *A. kotchi, A. sylvestris* subs. *sylvestris, A. sylvestris* subs. *nemarosa* and *A. lamprocarpa'nın* provided hybridization among themselves but they did not complete their speciation. It was determined that *A.lamprocarpa* var. *chelikhii* which is one of the two different varieties of *A. lamprocarpa* is actually a new sub-species. This fact was supported by molecular data obtained from the study we made after morphologic data.

P-MIS-115
Comparison of immunoassay and liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods in the measurement of serum androstenedione levels

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Introduction: Excessive production of androstenedione can becaused by defects of adrenal steroid biosynthesis, tumors of ovarian and adrenal origin, polycystic ovarian syndrome, increased peripheral sensitivity to androgens, and increased peripheral production of androgens. Most epidemiologic studies use enzyme-linked immunosorbentassay (ELISA) to measure sex steroid hormones because they have acceptable turnaround times and arerelatively inexpensive. Mass spectrometry-based methods are currently the most specific quantitative analytical methods for steroid determination. Mass spectrometry methods are independent of matrix effects or cross-reactivity. In this study, a new liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed.

Materials and Methods: For serum androstenedione measurement, 50 μL of internal standard (d5-11 deoxycortizol) in methanol was added to 250 μL standart or serum and centrifuged at 4.500 rpm for 10 minutes to remove the precipitated proteins. Supernatant was transferred to clean tubes and this procedure was performed twice. The supernatant was collected and dried under a nitrogen gas flow at 60 °C and dissolved in mobile phase. 60 μL was injected in to the ultra performance liquid chromatography analytical column for chromatography.

ELISA study was conducted with DRG (Lot. No. 50K074) brand kit.

Results: Method comporison between LC-MS/MS and ELISA was found slope value 18,412, intercept value −22.87 and r^2 value 0.1033. The regressione quation was ELISA= −2.861782 + 4.905103 LC-MS/MS.

Discussion and Conclusion: Method comparison study presented higher results in ELISA compared to LC-MS/MS. In our opinion, this might due to the interference in ELISA systems. Our LC-MS/MS method allows rapid, sensitive and specific determination of androgens in plasma and serum.The specificity of liquid chromatography–tandem mass spectrometry (LC-MS/MS) offers advantages over immunoassays.

P-MIS-116
Heparin treatment increases thioredoxin interacting protein expression in hepatocellular carcinoma cells

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Heparins play an important role in cell growth, differentiation, migration and invasion. However, the molecular mechanisms of heparin mediated cellular behaviors are not well defined. To determine the effect of heparin on gene expression, we performed a cDNA microarray in a hepatocellular carcinoma cell line and found that heparin regulates transcription of genes involved in glucose metabolism. In this study, we showed a new role of heparin in the regulation of thioredoxin interacting protein, which is a major regulator of glucose metabolism, in hepatocellular carcinoma cell lines. We determined the importance of a unique carbohydrate response element located on its promoter for the heparin-induced activation of thioredoxin-interacting protein and the modulatory role of heparin on nuclear accumulation of carbohydrate response element associated proteins. We showed the importance of heparin mediated histone modifications and down-regulation of Enhancer of zeste 2 polycomb repressive complex 2 expression for heparin mediated overexpression of thioredoxin-interacting protein. When we tested biological significance of these data; we observed that cells overexpressing thioredoxin-interacting protein are less adhesive and proliferative, however they have a higher migration and invasion ability. Interestingly, heparin treatment increased thioredoxin-interacting protein expression in liver of diabetic rats. In conclusion, our results show that heparin activates thioredoxin-interacting protein expression in liver and hepatocellular carcinoma cells and provide the first evidences of regulatory roles of heparin on carbohydrate response element associated factors. This study will contribute future understanding of the effect of heparin on glucose metabolism and glucose independent overexpression of thioredoxin-interacting protein during hepatocarcinogenesis.

P-MIS-117
Prolidase activity in chronic obstructive pulmonary disease and asthma

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Chronic obstructive pulmonary disease (COPD) is a consequence of an underlying chronic inflammatory disorder of the airways that is usually progressive and causes dysregulation in the metabolism of collagen. And Asthma is a disease where there is an accumulation of collagen in the reticular basal membrane of the airway leading to chronic inflammation. Prolidase has an important role in the recycling of proline for collagen synthesis and cell growth. We measured and compared prolidase activity in healthy individuals with COPD and asthma patients to find out that whether its activity might reflect disturbances of collagen metabolism in the patients.

60 patients with COPD, 60 patients with asthma and 20 healthy control subjects with similar age range and sex were included in our study. The patient and control groups do not have any other chronic disease. Serum prolidase activity was

measured in the patient and control groups. Ferritin and alpha-1 antitrypsin concentrations were also compared.

There was no significant difference between serum prolidaz activities of asthma and COPD patients. Serum prolidase activities of both COPD and asthma patients were significantly lower than those of the control subjects ($p < 0.05$). There was no significant difference for ferritine and alpha-1 antityripsin levels between the groups.

The prolidase activity is significantly lower in asthma and COPD patients comparing with control subjects. The collagen metabolism may be undergone to a change in these patients. Hence, there may be an effect on the accumulation of collagen in the reticular basal membrane. The results suggest that collagen turnover are altered by the development of COPD and asthma in human lungs, and prolidase activity may reflect disturbances of collagen metabolism in these pulmonary diseases. Monitoring serum prolidase activity may be useful in evaluating fibrotic processes and in the chronic inflammatory lung diseases in human.

P-MIS-118
Acyclovir molecule in the active site of *E. coli* purine nucleoside phosphorylase (on the basis of x-ray study)

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E. coli purine nucleoside phosphorylase (PNP), which catalyzes the reversible phosphorolysis of purine ribonucleosides, belongs to the family I of hexameric PNPs. Due to key role in the purine sulvage pathway PNPs are attractive targets for drug design against some pathogens. They also used widely in biotechnology for the synthesis of nucleoside analogues as well as for the activation of the prodrugs in anti-cancer gene therapies. The acyclovir (ACV), acyclic derivative of guanosine, is antiviral drug for the treatment of some human viral infections. The crystalline complex of *E. coli* PNP with acyclovir was prepared by co-crystallization using counter diffusion in capillary through the gel layer. The set of X-ray data at 100 K from single crystal grown in space (sp. group $P6_122$) was collected on the Spring-8 synchrotron-radiation facility (Japan) and the structure was solved at 2.32 Å resolution, using the molecular replacement method (pdb ID 5i3c). ACV molecule was located in the nucleoside binding pocket of the enzyme in two conformations. The phosphate binding site was occupied by SO4 ion. The hydrogen bonds network and hydrophobic interactions stabilising ACV molecule in the active site as well as the conformational changes upon ligand binding were described. The comparison of *E. coli* PNP/acyclovir complex and the similar complexes of *Bacillus subtilis* PNP (pdb ID 4da7) and human PNP (pdb ID 1pwy) allowed to establish the peculiarities of ACV binding of in the *E. coli* enzyme.

P-MIS-119
Bacmam delivered camp biosensor assay for quantification of gonadotropins

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Gonadotropins are glycoprotein hormones that regulate normal growth, sexual development, and reproductive function. These are large, up to 40 kDa proteins, which are synthesized and secreted by the gonadotropic cells of the anterior pituitary gland. These hormones may vary in the level of glycosylation depending on the tissue and the metabolism cycles. Follicle-stimulating hormone (FSH) and upon binding to FSH receptor, a G-protein coupled receptor (GPCR), regulates the development, growth, pubertal maturation, and reproductive processes of the body. Human chorionic gonadotropin (hCG) and luteinizing hormone (LH) act via a shared GPCR (LH receptor) and regulate mechanisms essential for ovulation, early pregnancy and placental function in females as well as spermatogenesis and testosterone production in males.

Activation of GPCRs by these hormones can be measured by monitoring formation of cellular cyclic adenosine monophosphate (cAMP). The level on cAMP was measured using a Förster resonance energy transfer (FRET)-based biosensor TEpacVV (H74) kindly provided by Dr, Kees Jalink. The biosensor was expressed using the developed BacMam gene delivery system (recombinant baculoviruses carrying the transgene under a strong mammalian promoter). KGN cells expressing the FSH receptor and COS7 cells expressing the LH receptor served as study objects. Monitoring of specific GPCR activation in living cells, allows detection of only the biologically active agonists, which has real impact in quantification of large hormones. Differences in levels of hormone glycosylation may affect their biological function. Investigation of this phenomena is planned for near future.

Detection of biological activity of gonadotropins is of importance for pharmaceutical industry, where today the concentration of recombinant proteins is mostly estimated using immunological assays only.

P-MIS-120
Development of a colorimetric aptasensor for the detection of peanut allergen protein Ara h 1 in food samples

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Food allergy, especially peanut allergy is a life-threatening problem, and severe reactions against these foods can be observed. Since unintnded consumption of non-labeled foods is the most dangerous risk, any residual allergen protein should be tested and labeled by the manufacturers. An aptamer based colorimetric test is a powerful alternative to commercially available RT-PCR and ELISA test kits. The main objective of this study is to develop an aptamer based colorimetric test fort he detection of major peanut allergen protein Ara h 1.

Ara h 1 aptamer was used to recognize any residual peanut major allergen protein Ara h 1 in food samples. Recombinant Ara h 1 protein was produced and puirifed to be used as a target. Ara h 1 aptamer was used in combination with a blocking sequence, to prevent non-specific binding event, a biotinylated complementary strand to the blocking sequence, and finally Strp-HRP interaction in order to facilitate colorimetric reaction. Optimal blocking sequence length was optimized and introduced to the 3′site of aptamer sequence to construct an aptamer-hairpin structure. Liberation of the blocking sequence allows biotinylated complementary strand to bind to the blocking sequence and consequently Str-HRP conjugate to achieve color development that is proportional to the target concentration.

Since, the aptasensor will be used for the detection of Ara h 1 in food samples, total protein extraction from chocolate samples was also optimized. In order to lower the detection limit of aptasensor, aptamer coupled magnetic bead based pre-enrichment assay was aslo optimized for the total protein extraction. As a

result, a sensitive, fast and reliable aptamer based colorimetric assay was developed for the detection of peanut allergen protein from food samples. Moreover, the assay has the advantages like ease of application and low cost which makes the assay a promising and a powerful alternative to commercially available RT-PCR and ELISA tests.

P-MIS-121
The association between lipid parameters and waist circumference in female university students in Turkey

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A high waist circumference is associated with an increased risk for type 2 diabetes, dyslipidemia, hypertension, and CVD in patients with a BMI in a range between 25 and 34.9 kg/m^2. Monitoring changes in waist circumference may be helpful, in addition to measuring BMI, since it can provide an estimate of increased abdominal fat even in the absence of a change in BMI.

Objective of the study was to find an association between plasma lipid profile and anthropometric parameters (waist circumference percentage of body fat and body mass index (BMI)) in abdominal obesity in Turkish university students. Lipid profile and anthropometric parameters of obesity were studied in a sample of 30 women.

Students with high BMI (>24) had higher values of low-density lipoprotein (LDL), triglycerides (TG) and cholesterol (C) than students with low BMI (<24) but these differences were not significant. High-density lipoprotein (HDL) levels were non-significantly higher in low BMI (<24) student group. Waist circumference, percentage of body fat was higher in high BMI (>24) group than low BMI (<24) group. Waist circumference, percentage of body fat was positively correlated with BMI in both samples (BMI (>24) and BMI (<24)). Students were grouped depend on their waist circumference. Healty individuals who had lower than 80 cm waist circumference had decreased TG levels compared to cardiovascular risk group who had higher waist circumference than 80 cm.

This study shows an association between waist circumference, percentage of body fat, body mass index and lipid parameters in young female university students.

With regard to the relationship, the screening females for central obesity to prevention of cardiovascular disease are recommended.

P-MIS-122
A new biotechnological product from propolis with low allergen: anti-inflammatory effect

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Propolis is extensively used in food industry due to its special medical properies (antioxidant, antimicrobial, antiseptic, antibacterial, anti-inflammatory and antimutagenic effects). Even these positive properties it may cause some allergic reactions in consumers with allergic predispositons. Previously, we demonstrated that biotransformation of propolis by some special strains of *Lactobacillus plantarum* (10, 8014, AATC strains) might decrease the allergenic molecules in propolis. In this study, we aimed to investigate the effect of biotransformation of popolis on it's anti-inflammatory activities.

Before biotransformation, propolis samples were treated with different solutions (10% ethanol and polyethylene glycol – PEG 40%) and different method (ultrasonic treatment 300 W/25 ^{O}C/ 30 minutes) in order to facilitate solvation of solid samples which are very dense and not suitable for fermentation. Fermantations were performed at 30 ^{O}C/48 hours under constant agitation conditions. The anti-inflammatory activity was determined in-vitro conditions using hyaluronidase's analysis and the xanthine oxidase activity.

The highest inhibition (%) of radicals produced by xanthine oxidase was determined in solid samples treated by PEG prior to biotransformation and using of *L.plantarum* 8014 strain during fermentation (88.43%), followed by liquid samples treated by ultrasonic method prior to transformation (86.21%). Concernig the results of hyaluronidase activity (%) inhibitions, the best value were determined in the solid sample treated by PEG prior to biotransformation and using of *L.plantarum* 8014 strain during fermentation (93.93%).

Results indicated that the anti-inflammatory activities of analysed samples are quite high and depending of used extraction methods prior the biotransformation and used specific strain of *L.plantarum* could be optimized in terms of other required parameters.

P-MIS-123
Faceanti-mullerian hormone is not predictive for poor neonatal outcome

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Aim: Anti-Mullerian hormone (AMH) is a growth factor specific to ovaries. It is commonly used to predict ovarian reserve and outcomes of fertility treatments. Recently, low levels of AMH have been shown to be related to hypertensive diseases of the pregnancy and the risk of preterm labor. The aim of this study was to investigate the diagnostic performance of AMH levels of mothers to predict poor neonatal outcome in term pregnancies and the relationship between AMH and birthweights of the newborns.

Materials and Methods: 187 patients, having delivery beyond 37 weeks, and who did not have any other medical problems were included in the study. The patients had normal 50 g. oral glucose tolerance test results. They were divided as 3 groups, based on their newborns' birthweight as "2500 g. and 4000 g.". Level of AMH was determined by ELISA method.

Results: There was not any relation with the AMH of the mothers and the poor neonatal outcome of the newborns, in all 3 groups. Also no siginificant difference was observed in AMH levels of the patients having delivery in early term and late term periods. When the patients of the same group were evaluated; AMH levels were irrelevant to age, gravidy, delivery week, body mass index, the weight gain during pregnancy, and poor neonatal outcome.

Conclusion: AMH is not a predictive factor for poor neonatal outcome and it is not a determinant of the weight of the newborn.

Keywords: Anti-Mullerian hormone, poor neonatal outcome, newborn, birthweight

P-MIS-124
Effects of hemolysis on the assays of serum high sensitivity troponin I, CK-MB (mass) and myoglobin measurements

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Objectives: The aim of the study was to investigate the effects of differing amounts of hemolysis on serum high sensitvity troponin I (hs-TnI), CK-MB mass and myoglobin measurements.
Materials and Methods: We prepared serum pools having troponin I, CK-MB and myoglobulin concentrations at low (5.33 ng/l,1.5 ng/ml and 34.3 ng/ml respectively), normal (39.25 ng/l, 3 ng/ml, 197.9 ng/l respectively) and high (7345 ng/l, 22 ng/ml, 574 g/ml respectively) values. The osmotic shock method was utilized to prepare a hemolysate. Hemolysate was added into serum pools increasing concentrations of hemoglobin (0.65, 1.3, 2.5, 5, 7.26 and 9.42 g/l hemoglobin). Troponin I, CK-MB (mass) and myoglobin concentrations were measured in duplicate by Beckman Coulter Access 2 analyzer. The hemolysis indices were measured on Beckman Coulter AU 5800. A change of 10% from baseline results was taken as evidence of interference and the interfered tests were also evaluated according to total analytical error based on analytical imprecision and intraindividual biological variation.
Results: We found a positive interference due to hemolysis for CK-MB (mass) at low concentrations (1.5 ng/ml), and a negative interference for myoglobin at low concentrations (34.3 ng/ml) and high concentrations (574 ng/ml).
Conclusions: CK-MB increase and myoglobin decrease in hemolyzed samples with hemoglobin ≥9.42 g/l, but the bias might not be clinically significant (< total analytical error) in samples.

P-MIS-125
A retrospective study to determine a reliable marker for selective screening of pompe disease

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Lysosomal Storage Diseases (LSD) are rare inherited metabolic disorders caused as consequence of a deficiency in a specific enzyme required for lysosomal function. Pompe disease is one of these disorders with deficiency of α-1,4 glycosidase enzyme with an incidence of 1:4,500–1:33,000.

As enzyme replacement therapies are available nowadays, early diagnosis is crucial and selective screening is a rational method to reach Pompe patients among people who administer to healthcare with LSD suspected symptoms.

This study aims to examine the relationship between basic biochemistry parameters and α-1,4-glycosidase activities retrospectively, in order to find a key parameter for selective screening of Pompe Disease.

For this reason α-1,4glycosidase, creatine kinase (CK), creatine kinase-MB (CK-MB) activities calcium, phosphate levels of those who had been suspected to be LSD patients and administered to our laboratory for analysis are examined retrospectively.

Out of 134 patients's examined,14 of them were diagnosed with Pompe disease depending on clinical findings & low α-1,4-glycosidase activity. Enzyme activities of Pompe patients were 0.337 nmol/ml/hour as LSD suspected patients'activities had a mean of 2.78 nmol/ml/hour ($p = 0.00$).Comparison of CK activity was compared results showed significant difference between Pompe patients and LSD suspected patients. Even though CK activity levels of the LSD suspected patients were much higher (400vs41-171U/L) than reference interval, the levels of the Pompe disease patients' were still more than twice of the LSD suspected group (956vs400U/L, $p = 0.02$). CK-MB, Ca, P levels didn't show a significant difference. A strong (-) correlation ($p = 0.005$ $r=-0.240$) was observed between α-1,4-glycosidase and CK activities (n:134).

Selective screening is a rational way to diagnose rare diseases. This study's results show that CK activity can be used as a key parameter to determine patients for selective screening of Pompe disease within LSD suspected population.

P-MIS-127
The functional effect of stem cells on the reproductive organs

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Infertitility is considered as a major health problem of recent century. Importance of stem cell is increasing so it is searched new features and supposed to be involved in the infertitility treatment where oxidative stress and apoptosis play importany role. We aimed to investigate the beneficial effect of the stem cells related to free radicals and cell death on testis and ovary. Biopcy model of wound healing was created in the rat testis and ovary with PPD syringe where stem cells were delivered by injection. Rats were divided into four groups including controls, sham, wound healing and wound healing with stem cell. After the creation of the wound, bone marrow-derived mesenchymal stem cells from the tibia of the mature rats and medium were administered to ovaries and testes. Following the applications, ovary and testis samples were investigated for oxidative stress and apoptosis by immunohistochemistry. In comparison with the medium and stem cell applications without a medium support, it was meaningfully determined that healing effect in testicles and ovaries were spotted specifically on the seven day. Tissues were analysed for these staining by H-score and H-score results were determined using One-Way ANOVA test statistically. Our results show the positive effects which clinic applications can bring by displaying the great contribution of the stem cell application in the treatment of testicle and ovary damage. These findings suggest that transplantation of the mesenchymal stem cells may help to promote better enviroment for the reproductive organs by the effect on oxidative stress and apoptosis. The further studies of these results in the molecular level can lead the way to solve the problem of infertility, to increase the percentage of success in the IVF and ICSI techniques and more importantly to perform a differentiation from a somatic cell to a germ cell.

P-MIS-128
The antimicrobial activity of 2 (5H)-furanone derivative on *Staphylococcus aureus*

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Nosocomial infections caused by methicillin-resistant *Staphylococcus aureus* strains are known to be a reason of many infectious diseases like osteomyelitis, endocarditis, sepsis etc. Being

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organized in biofilms these bacteria become extremely resistant to antimicrobials and host immune system leading to difficulties in treatments. Here we report the effect of 2 (5*H*)-furanone derivative possessing sulfonyl group and *l*-menthol moiety (F105) on biofilms formed by *S. aureus* ATCC29213 and MRSA cells. While exhibiting relatively high minimal inhibiting concentration – MIC (16 mg/l), clear synergy with a number of antibiotics was found in the checkerboard assay. Thus, in the presence of 2 mg/l of F105 the MIC of kanamycin was decreased 4-fold, and the MICs of both erythromycin and ampicillin were lowered 2-fold. At the concentration of 80 mg/l F105 also completely inhibited the biofilm formation by *S. aureus*; the cell growth was suppressed by two orders of magnitude as judged by differential fluorescent staining with Syto9 and propidium iodide. The addition of F105 to preformed 24 h-old biofilms increased the fraction of red-stained (dead) cells of both *S. aureus* ATCC29213 and MRSA strains uniformly throughout the whole profile of the biofilm. The quantitative analysis of CLSM microphotographs revealed that F105 at concentration of 80 mg/l led to death of up to 98% of biofilm-embedded cells. This fact suggests that F105 efficiently penetrates into the biofilm matrix and kills the cells without visible damage of biofilm structure. In summary, furanone F105 seems to be a promising compound for drugs design to treat biofilm-embedded *S. aureus*.

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P-MIS-129
C-reactive protein and procalcitonin levels in elderly patients with pneumonia

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Pneumonia is an inflammatory lung disease which can be associated with inadequacy of host defense system and the proliferation of various pathogenic microorganisms into the lower respiratory tract. Community acquired pneumonia (CAP) is one of the leading causes of death in elderly. The incidence of pneumonia in people aged 65 and over is 3–5 times more than young adults. C-reactive protein (CRP) is an acute-phase protein of hepatic origin that increases following interleukin-6 secretion by T cells and macrophages. Procalcitonin (PCT) is a peptide precursor of the hormone calcitonin, the latter being involved with calcium homeostasis. It is composed of 116 amino acids and is produced by parafollicular cells (C cells) of the thyroid and by the neuroendocrine cells of the lung and the intestine. The level of PCT rises in a response to a proinflammatory stimulus, especially of bacterial origin. The aim of this study was to compare CRP and PCT levels in young and elderly patients with pneumonia. Recently diagnosed 43 young and 46 elderly patients with pneumonia and their respective aged matched controls (n = 41, n = 42) were enrolled this study. CRP and PCT levels were by immunoturbidometric and by ELISA methods respectively. CRP and PCT levels for young control and patients and elderly control and patients respectively are 2.32 ± 2.25 mg/l, 0.26 ± 0.13 ng/ml, 20.71 ± 34.61 mg/l, 0.64 ± 1.09 ng/ml, 2.32 ± 2.14 mg/l, 0.27 ± 0.07 ng/ml and 31.41 ± 31.0 mg/l, 0.49 ± 0.83 ng/ml. Young patients with pneumonia have significantly higher CRP and PCT levels than their controls ($p < 0.001$ and $p < 0.028$). Elderly patients with pneumonia have significantly higher CRP levels than their controls ($p < 0.001$). CRP and PCT are important markers in the diagnosis of pneumonia.

P-MIS-130
Effect of serum albumin concentration on total and ionized calcium

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Objective: The aim of the study is to investigate *in vitro* effect of albumin concentration on total and ionized calcium concentrations.

Materials and Methods: A serum pool with low albumin (3.10 g/dL) and normal calcium (9.79 mg/dL) concentrations was prepared from leftover sera. From this serum pool, two parts, each of 10 ml were aliquoted. Purified albumin, 0.3 g, was added to one of these pools and albumin concentration was determined as 6.1 g/dL. The low and high albumin pools were mixed at different ratios and pools with 3.89, 4.78, and 5.37 g/dL albumin concentrations. Total calcium and albumin concentrations of these 5 pools were measured at a Beckman-Coulter AU5800 analyzer and ionized calcium was measured at a Radiometer ABL 800 blood gas analyzer in triplicate. Total and ionized calcium concentrations were evaluated as compared to those of the original pool with an albumin concentration of 3.10 g/dL.

Results: Total calcium concentrations are increased with the increasing albumin concentrations: 1.2%, 1.58%, 2.85%, and 3.59%, respectively. Whereas, ionized calcium concentrations were decreased with increasing albumin: 3.0%, 6.1%, 7.4%, and 8.6%, respectively.

Conclusions: When total allowable error limits based on biological variation were considered, total calcium concentrations are significantly increased at >5 g/dL albumin concentrations. Ionized calcium is significantly affected by 0.8 g/dL and over albumin concentrations. A regression equation based on albumin concentration may be useful for corrected ionized calcium concentrations.

P-MIS-132
Relationship between Lipoprotein (a) and HbA1c in patients with type II diabetes

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Lipoprotein (a) {Lp(a)], is a complex lipoprotein consisting of LDL and apolipoprotein(a). Lp(a) is a risk factor for coronary artery disease and stroke. The relationship between Lp(a) and diabetes mellitus is not clear. In this study, the relationship between Lp(a) and glycemic parameters such as HbA1c and fasting glucose concentration was investigated.

Lp(a), HbA1c, fasting glucose, triglyceride, total cholesterol, LDL- and HDL-cholesterol concentrations were screened retrospectively from July 2013 to July 2016. There were 1831 patients with these test results at the same time. The patients were grouped according to HbA1c values: Group I < 5.7% (n = 468), Group II 5.7–6.4% (n = 739), and Group III >6.5% (n = 624). The relationship between these parameters were statistically within each group and all groups.

There was not a statistically significant difference between the Lp(a) concentrations of Group I and Group II. Lp(a) concentrations of Group I and II were significantly higher than those of Group III.. İn total, Lp (a) was negatively correlated with HbA1c ($r = 0.09$; $p < 0.01$), but there was not a significant

correlation with fasting blood glucose. İn groups, there was a significant and negative correlation between Lp(a) and fasting glucose in only Group I.

The negative correlation between Lp(a) and glycemic parameters is interesting in patients with diabetes. Despite Lp(a) is an independent risk factor for cardiovascular diseases, on the contrary to expectations, Lp(a) concentrations are decreased in diabetes.

P-MIS-133
Effect of blood collection through intravenous lines on hemolysis

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Erroneous results are one of the most important causes of medical errors and may lead to unnecessary investigations or inappropriate interventions. Total testing process consists of preanalytical, analytical and postanalytical phases. Hemolyzed specimens that one of the most common source of preanalytical errors are frequently observed in laboratory practice and associated with incorrect laboratory results. Blood collection through intravenous lines frequently results in hemolysis especially at EDs and ICUs. In this study, we aimed to compare the effect of blood drawing by using BD luer-lock adapters and injector on the hemolysis rates at the ED.

60 patients who has been admitted to the ED were included in this study. All samples were drawn from newly inserted IV lines. The first blood sample was drawn with injector and the second one was drawn with luer-lock adapters to vacuum tubes. After the centrifugation routine chemistry tests and hemolysis indices were analysed on a Beckman Coulter AU680 analyzer for each serum tube. The statistical significance of differences between two tubes was calculated with paired samples t test and statistical significance was accepted as $p < 0.05$.

There were statistically significant differences between the two groups of tubes for the following parameters: LDH, CK, AST, K^+, total bilirubin, protein, albumin, ALP, calcium and hemolysis index ($p < 0.05$).

The use of luer-lock adapters instead of injector could reduce the hemolysis rate. Because of it reduces false results and unnecessary investigations, this approach will be more appropriate and cost-effective in ED.

P-MIS-134
Hemolysis and test rejection: are we following a reliable process?

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Introduction: In laboratories, some blood samples are rejected due to hemolysis. We usually cancel only some of the tests that are affected by hemolysis. However, the frequency of the test cancellation may be relative. Each test is affected in different degrees of hemolysis; some of them are not even affected at all. In this study, we aim to investigate unnecessary cancellations and explain the relationship between hemolysis and test results according to their kit inserts.

Materials and Methods: We measured Hemoglobin levels of 50 hemolyzed serum using Drabkin method (Abbott). Interference studies are conducted using CLSI protocol NCCLS EP7-P is written in kit inserts. Target values (100%) and their change due to different degree of hemolysis have been defined.

Results: Hb concentration ranges of hemolyzed sera were found from 44 to 849 mg/dl. According to kit inserts, Aspartate aminotransferase (AST) test results deviate 5.7% from the target when the degrees of Hb are 62 mg/dl. When the degree of hemoglobin is 125 mg/dl, the test strays about 11.6%. Potassium levels increase (108%) at 125 mg/dl Hb while this increase reaches to 17.9% at 250 mg/dl Hb. Sodium, calcium, CK, crea, total bil, lipase are not significantly affected even at 2000 mg/dl. In lactate dehydrogenase (LDH) tests, test reporting is not allowed at any hemolysis level. ALT increases 11%, at the 1000 mg/dl Hb.

AST and potassium results were excluded from patients' reports even though those samples had low Hb. Some of them were reported despite of excess hemolysis. Some tests are even blocked without ever being studied.

Discussion: Prior to the approval of the lab specialist, technicians decide whether to cancel the tests affected by the hemolysis according to the visible hemolysis based on their personal knowledge.

Conclusion: We should use the hemolysis index, in which standards would be defined via guidelines. This way, all technicians and specialists could know which results are false.

P-MIS-135
X-ray diffraction study of the crystals of HU-protein from *Mycoplasma gallisepticum*

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The DNA-binding HU-proteins are present in all bacteria and belong to the family of nucleoid-associated proteins. These proteins can be considered precursors to eukaryotic histones. Gene knockout of HU-proteins partially inhibits the growth of bacteria, their ability to resist various stressing factors and in some cases leads to their death. Since the spatial structure of HU-proteins is highly conserved it is possible to create inhibitors that will affect them in a broad spectrum of pathogenic bacteria.

In the present work the preparation of the recombinant HU protein from Mycoplasma gallisepticum, crystallization of this protein, and X-ray diffraction study of this protein has been reported. The crystallization conditions for studying protein were found by the hanging-drop vapor-diffusion method. Found conditions have been adapted to the counther-diffusion method in the capillary. The X-ray diffaction dataset from grown crystals have been collected using synchrotron radiation. 3D-structure of the HU protein from Mycoplasma gallisepticum have been determined with 3A resolution. Structural features of the investigated protein are described.

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P-MIS-137
A new, sensitive, low-cost, disposable ITO based electrochemical immunosensor for detection of SOX2, a biomarker of cancer

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A novel sensitive disposable indium tin oxide (ITO)-based electrochemical immunosensor was developed for simple, rapid and sensitive biomonitoring of SOX2. SOX2 is a cancer biomarker and used for detecting of small cell lung cancer, lung adenocarcinoma, squamous cell carcinoma, skin cancer, prostate cancer, and breast cancer.

In this study, indium thin oxide (ITO) thin film was used as working electrode. Carboxyethylsilanetriol was also used for electrode modifying so as to obtain self-assembled monolayers. The formed self-assembled monolayers were activated with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC)/N-hydroxysuccinimide (NHS) chemistry. EDC was used as a heterobifunctional crosslinker. NHS was used in conjunction with the crosslinker EDC. Anti-SOX2 antibody was used as a biorecognition element and it was covalently immobilized onto the ITO electrode modified with carboxyethylsilanetriol. Immobiliztion steps were characterized by cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), and scanning electron microscopy (SEM).

The optimal immobilization conditions for the best sensitivity of the new immunosensor were investigated. Under optimal conditions, this immunosensor demonstrated a wide linear range (0.0025–2 pg/ml) with a detection limit as low as 0.02 ng/ml SOX2. Furthermore, the developed SOX2 immunosensor had good storage stability, repeatability and reproducibility.

In this work, we successfully fabricated disposable ITO thin film based electrodes for sensing the interaction between SOX2 antigen and anti-SOX2 antibody by electrochemical impedance spectroscopy and cyclic voltammetry. And our developed immunosensor has an acceptable performances for the detection of SOX2 antigen, exhibits low detection limit, has selective and reproducible results in immunoreaction analysis. We are thankful for the support from TÜBİTAK (The Scientific and Technological Research Council of Turkey, Project number: 113 Z 678).

P-MIS-138
Applying multiple linear regression model to determine the relationship between anti mullerian hormone with age, luteinizing hormone, follicle stimulating hormone and estradiol: a data mining study

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Introduction: Anti mullerian hormone (AMH) has a widely used in our life because it is a good indicator of reproductive age to estimate the time of menopause. The purpose of this retrospective data mining study is the estimate of ovarian reserve by using AMH and determines relationship between other indicators which are luteinizing hormone (LH), follicle stimulating hormone (FSH), estradiol and age.
Materials and Methods: 25.294 women members were included this retrospective data mining study who were applying to Acıbadem Labmed laboratory. Multiple regression analysis of age related changes of AMH (18–45) and LH, FSH and estradiol were investigated. Beckman Gen II ELISA kit was used for AMH and the technique of electrochemiluminescence and Roche Elecsys Cobas analyzer were used for the measure of other hormones.
Results: AMH shows meaningful correlation between LH, FSH, estradiol and age but also seen there is no correlation between progesterone. After the multiple linear regression analysiz AMH= 11.018- (0.220 × age)−(0.066 × FSH) + (0.044 × LH)−(0.004 × Estradiol) is detected and the model's $R^2 = 0.627$ is also detected.
Conclusion: Nowadays there are lots of methodology were developed the estimate the function of ovary and biological age of ovarian. Age, FSH, LH and estradiol show ovarian reserve by indirectly. This study shows the mathematical relationship between AMH and the other indicators and results are thought to lead to future developments.

P-MIS-139
Antioxidant and anticancer effect of Artemisia absinthium extract on colon and endometrium adenocarcinoma cells

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Plants have always been among the common sources of medicines that have many phytochemicals with various bioactivities, including antioxidant and anticancer activities Artemisia absinthium (AR) has been used as an antipyretic, antiseptic, anthelmintic, tonic, diuretic, and for the treatment of stomachaches in Turkish folk medicine. This study aimed to investigate antioxidant, cytotoxic, genotoxic and apoptotic effect of methanol extracts of AR activities on the human colon (DLD-1) and endometrium (ECC-1) adenocarcinoma cell line. Total phenolic, flavonoid content, and antioxidant activities were determined using suitable methods (ABTS, CUPRAC i.e). Cytotoxic effects of AR on cells were determined by MTT and neutral red uptake assays. Genotoxicity was evaluated by Comet assay and, apoptosis induction were detected by apoptosis ELISA and acridine orange staining methods at the half maximal inhibitory concentrations (IC50) levels. It was determined that extract have shown antioxidant activity in all tests and that they could be considered as a source of natural antioxidants. Cytotoxic effects were concentration-time dependent. Specifically, apoptotic and genotoxic effect increased at 100 and 200 μg/ml concentrations by 48 hours. We found that AR extract had antiproliferative, genotoxic and apoptotic effects on the human cancer cell lines DLD-1 and ECC-1. However, further studies at molecular level are required to support our findings and to elucidate chemopreventive and chemotherapeutic effects of AR on colon and endometrium cancers.
Keywords: Artemisia absinthium, Antioxidant, Anticancer, Apoptosis, Genotoxicity

P-MIS-141
Design and expression of recombinant CE-CA antigen as a marker for colorectal cancer

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Introduction: Colorectal cancer is considered as a major gastrointestinal. This cancer is the second cancer related cause of death after lung cancer in worldwide. We designed a vaccine chimeric including CEA and CA19-9 against colorectal cancer (CE-CA).
Materials and methods: The construct were analyzed by bioinformatics softwares. In this study, the CE-CA gene was optimized using the codon bias of *E.coli* and synthesized by Biomatik Company. Then construct (CE-CA) was cloned into an expression vector and recombinant constructs transferred to *E.-coli* BL21DE bacterium and desired recombinant protein was expressed. Recombinant protein was purified using Ni-NTA affinity chromatography. The content of secondary structures was obtained by circular dichroism (CD) spectrum. Then Recombinant protein was confirmed using western blot analysis and indirect ELISA method.
Results: SDS-PAGE analysis showed that the recombinant protein was highly expressed and purified. Western blot analysis confirmed recombinant protein. Also CD spectrum confirmed predicted structures by bioinformatics tools. The ELISA results showed significantly high affinity toward recombinant CE-CA protein.
Discussion: Based on many studies, CEA as potential immunogenic candidate could be considered in vaccine studies. Also CA19-9 is a cell-surface antigen that has significant increase of expression in colorectal cancer, thus as marker of colorectal cancer. Based in available data, these two antigens, in combination can provide specificity for production of colorectal cancer vaccine.
Conclusion: These findings suggest that CE-CA as potential immunogenic candidate which could be considered in future vaccine studies and detection of colorectal cancer.

P-MIS-142
Flow cytometric cell cycle and apoptosis analyses of some wild animal species

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Cell biobanking; more specifically cryopreservation of biological diversity, is promising as a tool to preserve wild animals as well as domestic ones via nuclear transfer. In this study, we investigated the viability and cell cycle characteristics of 5 wild animal species (fallow deer, red deer, wild sheep, wolf, wild goat). Auricular tissue samples were maintained in PBS+2%PSA. Tissues were seeded on 35 mm petri dishes containing DMEM/High glucose supplemented with 20% (v/v) FCS and incubated 5%CO2 in air at 95% relative humidity and at 37°C. After seeding, the medium was unchanged for 7 days and then it was changed in every 2 days for 25 days at maximum. Once the cells were obtained; flow cytometric cell cycle and apoptosis analyses were done. In terms of apoptosis, all the groups showed high viability rates (over 95%) in culture when compared with the negative control (38%). The cell cycle comparisons were made between serum-starved cells and roscovitine treated cells, both for which untreated cells were used as control, which revealed different results for different species. There was no difference found between serum-starved cells and roscovitine treated cells for red deer and wolf. The serum-starved cells resulted in higher G0/G1 phase for fallow deer and wild goat. On the contrary, roscovitine treated cells resulted in higher G0/G1 phase for wild sheep. As a result; the cells obtained from wild animals had high viability and G0/G1 phase rates. Therefore, they may serve as a donor cell source for nuclear transfer studies.(Grant: TUBITAK KAMAG, Turkey, 109G016).

P-MIS-143
The interaction of different types of antibiotics with endothelial cells in the presence of nanoparticles

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The interaction of nanomaterials with cells and lipid bilayers is critical in many applications such as phototherapy, imaging, and drug/gene delivery. The aim of this study was to investigate the interaction of nanoparticles (Fe_3O_4) or nanoparticles fused with different antibiotics with cell membranes in order to reveal changes in the membrane organization. Endothelial cells were used to determine the effect of different antibiotics (gentamicin, kanamycin, amikacin, penicillin, polymyxin, neomycin, cefotaxime, bacitracin, moxicillin, erythromycin, streptomycin and vancomycin) on the membrane organization. For recording the anisotropy of cell suspensions treated with antibiotics or nanoparticles fused with antibiotics we used 1-4-trimethyl-6-phenyl 1, 3, 5 hexatrien p-toluenesulfonate (TMA-DPH). We decided to use nanoparticles fused with antibiotics because they contain small amounts of antibiotics which makes them less toxic than simple antibiotics,which is very important in patients with genetic diseases such as cystic fibrosis, that should be treated with antibiotics for a long time. Our results showed that at temperatures between 31 and 35°C simple nanoparticles decreased the membrane fluidity. At physiological temperatures (37–39°C) nanoparticles fused with antibiotics (gentamicin, vancomicin, cefotaxim, bacitracin, amoxicillin) increase more the membrane rigidity compare with simple antibiotics or nanoparticles.Erythromycin, polymyxin and penicillin increase the membrane rigidity at 37°C, and at 39°C the same effect was obtained in the presence of nanoparticles fused with these antibiotics,suggesting that the nanoparticles are dependent to temperature for penetrating the membrane. In conclusion the membrane fluidity does not depend on antibiotics types, the modification are present in many antibiotics irrespective of class type.The presence of nanoparticles fused with antibiotics is very important for long term treatment.

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P-MIS-144
Levels of in monoamine oxidase and 8-isoprostane in newly diagnosed stage 1 hypertensive patients

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Objectives: Hypertension is an important cardiovascular risk factor for the development of atrial fibrillation (AF). Increased atrial electromechanical coupling time interval measured by tissue Doppler is accepted as an important factor for prediction of AF development in hypertensive patients. Monoamine oxidases (MAOs), are enzymes which catalyze the oxidation of monoamines. 8-isoprostane is considered as an indicator of oxidative stress. MAO activity and 8-isoprostane levels were measured in some diseases. However, there are no information on 8-isoprostane levels and MAO activity in newly diagnosed patients with stage 1 hypertension has not been observed in a study of literature.
Aim: This is the first study, we aimed to evaluate the levels of MAO and 8-isoprostane in newly diagnosed patients with stage 1 hypertension.
Materials and Methods: The study included 60 newly diagnosed stage 1 hypertensive patients with no other systemic disease. 30 patients were selected as randomized (17 women, 13 men; range of age 46–74 years) and 30 healthy individuals as control (15 women, 15 men; range of age 44–72 years). All the underwent tissue Doppler echocardiographic examination. Blood samples were taken from patients and controls and, the levels of MAO and 8-isoprostane in serum samples were measured by ELISA.
Results: Baseline blood pressures, electrocardiographic and echocardiographic findings, and atrial electromechanical coupling were similar in both groups ($p > 0.05$). Compared to the control group, the activity of MAO and 8-isoprostane levels were found significantly higher in patients ($p < 0.05$).
Conclusion: Increased 8-isoprostane level indicate that there is oxidative stress in newly diagnosed patients with stage 1 hypertension. Also, increased MAO activity may be biochemical biomarkers for the diagnosis of hypertension.
Keywords: Hypertension, monoamine oxidase, 8-isoprostane

P-MIS-147
Determining the indirect reference intervals for complete blood count parameters in Bursa, Turkey

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Reference intervals (RIs) for laboratory test results are defined as the most commonly used diagnostic tool in medicine. Therefore, careful determination of RIs by the laboratory for use is a very important task. Although C28-A3 Guideline recommends the direct RIs (dRIs) calculated from healthy subjects, RIs can be calculated from laboratory data which are called as indirect RIs (iRIs).

The study was carried out at the Central Laboratory for Clinical Chemistry, Teaching and Research (Uludag University, Bursa, Turkey). The results of the laboratory analyses from 142,591 males, 215,658 females, stored for approximately one year, were used for statistical analysis. Data for hospitalized patients and for ambulatory patients from the intensive care unit were eliminated. Furthermore, we used evidence based criteria to enrich the health-related values. A modified Bhattacharya procedure was used to estimate the iRIs from hospital patient data. The nested ANOVA was used to evaluate variations among genders and ages. Cell Dyn analyzer (Abbott Diagnostics, IL, US) was used for the measurements of Complete Blood Count. The obtained iRIs were also compared the dRIs determined in our previous RI study and the RIs suggested by the manufacturer.

We found that the RIs of RBC, Hb and Hct required strong gender partition and calculated the RIs of RBC, Hb and Hct separately. The observed iRIs for WBC, sub-fractions of WBC and PLT in both genders are in good accordance with the dRIs reported in previous study. Age-related changes were noted for RBC, Hb, and Hct. The calculated iRIs for RBC, MCV and RDW are different from the RIs suggested by the manufacturer.

We believe that, using this relatively easy technique, every laboratory can produce its own iRIs, divided, where possible, according to sex and age and according to local conditions. These ranges can be complementary to dRIs obtained for reference individuals according to the IFCC recommendations.

Monday 5 September 12:30–14:30

RNA biology, biogenesis and processing

P-01.02.2-001
Transcriptional pausing induced by alternative sigma subunits of *Escherichia coli* RNA polymerase

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The principal sigma ^{70} subunit, involved in transcription of most house-keeping genes in *Escherichia coli*, was also shown to induce RNAP pausing during transcription elongation, by interacting with promoter-like motifs in the transcribed DNA. Such pauses were proposed to play important roles in the regulation of phage and cellular genes. *E. coli* contains six alternative s subunits but little is known about their ability to induce transcriptional pausing.

We expressed and purified alternative s subunits of the sigma^{70} family and tested their effects on transcription elongation *in vitro* on natural and synthetic DNA templates containing consensus promoter motifs. The structure of the paused complexes was analyzed by DNA footprinting methods. *In vivo* analysis of transcription was performed using reporter genes placed under the control of corresponding promoters.

We demonstrated that the stationary phase sigma^{38} subunit induced efficient RNAP pausing on both synthetic and natural DNA templates containing promoter-like motifs in initially transcribed regions. In contrast, the sigma^{32} and sigma^{28} subunits did not affect RNA elongation. We showed that the sigma^{38}-induced pausing depends on sigma contacts with both nontemplate DNA strand and RNAP core. The pausing results in formation of backtracked transcription elongation complexes which can be reactivated by Gre factors that stimulate RNA cleavage by RNAP.

Our results for the first time reveal transcriptional pausing induced by an alternative s subunit. Analysis of sigma^{38}-dependent promoters shows that a substantial fraction of them contains potential pause-inducing motifs suggesting that such pausing may be a widespread phenomenon. We propose that

sigma[38]-dependent pauses may play important roles in genetic regulation and modulate the binding of transcription repressors or activators to promoter regions.

P-01.02.2-002
The crosstalk between *Streptococcus pneumoniae* RNase R, ribosomes and translation

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Ribonucleases (RNases) are enzymes that ensure maturation, degradation and quality control of RNA thus, contributing to the maintenance of the optimal amount of each transcript in the cells.

Escherichia coli RNB family of enzymes is present in all domains of life and includes RNase R, RNase II and the eukaryotic Rrp44/Dis3, Dis3L1 and Dis3L2 proteins. In *Streptococcus pneumoniae* only RNase R was identified. RNase R, encoded by the *rnr* gene, hydrolyzes RNAs starting from the 3′ end. RNase R level is increased in several stress conditions such as heat shock, stationary phase or cold shock, conditions in which most of the proteins translation is blocked. Moreover, RNase R is the only exoribonuclease able to degrade highly structured RNAs without the help of a helicase which is critical at low temperatures.

Here, we investigated the role of this enzyme by comparing the wild type strain with an *rnr* mutant strain. For that purpose we performed Northern blots analysis of transcripts involved in translation. Also, we investigated RNase R connection to the ribosome and polysome fractions using sucrose gradient polysome separation and Western blots.

In this study, we highlight the importance of *S. pneumoniae* RNase R in translation. We show that this enzyme interacts with ribosomes mostly with the 30S subunit at 37°C. Moreover, in the absence of this enzyme we have observed a decrease in the amount of the 70S ribosomal subunit, concomitantly with the increase of 30S and 50S subunits. RNase R seems also to modulate the amount of the elongation factors EF-Tu and EF-G transcripts. Nevertheless, preliminary results further suggest other roles of RNase R in translation.

P-01.02.2-003
Discovery of a novel gene, involved in the conversion of 2-thiouracil into uracil

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Modified nucleotides are present in many RNA species in all Domains of Life. The biosynthetic pathways of such nucleotides are well studied. However, much less is known about the degradation of RNAs and the salvage of modified nucleotides, their respective nucleosides or heterocyclic bases. Using an *E. coli* uracil auxotrophic strain, we screened the metagenomic libraries for genes, which would allow the conversion of 2-thiouracil to uracil and thereby lead to the growth on a defined synthetic medium. We show that a novel gene encoding previously uncharacterized Domain of Unknown Function (DUF) is responsible for such phenotype. We have purified this recombinant protein and demonstrated that it contains a Fe-S cluster. The substitution of cysteines, which have been predicted to bind such clusters, with alanines abolished the growth phenotype. We conclude that this domain is required for conversion of 2-thiouracil into uracil *in vivo*. This work is supported by the Research Council of Lithuania (LMT, MIP-103/2015).

P-01.02.2-004
Discovery of bacterial genes encoding isocytosine deaminases

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Modified nucleotides are present in almost all classes of RNA. They have great chemical diversity and are critical for RNA folding, stability, interaction with cellular proteins and thereby for various cellular processes such as translation, stress response, and signaling pathways. Biosynthesis of pyrimidine nucleotides and their modified derivatives in RNA is well studied. Nonetheless, not much is known about the cellular degradation of these compounds and the enzymes catalyzing such processes. Using an *E. coli* uracil auxotrophic strain, we screened metagenomic libraries for genes encoding isocytosine deaminases. Three novel genes were obtained, one of which encodes a protein similar to 8-oxoguanine deaminases. The other two encode proteins resembling hydroxydechloroatrazine ethylaminohydrolases. We confirmed that these proteins are functional *in vivo*, allowing growth of *E.coli* on minimal medium with isocytosine. We also demonstrated that such purified recombinant enzymes catalyze the conversion of isocytosine, but not cytosine, into uracil *in vitro*. This work is supported by the Research Council of Lithuania (LMT, SEN-07/2015).

P-01.02.2-005
Extracts from Limnio red-wine grape skin trigger apoptosis in ovarian malignant adenocarcinoma cells through activation of the intrinsic apoptotic pathway

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Natural products display special attributes in the treatment and prevention of various human diseases, including cancer. A significant number of organic compounds from plants exhibit anticancer properties as attested by *in vitro* and *in vivo* studies. Emerging evidence supporting the antineoplastic activity of natural compounds has rendered them promising agents in the fight against cancer. In this study, skin from Limnio grape, a red Greek grape variety that is indigenous to the Greek island of Lemnos, was extracted using mixtures of methanol, water and acetone; the apoptosis-inducing properties of these extracts were studied in the human ovarian malignant adenocarcinoma cell lines TOV-21G and TOV-112D. For this purpose, TOV-21G and TOV-112D cells were treated with Limnio grape skin extracts at a range of concentrations, at 37°C, for 24, 48 and 72 hours.

Untreated cells incubated for the same time intervals served as controls. Cell viability was determined by measuring metabolic activity (colorimetric MTT assay) and observing cell membrane integrity (cell staining with trypan blue). After the determination

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of the optimal concentration of the extract, total RNA was extracted from treated and untreated (control) TOV-21G and TOV-112D cells. After determination of RNA concentration and subsequent first-strand cDNA synthesis, mRNA expression analysis of apoptosis-related genes was performed with RT-PCR using gene-specific primers.

An increasing percentage of non-viable cells was observed by increasing cell exposure time and extract concentration. Distinct modulations of the expression of apoptosis-related genes at the mRNA level were also observed, mainly concerning *BCL2*, *BCLX*, *BAX*, *BAK1* and *BCL2L12*, along apoptosis induction.

In conclusion, the cytotoxic properties of Limnio grape skin extracts against ovarian malignant adenocarcinoma cells merit further investigation. The intrinsic apoptotic pathway seems to be the major mechanism of action induced by these plant extracts.

P-01.02.2-006
3′UTR lengths change in connection with estrogen receptor-alpha in breast cancer

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Almost all eukaryotic mRNAs are polyadenylated by a complex machinery that recognizes the poly (A) signal, cleaves the mRNA and adds the poly (A) tail. 70% of human genes harbor multiple poly (A) signals. Alternative polyadenylation (APA) generates transcript isoforms with different 3′UTR (untranslated region) lengths due to the use of proximal or distal poly (A) signals. Hence, tightly regulated APA has been observed in normal physiological settings as well as in diseases. Considering that 3′UTR shortening cases have been linked to increased protein levels, we hypothesized deregulated APA to be one of the potential cancer related mechanisms.

We investigated the 3′UTR alterations in ER(+) breast cancer patients and cell models compared to normal breast tissue, using gene expression data and a probe-based quantification tool, APADetect. Based on means of proximal to distal probe sets, SLR (short-long ratio) were calculated as an indication APA. Significance Analysis of Microarrays (SAM) determined significant genes. The GSE numbers of the datasets are GSE2034, GSE7390 and GSE9761.

We analyzed two datasets of ER(+) breast cancer patient samples (n = 209, n = 135) compared to normal breast tissue (n = 82) using APADetect and SAM. A total of 184 3′ UTR shortening and 253 3′ UTR lengthening events were detected in breast cancer samples compared to normal breast tissue. Ontology analysis suggested almost all the 3′UTR shortening genes were proliferation related and were indeed reported to be upregulated in breast cancer. To further investigate the connection between APA and ERα status, we used data from a cell line model; wild type or ERα transfected MDA-MB-231 cells that are otherwise of triple negative nature. Our results suggested that most of the genes are 3′UTR shortened or lengthened via direct binding of ERα to DNA.

Our results suggest involvement of APA mechanisms in ERα action mechanisms. Possible link between ERα regulated transcription and APA remains to be elucidated.

P-01.02.2-007
Determination of nucleic acid purity by HPLC analysis

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Contamination of nucleic acids (NA) as a result of NA extraction protocols may result an inaccurate measurement of DNA copy number. Agarose gel electrophoresis and spectrophotometric methods are commonly used to check DNA purity. However, the resolution of these methods may not be good enough for special applications such as determination of DNA copy number and separation of base pairs (bp) that are close in their bp number. In this study, we have developed a new method for separating NA's ranging between 75-20000 bp also detecting the impurities in DNA solution in 1%, 5% and 10% ratios to the DNA of interest. The developed method was validated using the in-house DNA fragments of 100, 150 and 200 bp.

The DNA mixture analyzed using analytical HITACHI Elite LaChrom HPLC using the guard and analytical columns TSKgel DNA-NPR, 2.5 μm, 4.6 mm ID × 0.5 cm and TSKgel DNA-NPR, 2.5 μm, 4.6 mm ID × 7.5 cm, respectively. The validation of the analysis was performed by running each sample five times on three different days. The linearity of the detector response was established by plotting a graph to quantity versus area of 200 bp DNA. The LOD and LOQ were then measured by calculating the minimum level at which analyte can be readily detected and quantified. The ratios calculated with HPLC were compared to the ratios calculated by Quant-it kit. Recovery values were calculated for each measurement and the uncertainty were calculated for each ratio.

The method was found linear for 200 bp in the range of 0.4 ng to 800 ng DNA with the regression coefficient of R^2=0. 9992. LOD and LOQ for the 200 bp DNA was found to be 0.39 ng and 1.56 ng, respectively. The recovery values for the 1%, 5% and 10% impurity ratios were found 101.74. 97.41 and 99.47, respectively.

The purity of the synthetic DNA was determined by HPLC and related uncertainty was calculated. The developed method is a simple alternative to electrophoresis and spectrophotometric methods with higher resolution and separation range.

P-01.02.2-008
Post-transcriptional regulation of cellular response to unfolded proteins: tiRNA involvement

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Physical and chemical factors can disturb the conformation of proteins maturing within the cellular secretory pathway. In response to unfolded proteins the cell activates several stress signaling and adaptive response mechanisms. The aim of our study was to investigate small non-coding RNAs as the potential regulators of cellular response to unfolded proteins (UPR).

For this, we conduct the next generation sequencing of small RNA and transcriptome analysis of mRNA from Jurkat cells exposed to dithiothreitol (DTT), which reduces protein disulfide bounds.

Analysis of miRNAs reveals the differential expression of 104 miRNAs. We observe a decrease in the normalized amount of reads aligned to miRNA loci in stressed cells. Affymetrix analysis with subsequent GSEA reveals downregulation of Reactome miRNA biogenesis pathway (FDR = 0.096).

The length distribution of small RNAs revealed 32 nt-peak corresponding to tRNA-derived fragments, amount of which was increased by 2.6-fold under DTT treatment. The tRNA isotypes that gave rise to almost 76% and 86% of all 32nt RNA fragments in stressed and control cells, respectively, include glycine, glutamic acid, aspartic acid and valine. The vast majority of 32nt fragments produced from these tRNAs are precisely phased 5′ halves with the characteristic cleavage patterns generated by RNase A Angiogenin (ANG). Observed upregulation of tiRNA in stressed cells is accompanied with upregulation of *Ang* mRNA and down-regulation of angiogenin inhibitor 1 (RNH1). We speculate that translational repression, associated with observed tiRNA, is an additional mechanism of reducing global protein synthesis in response to DTT-induced stress.

Collectively, our findings reveal the increase in tiRNA, the differential regulation of miRNA expression together with the global miRNA downregulation as the most prominent small RNome reprogramming events and possible fine-tuned levels of post-transcriptional regulation upon DTT-induced cellular stress response.

P-01.02.2-009
Global gene expression changes after spinal cord injury

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The neuronal regeneration is hardly achieved spontaneously after spinal cord injury (SCI), and the restoration of somatic and autonomic functions after SCI is also challenging in the clinical field. The pathophysiology of SCI is extremely complex and many *in vitro* and *in vivo* studies continued to report opposite results each other in spite of the same treatments, therefore a fundamental analysis such as an extensive assay of global gene expression is required to find a way for spinal cord regeneration. In this study, we aimed to detect the changes of global gene expression after spinal cord contusion in rats according to the time sequence. The spinal cord tissues at contusion site were sequenced after spinal cord contusion in rats using RNA-sequencing technology. For time sequence analysis, five time points was determined; 1 hour, 1 day, 1 week, 1 month and 3 months after spinal cord contusion, and sham operated rats at each time point were used as controls. Quantitative RT-PCR analysis was also performed to validate expression changes of candidate genes in each category. We found that the pattern of changes in gene expression at acute and subacute stages was quite different from that at chronic stage, especially genes associated with with neurotrophin signaling and apoptosis pathways. Most of gene expression levels of inflammatory cell markers were increased and peak during acute stage (1 hour to 1 week) and maintained until chronic stage. Some of regeneration-associated genes (RAGs) including brain derived neurotrophic factor, glial cell derived neurotrophic factor and ciliary neurotrophic factor were increased at 1 hour or 1 day after SCI. We concluded that the information of gene expression level according to the time sequence after SCI might be useful to determine treatment strategies for spinal cord regeneration especially in chronic stage.

P-01.02.2-011
3′UTR length isoform generation profile in a differentiation model

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Alternative polyadenylation (APA) is the regulated selection of a specific poly(A) signal among other proximal and/or distal signals on the 3′ UTRs (untranslated region) for the endolytic cleavage and addition of a poly(A) tail to form the mature mRNA. Consequently, position of the poly(A) site determines the length of the 3′UTR which is known to harbor microRNA and RNA binding protein sites. Such APA isoforms have already been linked to altered protein levels and even functions. Therefore we hypothesized APA to be one of the mechanisms to generate isoform diversity in proliferating and differentiated cells to better understand the molecular basis of cancer.

We used a combinatorial *in silico* and *in vitro* approach to analyze a well known enterocyte differentiation model; Caco-2 cells. Initially we analyzed gene expression datasets for the proliferative and differentiated Caco-2 cells using a probe based APA detection tool. To better understand the significance and to validate these results, we used proliferating and differentiated (Day10) Caco-2 cells and tested sample APA events by RT-qPCR. 3′UTR Isoforms were identified by using 3′RACE PCR.

We identified 43 genes (32% of all APA events) to undergo 3′ UTR shortening in differentiated cells compared to proliferating cells. On the contrary 91 genes (68% of all APA events) went through 3′ UTR lengthening events. Several genes have been validated to follow the pattern that was seen in APA detection tool so far.

To begin understanding the mechanism behind these observations, we are investigating potential inducers of APA during the complex events of differentiation. Our next aim will be to further validate and investigate the consequence of such isoform generation events both in the context of differentiation in colon cancer cells.

P-01.02.2-012
Recognition of phosphorylated threonine-4 of RNA polymerase II C-terminal domain by 3′-end processing apparatus

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RNA polymerase II has evolved an array of heptad repeats with the consensus sequence Y1-S2-P3-T4-S5-P6-S7 at the C-terminal domain (CTD) of its largest subunit, Rpb1. Phosphorylation of serines (S2, S5, and S7) and tyrosine-1 orchestrate the binding of RNA processing and transcription factors in the site of transcription. Several recent studies showed that also threonine-4 site can be phosphorylated which has a number of functional consequences. To reveal the structural basis for the recognition of threonine-4 phosphorylated CTD, we set out to investigate several proteins factors that were implicated with a high levels of threonine-4 CTD phosphomarks using integrative structural biology. One of them, a factor involved in the 3′-end processing and transcription termination, showed a high affinity to the phosphothreonine CTD. Using nuclear magnetic resonance spectroscopy (NMR), we determined its structure bound to the CTD phosphorylated at threonine-4 that reveals a direct read-out of the phosphothreonine. Altogether, our data provides the first insights into the recognition of this poorly understood CTD mark that plays important role in the CTD code of RNA polymerase II. The

The FEBS Journal **283** (Suppl. 1) (2016) 129–417 DOI: 10.1111/febs.13808

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Monday 5 September 12:30–14:30

Proteins in action

P-02.02.2-001
Indomethacin increased telomerase activity in the treatment of glioblastoma *in vitro*

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Introduction: The treatment of brain tumor glioblastoma (GBM) is still one of the greatest challenge. Anti-inflammatory drug Indomethacin (IND) mainly acting through the inhibition of cyclooxygenase (COX) has also anti-cancer activity including brain tumors. The aim was to investigate how IND effects an immortality enzyme telomerases' activity.
Materials and Methods: Monolayer and spheroid cultures of T98G human GBM cell line were used to evaluate the effects of IND (100 μM) on cell proliferation, viability, apoptosis, cell cycle, cAMP levels, the levels of apoptotic and anti-apoptotic proteins, morphology (SEM) and ultrastructure (TEM) for 72 hours. Results were analyzed using the Student's t-test.
Results: IND decreased cell proliferation ($p72 < 0.01$), cell viability ($p72 < 0.000001$), cell rate at S phase ($p72 < 0.000001$) and G2 + M phase ($p72 < 0.001$), cAMP levels ($p72 < 0.01$), the levels of PDGFR- α ($p72 < 0.001$), MRP-1 ($p72 < 0.05$), nf- κB ($p72 < 0.01$) and COX-2 ($p72 < 0.01$) in comparison to control group. IND mildly increased apoptosis ($p72 < 0.001$) and caspase-3 levels ($p72 < 0.01$). Interestingly, IND increased hTERT levels (142%, $p24 < 0.001$; 154%, $p72 < 0.0001$). SEM evaluation showed that IND led to decreased and shortened microvilli, the lost of cell interactions and the conversion of many cell shapes from spindle to oval. Many cell remnants in the intercellular area, intact cell membranes, many dense lipid droplets and few autophagic vacuols in the cytoplasm were observed under TEM.
Discussion and Conclusions: The effect of IND on telomerase activity can only be found in 8 publications at Pubmed research that they only showed its' inhibitory effect in colon, gastric, head and neck cancers. In contrast to previous studies, it was shown for the first time that IND increased telomerase activity in GBM cells and this increase was independent from COX-2 and other tested factors.

P-02.02.2-002
Interaction between fibrinogen and insulin-like growth factor binding protein-1 under physiologic conditions and influence of diabetes mellitus type 2 on this interaction

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Fibrinogen is plasma glycoprotein and principle participant in blood coagulation. It interacts with many proteins, including insulin-like growth factor binding proteins (IGFBPs). One of them, IGFBP-1, is controlled by insulin. Metabolic changes due to diabetes mellitus (DM) affect IGFBP-1. Besides glucose regulation, IGFBP-1 stimulates wound healing. We have investigated complexes formed between fibrinogen and IGFBP-1, their change in DM type 2 (DM2) patients and involvement in fibrin clot.

Samples from adult healthy persons and DM2 patients were studied: plasma, isolated fibrinogen and fibrin. The amount of IGFBP-1/fibrinogen complexes was determined using immunoblotting. Immunoprecipitation and lectin affinity chromatography were used to confirm interaction between fibrinogen and IGFBP-1. *In vitro* incubation of fibrinogen with excess glucose or methylgyoxal (MGO) was employed to demonstrate influence of glyco-oxidation on complexes.

Results have shown that IGFBP-1/fibrinogen complexes can be differentiated from IGFBP-1 oligomers and IGFBP-1/alpha-2-macroglobulin complexes. The amount of IGFBP-1/fibrinogen complexes was lower in patients with DM2. Complexes participated in fibrin clot formation, the amount being significantly lower in patients' samples. The quantity of IGFBP-1 monomer in fibrin clot was greater in patients' samples. *In vitro* experiments revealed that complexes undergo glyco-oxidative modifications leading to their reduced formation, cross-linking and increased acidity (faster electrophoretic movement). Isolated fibrinogen from patients with DM2 was additionally able to bind exogenous IGFBP-1.

Since IGFBP-1 stimulates wound healing, directly and by delivering IGFs, IGFBP-1/fibrinogen complexes may be seen as IGFBP-1 storage instrument, ready to participate in fibrin formation and to assist in damage repair. Reduction of complexes due to glyco-oxidative stress in patients with DM may be part of the mechanism responsible for impaired coagulation process.

P-02.02.2-003
New approach for fine regulation of the endogenous human interferon gamma activity by competitive inhibition with interferon mutant analogues

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Human interferon gamma (hIFNγ) is a proinflammatory cytokine involved in the regulation of nearly all phases of immune and inflammatory responses. Its abnormal expression is associated with the aetiology of many inflammatory and autoimmune diseases. Recently we have been exploring the idea to counteract the over-expression of the endogenous hIFNγ by competitive inhibition with inactive hIFNγ mutants. They are designed to

have preserved affinity to the hIFNγ receptor, but to be deprived in their capability to trigger the intracellular signal transduction.

To this end a library of mutants was created and two potential hIFNγ antagonists were selected for further investigations: a single point mutant K88Q (Q substitution for K in position 88) and a double mutant with additional substitution in the N-terminus. Both mutants and the wild type hIFNγ were expressed in *E. coli* employing the established by us methodology for large scale production of aggregation-prone proteins in soluble native form. The purified mutants were screened for interferon activity (antiproliferative assay), binding affinity (isothermal titration calorimetry) and ability to compete with the wild type for the hIFNγ receptor (competition assay on WISH cells).

The selected mutants demonstrated 100 (single mutant) and 1000 (double mutant) times lower antiproliferative activity than the wild type. Measuring the binding thermodynamic parameters, we proved that the receptor binding affinity of both mutants was preserved, which is an indication for their potential to compete with the wild type hIFNγ for its receptor. Finally, the biological assay performed on WISH cells showed a distinct dose-dependent competition between the wild type hIFNγ and the mutants.

Based on the results presented in this study we conclude that the two hIFNγ mutants are potential candidates for autoimmune therapy based on selective suppression of the endogenous hIFNγ activity.

P-02.02.2-004
MANF may attenuate hypoxia/reperfusion-induced renal cell injury

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Mesencephalic astrocyte-derived neurotrophic factor (MANF) is an ER (endoplasmic reticulum) stress-inducible protein and widely expressed in mammalian tissues. It has been identified as a secretory protein that protects cells against ER stress-induced damage. ER-stress is one of the main mechanisms that play a role in ischemia/reperfusion (I/R)- induced renal injury. Recent studies demonstrated that MANF can protect cardiac myocytes and cortical neurons against I/R-induced injury. Moreover, it has been suggested that it has a restorative effect in ischemic injury. Nevertheless, the function of MANF in I/R-induced renal injury is still not known.

In the present study, we investigated the function of MANF by manipulation its expression level in ischemic acute renal failure model established in proximal tubular kidney cells (HK-2 cells).

For this purpose, the cells were transfected with either MANF siRNA or MANF encoding plasmids for silencing or over-expression of MANF, respectively. Then, the cells were exposed to hypoxia-reperfusion (H/R) induction for indicated times. Evaluations of cell viability were determined with WST-1 reagent. The changes in protein levels of H/R-induced stress markers were analyzed byimmunoblotting.

The results showed that the overexpression of MANF has provided a significant resistance to H/R-induced cell death, whereas silencing of MANF has rendered the cells more susceptible to death. It was also determined that the pretreatment of cells with MANF conditioned medium caused a decrease in cell death.

Additionally, oxidative/nitrosative stress (OS/NS) and ER stress levels were decreased with over-expression of MANF and increased by silencing of MANF in HK-2 cells.

Taken together, our study suggests that MANF may have a protective role against H/R-induced renal cell injury, possibly through the reducing effects on OS/NS and ER stress.

P-02.02.2-005
His-flag tag as a fusion partner in insect expression system – gain or loss?

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Human interferon gamma (hIFNγ) is a glycoprotein playing major role in the regulation of innate and adaptive immunity. Glycosylation is not essential for hIFNγ activity but is important for its stability, half-life and protease resistance in blood. The commonly used hIFNγ in therapy and research is produced in *E. coli* and therefore is not glycosylated. Bearing in mind the above mentioned shortcomings of the non-glycosylated hIFNγ we expressed it in mammalian cells and transgenic mice, however very low yields were achieved. To obtain glycosylated hIFNγ, here we employed a secretory expression of N-terminal HIS-FLAG fusion protein in baculovirus-infected insect High Five® cells. This small hydrophilic tag is designed to not affect the proper folding of the target protein and to facilitate the detection and purification procedures. In parallel the same fusion was expressed in *E. coli* cells. The fusion proteins were purified to high degree of purity by affinity and size-exclusion chromatography. Bioassay carried out on WISH cells showed that the antiproliferative activity of both fusion proteins was 500 times lower than that of the native hIFNγ. This result shows that, in contrast to the generally hold view, the N-terminal HIS-FLAG tag interferes with the biological activity of hIFNγ despite of the protein glycosylation. In order to restore the biological activity we attempted to remove the HIS-FLAG tag enzymatically. Surprisingly, we found that the fusion protein obtained from insect cells was resistant to enterokinase, independently of the enzyme source and experimental conditions, whereas the protein isolated from *E. coli* was susceptible and the tag-free protein showed fully restored biological activity. We are prone to explain the enterokinase resistance of the fusion protein from insect cells with either the specific conformation of the glycosylated protein or with the interaction of the carbohydrate residues with the enzymatic activity of the enterokinase.

P-02.02.2-006
Development of fluorescence assay for high-throughput screening system based on flow cytometry for directed evolution of cellobiose dehydrogenase

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Cellobiose dehydrogenase (CDH) is an enzyme produced by *Phanerochaete Chrysosporium* and it has been already successfully cloned in other organisms. One of the most important roles of CDH is removing products of cellulose degradation. CDH is very important for biofuel and biosensor industry. For improvements of enzyme properties we have used directed evolution. The most important step is to develop screening system that reflects properties of interest. Screening in microtiter plates (MTP) is expensive, time-consuming and has low throughput with a small number of variants detected (10^3–10^4 in months).

The aim of this work was the development of screening system for mutant libraries of CDH expressed on surface of yeast cells based on fluorescent enzymatic assay and flow cytometry. The screening method should be capable of screening cellobiose dehydrogenase variants mutated for higher activity and higher thermostability by error prone PCR. The fluorescent assay was

The FEBS Journal **283** (Suppl. 1) (2016) 129–417 DOI: 10.1111/febs.13808

evaluated in MTP and compared with DCIP assay. For further work the fluorescent assay will be tested using yeast cells with expressed active CDH on yeast surface using fluorescent activated cell sorter detection system.

P-02.02.2-008
Purification, characterization and gene cloning of beta-galactosidase from *Arthrobacter sulfonivorans*

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Beta-galactosidase (EC 3.2.1.23) also known as lactase is the enzyme that typically catalyzes hydrolysis of beta-1,4-D-galactosidic linkages in beta-D-galactosides, including disaccharide lactose, with glucose and galactose as end reaction products. This enzyme is able to catalyze synthesis of oligosaccharides, in particular galactooligosaccharides via galactosyl transfer reaction.

Arthrobacter sulfonivorans beta-galactosidase of unique for prokaryotes extracellular localization may find application in food industry for manufacturing lactose-free dairy products and in pharmacology as bioactive principle of medicines prescribed for patients suffering from lactase deficiency.

The study was aimed at cloning of the gene encoding *A. sulfonivorans* beta-galactosidase, purification and characterization of the enzyme.

A novel extracellular beta-galactosidase from *A. sulfonivorans* was recovered with an overall 207-fold purification, a 7.7% yield and specific activity 16 300 $U \cdot mg^{-1}$ protein. The subunit molecular mass of the enzyme determined by SDS-PAGE analysis equalled 125 kDa. It was found that the enzyme displays pI 5.35, prefers ortho-nitrophenyl-beta-galactoside as substrate (Km 27 mM) and shows maximum activity at 40°C and at pH 7.5–9.5.

The beta-galactosidase gene was isolated from the genomic DNA library of *A. sulfonivorans*, sequenced, cloned and deposited in the GenBank database under accession number KM277894.1. It was established that the gene carries an open reading frame consisting of 3132 bp (1043 amino acids) and encodes beta-galactosidase referred to Glycosyl Hydrolase Family 2 (CAZy database).

P-02.02.2-009
Different splice-forms of TDRD7 protein mutated in cataract's and glaucoma's interacts with S6K1/2

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Ribosomal S6 kinases (S6K) are important players in cellular PI3K/mTOR signalling network, deregulation of which has been associated with methabolic disorders, inflammation and cancer. Previously we had identified a novel binding partner of S6K1 – TDRD7 (Trap). TDRD7 is a scaffold protein detected in complexes involved in the regulation of cytoskeleton dynamics, mRNA transport, protein translation, non-coding piRNAs processing, transposons silensing. It was reported recently that mutations in human TDRD7 result in cataract and glaucoma formation, defined by elevated intraocular pressure (IOP) and optic nerve damage.

The aim of our study was to confirm S6K-TDRD7 interaplay and study its role in cells.

Bioinformatical analysis of TDRD7 sequence revealed the presence of potential phosphorylation sites of S6K2. Using *in vitro* kinase assay, we have demonstrated that recombinant S6K2 phosphorylate 3 from 5 fragments of TDRD7. Formation of S6K2-TDRD7 complexes *in vivo* was further confirmed by co-immunoprecipitation using anti-S6K2 and anti-TDRD7 antibodies generated previously in rat brain lysates. This interaction was further confirmed by confocal microscopy, Oleksandr had shown that TDRD7 co-localize with S6K2 in HEPG2 cells, predominantly in perinuclear region, enreached for one of the TDRD7 isoforms identified previously.

Moreover, we have detected that C-terminal synthetic peptides of S6K2 with methylated Arg interfere with TDRD7 from HEPG2 lysates. The physiological characteristics of S6K2-TDRD7 interaction and the role of this complex formation in neuropathology's development need further investigation.

P-02.02.2-010
Very stable high molecular mass multi-protein complex from human placenta

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Many biological function of placenta are performed not just a set of individual proteins, but also different oligomeric structures and complexes. Herewith, activities of complexes may considerably differ from activities of individual proteins. Therefore, identification and characterization of placental multi-protein complexes is an important step to understanding the placenta function.

The aim of the present work was to investigate a composition and biological functions of the very stable high molecular mass multi-protein complexes (SPC) from placenta of healthy mother.

We isolated SPCs (~1000 kDa) from the soluble fraction of three human placentas. Light scattering measurements and gel filtration showed that the SPC is stable in presence salts, acetonitrile and Triton X-100 in high concentrations, but efficiently dissociates in the presence of 8 M urea and 50 mM EDTA. Such a stable complex is unlikely to be a random associate of different proteins. It was shown the SPC includes a number of proteins with molecular weights of 2 to 180 kDa. Several protein components of the SPC were identified, including serum albumin, transferrin, IgGs, annexin A5 and other proteins. Serum albumin, transferrin and protein with molecular weight 14,1 kDa are the main proteins of the complex. It was shown high the SPCs from three placentas possesses DNSase and catalase activities. An addition, investigation of cytotoxic effect on human cancerous cell lines has shown that the SPCs reveal high cytotoxicity.

P-02.02.2-011
Antibody-cytochrome b5 fusion protein, characterization and applications for antibody development process

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Antibodies have recently become an essential tool being a part of immunodiagnostics, therapeutics and as a valuable instrument in life science research. An enormous number of options utilizing a various tags were used to create a universal antigen-binding domain, which can be easily detectable, highly soluble and might be produced in high yields with low costs, but no multipurpose solution exists yet.

We addressed the question whether a single tag could be found for enhancing solubility of recombinant Fab antibody fragment and providing its detection and accurate quantification by rather simple method.

A new application for hemeprotein – cytochrome b_5 as the antibodies fusion partner were proposed. We have constructed of recombinant Fab antibody fragment cytochrome b_5 fusion protein. We have shown that cytochrome b_5 enhance expression of Fab antibodies fragments in bacterial system, and could be a versatile tool for recombinant proteins folding, redox (oxidation) state studies and for their precise concentration determination in the turbid solutions.

Fusion Fab-b5 protein has a stable red color and characteristic absorbance spectrum with the maximum absorbance at 413 nm in oxidized environment. Cytochrome b_5 change its spectrum maximum depending on environmental redox potential and its folded state, so one can track these events in real time spectrophotometrically.

Binding activities of Fab-b_5 fusion protein and hybridoma secreted immunoglobulin were measured by biolayer interferometry and ELISA. No significant difference between them was revealed.

Due to this feature we can distinguish the chimeric protein of interest in complex mixtures and control the process of recombinant proteins expression and purification in real-time. Besides, cytochrome b_5 fusion tags multiples recombinant antibody yield (from 2 to 3 times) and doesn't affect antigen-binding properties.

P-02.02.2-012
The Bβ125-135 site of fibrin molecule is the site of fibrin protofibrils lateral association

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Previously we showed that fibrin-specific monoclonal antibody I-3c (monAb I-3c) inhibited the fibrin protofibrils lateral association. We suggested that the epitope of monAb I-3c in Bβ118-134 of coiled-coil region of fibrin molecule coincides with the site involved in fibrin protofibrils lateral association. The aim of this study was to localize the site of protofibrils lateral association in fibrin molecule using the synthetic peptides Bβ121-138, Bβ125-135 and both their scrambled version, and Bβ109-126 peptide.

MonAb I-3c was isolated from hybridoma culture medium by affinity chromatography on fibrin-sepharose. Turbidity analysis was used to study the effect of synthetic peptides on fibrin polymerization. The interaction between peptides and monAb I-3c was investigated by SPR method using Plasmon-6 device.

We investigated the effect of synthetic peptides which corresponded to amino acide sequences of fibrin molecule Bβ109-126, Bβ121-138, Bβ125-135, and the scrambled versions of Bβ121-138 and Bβ125-135 peptides on a binding to monAb I-3c and on the fibrin polymerization process. In SPR analysis was showed that Bβ121-138 and Bβ125-135 peptides, but not their scrambled version, binds to monAb I-3c, immobilized to a chip. Turbidity data showed that only Bβ121-138 and Bβ125-135 peptides caused the 2-fold decrease of the rate of the lateral association of protofibryls at the concentration 2.2×10^{-4} M and 2.7×10^{-4} M, respectively. Both of them decreased the final clot turbidity.

Our data let us to suggest that the Bβ125-135 site is the site that involved in protofibryls lateral association.

P-02.02.2-013
İrisin immunoreactivity in hematological malignancies

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It has been recently shown that irisin immunoreactivity was altered in gastrointestinal cancers. As known hematological malignancies was one of the most common malignancies through world, but no study was present how irisin was changed in this type of cancers. Therefore, purpose of this was to investigate how immunoreactivity to irisin was altered in hematological malignancies (blood cancers). We used an antibody from Phoenix to demonstrate how a 12 kDa band after deglycosylation of irisin altered in hematological malignancies. Here we first time showed that irisin tissue immunoreactivity from acute lymphoblastic leukemia (ALL) and acute myelogenous leukemia (AML) patients was increased when compared with unaffected biological tissue parts. From the immune-histochemical (IHC) investigations it is concluded that hematological tissue and blood cells may be another source of irisin and increased with cancer, thus this finding might help to enlighten pathophysiology of hematological malignancies.

P-02.02.2-014
The value of urine neutrophil gelatinase-associated lipocalin (NGAL) in acute heart failure

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Introduction: Renal dysfunction is very common in heart failure (HF) and neutrophil gelatinase-associated lipocalin (NGAL) is used as an early marker of acute renal tubular injury. Recent studies have been reporting that NGAL is inhibitor of inactivation of matrix metalloproteinases (MMP-9) which results in enhanced proteolytic activity with prolonged effects on collagen degradation. Due to its relation to extracellular matrix degradation in myocardium and infammation, we hypothesized possible increased NGAL expression in HF besides it renal dysfunction etiology.
Patients and methods: In study were included 30 patients hospitalised with signs and symtoms of AHF. Urine samples for NGAL analysis were collected at admission and analysed by the chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of neutrophil gelatinase-associated lipocalin in human urine (Abbott, ARCHITECT Analyzer). Refferent range for urine NGAL is 0–135 ng/ml. On admission blood samples for BNP (brain natriuretic peptide) analysis were drawn and tested by ARCHITECT BNP chemiluminescent microparticle immunoassay (CMIA), Abbott Laboratory.
Results: The mean age of the patients (male= 30, female= 30) was 68.86 years (SD 10.92 years). Among them 18 (30%) patients was diagnosed as a HF-PEF (HF with preserved ejection fraction) while 42 (70%) as a HF- REF (HF with reduced ejection fraction). Mean BNP values was 1616.71 pg/ml (SD 1511.15 pg/ml) and mean LVEF was 33.66% (SD 14.19%). Mean urine NGAL was 60.91 ng/ml (SD 78.72 ng/ml). We found significantly positive, but weak correlation among NGAL and

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BNP only by Pearson correlation test ($r = 0.139$, $p = 0.464$, Wilcoxon signed rank test $Z = -4.782$ $p < 0.5$).

Conclusion: BNP levels are elevated in HF with reduced and preserved ejection fraction. Urine NGAL is not elevated in acute heart failure, but it is slightly positively correlated with serum BNP values.

P-02.02.2-015
A proteomic study of memory after imprinting in the domestic chick

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Converging evidence implicates the intermediate and medial mesopallium (IMM) of the domestic chick forebrain in memory for a visual imprinting stimulus. A number of learning-related changes have been found in plasma membrane and mitochondrial proteins of IMM. For broader analysis of these changes we employed Two-dimensional gel electrophoresis/mass spectrometry approach and identified differentially expressed proteins in membrane-mitochondrial fraction of the IMM across chicks with different estimated levels of imprinting 24 h after training. We further inquired whether the amounts of those proteins in the IMM and a control region (posterior pole of the nidopallium, PPN) are correlated with memory for the imprinting stimulus.

Learning-related increase in the amounts of the following proteins was demonstrated in the left IMM, but not in the right IMM or left and right PPN: (i) membrane cognin;(ii) a protein resembling the P32 subunit of splicing factor SF2;(iii) voltage dependent anionic channel-1;(iv) dynamin-1; (v) heterogeneous nuclear ribonucleoprotein A2/B1. Obtained results indicate that the molecular processes involved in learning and memory of imprinting cover a wide range of cellular activities, including stabilization of protein structures, increased mRNA trafficking, synaptic vesicle recycling and specific changes in the mitochondrial proteome.

P-02.02.2-016
Substrate specificity of *E. coli* uridine phosphorylase: evidences of high-syn conformation of the substrate

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Nucleoside phosphorylases are involved in salvage pathways of nucleoside biosynthesis and catalyze the reversible phosphorolysis of nucleoside to a free heterocyclic base with the formation of α-D-ribose-1-phosphate. Thus, uridine phosphorylase (UP; EC 2.4.2.3) catalyzes the phosphorolysis of uridine. Also, this class of enzymes includes thymidine phosphorylase (EC 2.4.2.4), UP, and purine nucleoside phosphorylase (EC 2.4.2.1).

The aim of this work is to study the substrate and inhibitory properties of uridine derivatives in the reactions catalyzed by *E.coli* UP in order to shed some light on the substrate's conformation in the productive complex with the enzyme.

We studied the *E.coli* UP-catalyzed phosphorolysis of uridine and its derivatives modified in the heterocyclic base and the sugar moiety. The kinetic constants (*Km*, *Ki*, *kcat*) of the phosphorolysis reaction of near 30 uridine derivatives were determined.

The combined kinetic (NNNA, 35, 2016) and structural data (Acta Crystallogr., D70, 3310, 2014) provide clear evidence that UP binds uridine in the most energetically unfavorable conformation, which, to the best of our knowledge, has no precedents in the enzymes of nucleic acid metabolism. This is possible due to multiple interactions between the substrate and the protein environment (active site residues) mainly through hydrogen bonds. These results are important for understanding the mechanism of action of this class of enzymes.

An analysis of the conformations of nucleosides in solution and rotational barriers suggests that the energy difference between the ground state of uridine and uridine complexed with UP may be high as 63–69 kJ/mol. The binding in a high-energy conformation results in the weakening of the glycosidic bond. The observed conformation of uridine complexed with sulfate (mimetic of phosphate) may be very similar to its conformation in the transient state. The research was supported by the Russian Science Foundation, grant No 16-14-00178.

P-02.02.2-017
FOXP1 (forkhead box P1) enhances tumor cell migration by repressing of NFAT (nuclear factor of activated T cells) transcriptional activity

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Until now, FOXP1 (Forkhead Box P1) has been identified as a tumor suppressor in several correlation studies in breast cancer. Although, FOXP1 is defined as a transcriptional repressor that interacts with other transcription factors in various mechanistic studies, there is no study that explains its repressor functions in breast cancer biology. Here we demonstrate the repressor function of FOXP1 on NFAT (Nuclear Factor of Activated T Cells) and the migratory effect of this repression in MDA MB 231 breast cancer cells. We performed co-immunoprecipitation experiments for the investigation of protein-protein interaction between two transcription factors. Protein-protein interaction on DNA was investigated with EMSA and transcriptional effects of FOXP1 on NFAT, lusiferase reporter assay was performed. Wound healing assay was used to analyse the effects of overexpression of FOXP1 on tumor cell migration. Our results showed that FOXP1 has protein-protein interaction with NFAT on DNA and enhances breast cancer cell migration by repressing NFAT transcriptional activity and FOXP1 shows oncogenic function by regulating breast cancer cell motility.

P-02.02.2-018
Inhibition of phosphodiestrase 5 and Increasing level cGMP by hydroalcoholic Achillea: wilhelmsii extract in human breast cancer cell lines, MCF-7 and MDA-MB-468

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Introduction: Phosphodiesterase 5 (PDE5) is one of Phosphodiesterase lead to hydrolyzing cGMP.The cGMP signaling pathway has an important role in proliferation of cells. Previous studies showed PDE5 was increased in cell lines cancers thus PDE5 inhibitors can used as efficacious therapeutic option for treatment of cancers. The current study was to investigation the effect of hydroalcoholic Achillea.Wilhelmsii extract (HAWE) on the

PDE5 gene expression and cGMP signaling in the MCF-7 ER^+ and MDA-MB-468 ER^-.

Methods and Materials: The ED50 of the HAWE on both cell lines were examined by using MTT viability test then the expression of PDE5 and cGMP concentration were measured in time-dependent manner (in the ED50) by real-time RT-PCR and colorimetric assay respectively.

Results: Treatment with the HAWE showed, 25 μg/ml is ED50 for both cell lines and the HAWE lead to reduction in PDE5 mRNA expression and evaluation of intracellular cGMP showed an increase pattern in the time-dependent manner.

Conclusion: Our results showed that the HAWE has anti-proliferative property in the MCF-7 and MDA-MB-468, cell lines of breast cancer through the cGMP pathway, these data suggested that the HAWE can be potential source for the isolation of effective anti-proliferative molecules.

Keywords: Achillea.Wilhelmsii, Breast cancer, Anti-proliferative, Phosphodiesterase, cGMP signaling pathway.

P-02.02.2-019
pH-dependent conformational changes and thermal stability of outer membrane protein G mutants by Fourier transform infrared spectroscopy

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Outer membrane protein G (OmpG) is a stable monomeric porin having 14-stranded beta barrel form from *E.coli*. Its exact function is not fully understood; however, it allows the passage of molecules up to 900 Da in neutral pH but the pore is closed by going through a conformational change under pH 5.5. As being monomeric and having pH-dependent gating characters, it is suitable for biosensor and targeted drug delivery applications.

An attempt on OmpG is to create a larger pore while its stability is undisturbed. OmpG-16S is obtained by adding 38 amino acids to the primary chain in order to have a 16-stranded beta barrel porin. OmpG-16SL is formed by further adding 6 amino acids to loop L6 and by replacing 7 lysines with arginines. OmpG-16S and OmpG-16SL mutants are investigated by Fourier transform infrared spectroscopy (FTIR) and compared with OmpG-Wild Type (WT) in terms of pH-dependent conformational changes and thermal stability. Each mutant is prepared in Na-phosphate buffer pD 5.5/7.5 and infrared spectra are recorded. Further, temperature profiling are recorded for the range between 22 to 106 °C.

Results show that both mutants are responsive to pH changes. While turning the pH from acidic to neutral, beta sheet signals shift to lower wavenumbers showing difference in secondary structure, implying the existence of closed and open states. On the other hand, mutant proteins show structural differences compared with the WT protein. Porins are known for their remarkable thermal stability. The mutans retain this character by having transition temperature of ~75 °C, although this is less than the WT transition at ~85 °C.

In conclusion, two mutants show signs of open and closed states as OmpG-WT and even if the mutants are less stable than OmpG-WT. This study shows that the attempted alterations in OmpG structure are successful in terms of pH-response but it needs improvement in terms of stability when necessary.

P-02.02.2-020
A potential role of the Nudix pyrophosphatase NUDT13 in the regulation of the mitochondrial NAD contents

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NAD is a key factor in the regulation of mitochondrial metabolism. Besides its vital role as redox carrier, NAD serves as substrate for protein ADP-ribosylation and deacetylation, modifications which modulate enzyme activities in mitochondria. These functions depend on how NAD levels are maintained in this organelle. In human cells, mitochondrial NAD is segregated from the cytosolic pool and can be synthesized from NMN, which is probably imported into the matrix. Here, we tested whether the Nudix pyrophosphatase NUDT13 participates in the regulation of the mitochondrial NAD pool. This enzyme has a predicted NADH pyrophosphatase zinc ribbon domain and *a mitochondrial targeting* sequence at its N-terminus. However, it has not yet been functionally characterized.

We overexpressed NUDT13 endowed with a C-terminal FLAG-epitope in human cells. To evaluate changes in the mitochondrial NAD concentration, we used a reporter system which includes the overexpression of the catalytic domain of poly(ADP-ribose) polymerase 1 (PARP1) within the organelles (mitoPARP). Thereby mitochondrial NAD is converted into protein-bound poly(ADP-ribose) (PAR). The extent of PAR formation correlates with the mitochondrial NAD availability and is detected by Western blotting.

Our results established that NUDT13 is indeed a mitochondrial protein, as it was localized exclusively to these organelles. Moreover, when NUDT13 was overexpressed along with the mitoPARP detector system, a dramatic decrease of PAR was observed.

The obtained results indicate that NUDT13 is enzymatically active upon overexpression in the mitochondrial matrix and that it might cleave NAD, thereby modulating its organellar level. However, at this point we cannot exclude the possibility of direct PAR cleavage by NUDT13.

Further characterization of NUDT13 will define its substrate specificity and clarify its role in mitochondrial metabolism.

P-02.02.2-022
Effectiveness of anti- inflammatory agents in azoxymethane induced experimental colon cancer

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The incidence of increase in colorectal cancer (CRC) worldwide has become a major health problem. Early diagnosis and treatment of CRCs are of importance for improving survival. In the present study, it was aimed to investigate chemopreventive effect of rosmarinic acid and evaluate the angiogenesis process in azoxymethane (AOM)-induced CRC model.

Male Sprague-Dawley rats were randomly divided into a control group, AOM-induced rat colorectal cancer group (15 mg/kg body weight AOM; ip, weekly for four weeks), and rosmarinic acid (5 mg/kg body weight; oral, daily for four weeks)-treated

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group. In addition to the standart diet of the all groups 15.8% peanut oil was added throughout the experiment. The all rats were sacrificed at the end of 30 weeks. Biochemical examinations were performed in rat plasma.

Histopathological adenocarcinoma rates were observed in 87.5% of AOM group. The incidence of adenocarcinoma was showed a reduction in the treatment group. Significant increases in plasma TOS and MCP-1 levels were found in the AOM group compared to controls. These increases were reduced in the treatment groups but no significant. A significant increase was detected in TAS levels in the treatment group when compared to the AOM group. Significant decreases in plasma adiponectin levels were found in the AOM and the treatment groups compared to controls.

In conclusion, treatment with rosmarinic acid reduced the occurrence of inflammation and was helped to maintain the oxidant-antioxidant balance in the model of AOM-induced rat colon cancer.

Keywords: Colorectal cancer, rosmarinic acid, TAS, TOS, adiponectin

P-02.02.2-023
Aim23p is a multifunctional regulator of yeast mitochondrial translation

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Mitochondrial genome, while being strongly reduced in course of evolution, still codes for several proteins. The vast majority of them are components of the respiratory chain complexes. To produce these proteins, the system of mitochondrial translation is presented in the organelles, which is in common close to that in bacteria.

Translation initiation in bacterial cells is orchestrated by three protein factors called IF1, IF2 and IF3. The orthologs of the two latter proteins are commonly found in mitochondria. However, mitochondrial IF3 could not been identified in several groups of organisms, including *S.cerevisiae*, for a long time. Recently we have shown that baker's yeast protein Aim23p possesses a function of mtIF3. However, the mitochondrial translation has not been stopped in the yeast strain without Aim23p which is surprising taking into account the fact that IF3 is obligatory for the translation in bacterial systems. Instead of blocking of mitochondrial protein synthesis in absence of Aim23p, we observed the translational imbalance: the synthesis rate of the complex V subunits was increased while the synthesis rate of the complex IV subunits was repressed. Thus, in addition to its general role in translation initiation, Aim23p might specifically affect the biosynthesis of individual mitochondrial-encoded protein species. Our genetic experiments have revealed that, indeed, Aim23p is almost indispensable for Cox1p synthesis, and that it affects the translation of COX1 mRNA through its 5′-UTR, like classical mitochondrial translational activators. This is in accordance with our measurements of complex IV activity which is several times less in yeast lacking AIM23 gene than in the wild-type. Taken together, our results point on the multiple role of Aim23p in mitochondrial translation: in addition to its function as mitochondrial IF3, it specifically regulates the amount of complex IV subunits and its activity.

P-02.02.2-024
The circulating betatrophin and irisin levels in polycystic ovary syndrome patients with and without insulin resistance

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Introduction: Polycystic ovary syndrome (PCOS) is the most common endocrine/metabolic disease in women around the world, characterized by oligo- or anovulation, polycystic ovary, and/or hyper-androgenism. Insulin resistance (IR) and obesity are common findings in patients with PCOS. Irisin is a recently identified myokine secreted from skeletal muscle in response to physical activity. Irisin has been postulated to induce the differentiation of white fat tissue into brown fat tissue. Betatrophin is a currently discovered new hormone proposed to stimulate β-cell proliferation. In this study we investigated the levels of irisin and betatrophin in PCOS patients.

Materials and Methods: Our study group was consisted of 40 patients with PCOS and 20 healthy volunteers. Patients group was divided into two subgroups according to presence of IR. (PCOS+IR and PCOS-IR). The oral glucose tolerance test (OGTT) and the homeostatic model assessment (HOMA-IR) were performed to assess glucose tolerance and insulin sensitivity. Irisin and betatrophin levels were measured by ELISA method.

Results: Circulating irisin was significantly higher in the PCOS+IR subgroup than the control group ($p < 0.022$). Circulating betatrophin was significantly lower in both patients subgroups than the control group ($p < 0.008$). There was no negative or positive correlation between irisin and betatrophin levels.

Discussion: These data suggest that irisin and betatrophin may act a role together in the IR mechanism in PCOS patients.

Conclusion: Randomized controlled trials including larger sample group are needed to reveal the role of betatrophin and irisin in PCOS development.

P-02.02.2-025
New perspective on human butyrylcholinesterase activity: Lipid hydrolysis

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Butyrylcholinesterase (BChE) synthesized in liver has long been associated with hyperlipidemia, type 2 diabetes and obesity. There are also reports on BChE knockout mice becoming obese. The exact involvement of how BChE interacts with lipids is still not clear. Previously we displayed a correlation between leptin, waist circumference, fat mass and BChE levels. Recently, we have also shown that BChE overexpression in HepG2 cells is regulated by alpha linoleic acid. As the next approach on the analysis of lipid metabolism and BChE interaction, we considered the capability of BChE to hydrolyze lipids.

Human serum BChE was purified by subsequent DEAE-Tris-Acryl M and procainamide chromatography. The purified BChE was utilized in a modified acid lipase assay with the acid lipase substrate 4-Methylumbelliferyl palmitate (4-mU-palmitate). As the second alternative substrate trioleic acid was utilized. The triolein hydrolysis was measured by the NEFA kit. Verification that BChE hydrolysis of these lipid substrates was not due to another esterase was done by iso-OMPA inhibition studies. Also, lectin

binding studies with BChE and RCA were carried out to rule out non-specific esterase activity.

Using purified human serum BChE and hepatic lipase as control enzyme we found that BChE is able to hydrolyze the acid lipase substrate 4-Methylumbelliferyl palmitate (4-mU-palmitate). We found that BChE hydrolyses this molecule at pH 8 rather better than at pH 7.4. At pH values, purified human BChE has a Km value that was 10 times bigger than that of human pancreatic lipase. With the bigger molecule the triolein, the difference between the Km values of BChE and pancreatic lipase was smaller. BChE seems to hydrolyze triolein with an efficacy comparable to approximately 25% that of human pancreatic lipase.

Our results display that another function of BChE may be its lipid hydrolyzing activity.

P-02.02.2-026
Determination of regional reference ranges for erythropoietin with laboratory data mining

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Serum erythropoietin (epo) levels are the main regulator factor of erythrocyte production and increase in response to hypoxia. Our region is a location dominated by hypoxic conditions due to the high attitude. In this study we aimed to investigate the mean serum epo levels in the living conditions of our region.

Two hundred and eighty epo results from our laboratory data whose hemoglobin levels were normal were evaluated in the study. Mean serum epo levels were analyzed via chemiluminescence method in Beckman Coulter DXI 800 auto analyzer.

The epo levels of samples was 12.26 ± 9.32 mUL/ml (ranged between 3.00 and 48.57) mUL/ml. When we performed ± 1 SD for the studies population we determined normal serum epo levels were as 2.94–21.58 mUL/ml. The upper limit determined by our results was 17% higher than that of determined by the manufacturer as 18.5 mUL/ml and the lower limit determined by our results was 17% higher than that of determined by the manufacturer as 2.59 mUL/ml.

Normal serum epo levels were considerable for our region and the upper and lower limits were higher than those of determined by the manufacturer. More detailed studies considering the physical properties of participants including a higher number participants are necessary.

P-02.02.2-027
Assessment of serum apelin level in patients with subclinical hypothyroidism

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Subclinical hypothyroidism is the precursor to hypothyroidism because it has a tendency to transform into hypothyroidism. Subclinical hypothyroidism is considered one of the risk factors causing metabolic syndrome. Metabolic syndrome can be characterized by plasma level of apelin released from adipocytes. In the present study, we aimed to measure serum apelin level of patients with subclinical hypothyroidism and compare them with serum apelin level from healthy individuals.

Our study group included 31 patients diagnosed with subclinical hypothyroidism and 23 healthy volunteers. Serum samples were obtained from each participant for the measurement of apelin. These were then stored at −20∘C until the time of analysis. Serum apelin concentrations were determined using an enzyme-linked immunosorbent assay.

The mean serum apelin levels of subclinical hypothyroidism and control groups were 79 ng/l, control group 60 ng/l respectively. There was no statistically significant difference in terms of the mean apelin levels between the groups ($p > 0.05$). Apelin levels didn't show significant correlation with BMI ($p > 0.05$).

In the present study, no significant difference of serum apelin level was observed between patients with subclinical hypothyroidism and healthy control subjects. However, the apelin levels were higher in the patients with subclinical hypothyroidism than in the control group. The possible relationship between thyroid hormones and apelins is critical to understanding the etiopathogenesis of metabolic disorders.

P-02.02.2-032
The mitochondrial Erv1/Mia40 import system does not impact cytosolic Fe-S cluster protein maturation and iron regulation

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Erv1 is a sulfhydryl oxidase that partners with the import receptor Mia40 to import small cysteine-rich proteins into the mitochondrial intermembrane space. It has also been suggested that Erv1 has an additional role in maturation of cytosolic Fe-S cluster proteins and regulation of iron homeostasis in *S. cerevisiae*. However, these studies were performed on one particular *erv1* mutant strain (*erv1-1*) that we discovered has additional defects in glutathione (GSH) metabolism. Since GSH is required for iron regulation and cytosolic Fe-S cluster assembly, this complicates our understanding of Erv1′s role in these processes.

We discovered that the *erv1-1* strain originally tested for Fe-S cluster defects was the only strain to exhibit defects in the cytosolic Fe-S enzymes. Mitochondrial and cytosolic Fe-S protein activities in the other *erv1* and *mia40* mutants tested were similar to the WT control. In addition, while all the *erv1* and *mia40* mutants tested exhibit temperature-dependent defects in Mia40 oxidation, only the *erv1-1* strain has significantly reduced GSH levels and more oxidized GSH: GSSG redox state. We determined that the cause of GSH depletion in the *erv1-1* strain is an additional mutation in the gene encoding the glutathione biosynthesis enzyme (Gsh1) that compromises Gsh1 protein folding and/or stability. To address whether GSH deficiency in the *erv1-1* mutant is the underlying cause for the cytosolic Fe-S cluster defects and iron misregulation for this strain, we measured Fe-S protein activity, iron-regulated gene expression, and iron accumulation in *erv1* and *mia40* mutant strains. Only the *erv1-1* strain exhibited iron misregulation and accumulation of mitochondrial iron, while exogenous GSH rescued these defects.

These results demonstrate that the defects in cytosolic Fe-S enzymes and iron homeostasis in *erv1-1* are due to GSH depletion and neither Erv1 nor Mia40 play significant roles in cytosolic Fe-S cluster assembly and iron homeostasis.

P-02.02.2-033
Development and validation of PICAA method for the determination of the purity of synthetic human C-peptide

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Human C-peptide is a 31 amino acid polypeptide, which is secreted into blood from β-cells in the pancreas where pro-insulin undergoes a post translational modification and cleaved into insulin and C-peptide. Human C-peptide concentrations in blood plasma and urine reflect the level of insulin resistance associated β-cell function and can point out insulin secretory failure. The reference intervals in blood plasma and urine are 0.5–10.0 ng/ml and 40–150 ng/ml respectively. C-peptide measurement in urine and plasma provides a guide for therapy in diabetes. This study describes a method for the development and validation of PICAA (Peptide Impurity Corrected Amino Acid Analysis) method for the determination of the purity of the human C-peptide which could be used as a reference material to measure C-peptide concentrations in plasma.

Two different methods were performed for the PICAA; AAA-ID-MS/MS for quantification of constituent amino acids following hydrolysis of the material and RP-HPLC-ESI-TOF MS for determination of the peptide related impurities. The result of the AAA ID MS/MS method was corrected for the amino acids originating from the impurities. ID MS/MS-AAA was performed with Zivak® HPLC and Zivak® Tandem Gold Triple quadrupole MS equipped with a Phenomenex EZ:faast 4μ AAA column (250 × 2 mm i.d). The mobile phase was composed of, A: 20 mM ammonium formate (AF) in water, B: 10 mM AF in acetonitrile (ACN). The intact peptide analysis was performed by a Hitachi LaChrome Elite HPLC and Bruker microTOF-Q mass spectrometer equipped with a Capcell Pak MG-II C18 column (150 × 2 mm i.d., 5 mm particle size).

The purity of the synthetic C-peptide was determined by PICAA analysis and related uncertainty was calculated. Traceability to SI was established using the amino acid standards of which the purity was determined by TUBİTAK UME using qNMR analysis.

PICAA is a simpler alternative to the full mass balance approach which requires large quantities of the peptide material.

P-02.02.2-034
Heat shock proteins: complementary therapies in brain tumors with viscum album

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Cancer is one of the lethal diseases in the world. Different cancer types possess overexpressed Hsps levels. *Viscum album* extracts with their anticancer and antioxidant properties are being used in cancer therapies. Biochemical composition of this plant is known to vary its features depending on the host trees and time of harvest. In our previous study, it has been found that *V.album* inhibited Hsp27 expression and induced caspase-dependent apoptosis in C6 rat gliomas. The aim of the current study is to find out whether different *V.album* extracts have different effects on Hsps expression level and apoptosis in C6 glioma cell line or not.

In this study, three different extracts of *V.album* were compared for their potential inhibition effects on Hsps. The cytotoxic effects of extracts have been determined via MTT test. Different experiment groups were set up subjected to heat shock and/or incubated without any heat shock application. Overexpression of Hsps was induced by heat shock at 42°C for 1 h in C6 cells. Expression levels of Hsps were determined by Western blot analysis. The apoptosis inducing effect was also evaluated via caspase-3 activation in C6 glioma cells.

Pretreatment of the cells with non-toxic dose (10 μg/ml) of *V.album* extracts prior to heat shock, reduced significantly the expression levels of Hsps. Similarly, pretreatment with the extracts prior to heat shock increased apoptosis via caspase-3 activation in C6 glioma cells. These results will be utilized in the determination of the relation between extract composition and stress protein expressions.

These results suggest that different extracts of *V.album* are able to down regulate expression of Hsps, and induce apoptosis. This warrants further exploration as a potential resource of bioactive compounds that can be used in cancer therapy. Future studies targeting Hsps for the development of chemosensitizers may help improve the treatment of cancer in combinational therapy.

P-02.02.2-035
Functional derivatization of recombinant proteins: new strategies for sample preparation and structural characterization

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Biological drugs (biologics) are the fastest-growing category of therapeutics among those approved by the agencies for drugs regulation. Most biologics are proteins designed for parenteral use. However, proteins are characterized by poor pharmacokinetic and safety profiles. PEG-coating (poly-ethylene glycol coating) of biologics provides several benefits, including an increased half-life related to reduced renal clearance, an increased stability to degradation, and a reduced immunogenic/antigenic response. Preservation of the three-dimensional structure and activity of the PEGylated form is a strict requirement for human use.

The recombinant proteins used for this studies (AS-SOD, Superoxide Dismutase; MMP12, Matrix Metalloproteases 12; ANSII, L-Asparaginase II) were cloned and then over-expressed in Escherichia coli. PEGylation reactions were performed using commercial reagents. All the protein samples were purified and analyzed by solution and solid-state NMR (fields from 700 MHz to 950 MHz).

We developed new protocols to prepare samples of PEGylated proteins, demonstrating that solid-state NMR spectra of exceptionally good quality can be obtained for PEGylated proteins in the sedimented state (obtained by either ultracentrifugation or rehydration of freeze-dried samples); surprisingly, sedimentation of PEGylated proteins to this end has never been attempted. The spectral quality is comparable to – or better than – that of the corresponding crystalline samples.

The excellent quality of the solid-state NMR spectra would make it possible to perform extensive resonance assignment and even a conventional full structure determination of biologics. The proposed method is based on the comparison of a standard two-dimensional solid-state NMR spectrum of the sedimented PEGylated protein with that of the crystalline state of the native protein – for which the X-ray structure is available.

P-02.02.2-036
Antimicrobial peptide families expressed by human tissues

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All eukaryotic creatures hereditarily have natural defense mechanisms and are protected from the infections with this defense mechanism.

Antimicrobial peptides (AMP) contain 10–50 amino acid content, are positively charged with amphipathic feature. The antimicrobial activities of AMPs are thought to be depended on the microbiocidal effects by binding to the surface of microorganisms and creating pores in their membranes.

Defensins are both effector and mediator small antimicrobial peptides of the immune system. These peptides in cationic and amphipathic structure have broad spectrum antibacterial, antifungal and antiviral features. Defensins regulate the innate and acquired immune systems by suppressing proinflammatory responses during infection.

Mammals have three structural subfamilies of defensins. These show differences according to the trisulfide arrays in their structure and are classified as α,β, θ defensins.

Human beings have tissue-specific six functional α defensins. Human HNP-1 and HNP-4 encoded by DEFA1, DEFA3 and DEFA4 genes are firstly expressed in neutrophils. Human HDP5 and HDP6 encoded by DEFA5 and DEFA6 are firstly expressed in Paneth cells in the intestines and play important role in the defense and homeostasis. Human beings have many pseudogenes such as DEFAP and DEFTP in addition to these functional genes.

According to literature data, defensins play an important role in defense against microbial placements on mucosal surfaces. In addition, the antimicrobial spectra of defensins include Gram negative and Gram positive bacteria, fungi and viruses. In addition to their antimicrobial efficiency, they can accelerate the wound healing due to their mitogenic effects on epithelium cells and fibroblasts.

P-02.02.2-037
Functional characterization of Val58Met and Phe129Ile mutants of bile salt hydrolase from *Lactobacillus plantarum* in *E. coli* BLR(DE3) strain

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Bile salt hydrolase (BSH) enzyme catalyzes the hydrolysis of glycine and/or taurine-conjugated bile salts into amino acid residues and the free bile acids that reduce cholesterol. However, some intestinal bacteria have an excessive deconjugation of tauro-conjugated bile salts and production of secondary bile acid having potential harmful side effects to the host. The catalytic mechanism and substrate preference of such BSH enzyme is not clear.

In this study, *bsh* gene from *Lactobacillus plantarum* GD2 strain was cloned, expressed, characterized in *Escherichia coli* BLR(DE3) strain, and then Val-58 and Phe-129 amino acids, supposed to be responsible for substrate preference, were substituted for Met-58 and Ile-129 amino acids respectively by site directed mutagenesis. The hydrolysis activities and stability of the mutant recombinant BSH (mrBSH) enzymes were examined along with six different bile acids by ninhydrin assay and SDS-PAGE respectively.

Ninhydrin test results indicated that wild-type recombinant BSH (wrBSH) hydrolyzed six major human bile salts with an apparent preference towards glycine-conjugated to tauro-conjugated bile salts. However, the activities of mrBSH/Phe129Ile enzyme are 29%, 23%, 13%, 14%, 0% and 0% of the activity of wrBSH against to glycocholic acid (GCA), glycodeoxycholic acid (GDCA), glycochenodeoxycholic acid (GCDCA), taurocholic acid (TCA), taurodeoxycholic acid (TDCA) and taurochenodeoxycholic acid (TCDCA) respectively. The activities of Val58Met mrBSH enzyme are 79%, 93%, 74%, 43%, 44% and 28% of wrBSH against to GCA, GDCA, GCDCA, TCA, TDCA and TCDCA respectively.

Our findings support the suggestion that BSH enzymes recognize their substrates predominantly at the amino acid moieties and not at the cholate moieties. However, further PCR-based site-directed mutagenesis and structure-driven computational and theoretical approaches are required for the precise determination of their substrate specificities and the selection of probiotic bacteria.

P-02.02.2-038
Light-induced electrical properties of bacteriorhodopsin in purple membranes

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We deposited bacteriorhodopsin in purple membranes under applied electrical field onto ITO (Indium Tin Oxide) support. Purple membranes film, highly oriented in one direction, was placed between two ITO electrodes. We studied dependence of electrical properties of these films on light illumination. We argue that this setup can be used for functional studies of microbial rhodopsins. In opposite to already published results where this system was used as a photocondensor for studying functional properties of bacteriorhodopsin, we studied electric properties of such systems and we found strong light dependence of resistivity of bacteriorhodopsin in purple membranes films.

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P-02.02.2-039
Search of new optogenetics tools by means of structural and functional characterization of novel microbial rhodopsins which reproduce mutations of already known ones

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Optogenetics is already used in study of neuronal cells cooperation *in vitro* and *in vivo* by means of microbial rhodopsins – ion pumps and channels incorporated in membranes of neurons changing their electrical potential while receiving a light quantum by laser or LED source.

Best perspectives optogenetics will give after successful transfer to medical applications, such as the treatment of blindness, treatment of disorders like Parkinson's disease etc. But to achieve these we need a broad set of tools, optogenetics tools, highly specialized to solve specific problems of neurophysiology. To the creation of such tools our work is dedicated.

New optogenetic tools can be made by mutations in existing ones altering their properties (mainly spectral characteristics, selectivity and conductivity) or some promising mutations in conserved residues can be found in existing organisms. A halophilic archaeon *Halosimplex carlsbadens* is a host of protein of our interest. According to the theoretical data based on the alignment with bR and the 3D structure model of this novel protein, we suppose this protein functions like the light-driven H+ pump: all the key residues are the same or at worst have the similar properties, except one in the position 96 – leucine instead of the aspartic acid. A Gram-positive bacteria *Deinococcus-Thermus phylum* syntheses rhodopsin with substitution of this aspartic acid to alanine. *Sphingomonas paucimobilis* has rhodopsin where aspardic acid in position 96 is changed to serine residue. And one yet uncharacterised *Guillardia theta* rhodopsin even has the same as bR motif (D85, T89, and D96) but according to alignment is closer to ChR2 even the last one motif is E85, T89, N96. It is expected that all of them will show us new properties. Though the further experimental data are essential. The work is supported by RSF 16-15-00242.

P-02.02.2-040
Evaluation of some thymus proteins in patients with crimean Congo hemorrhagic fever

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Crimean Congo Hemorrhagic Fever (CCHF) is a tick-borne viral zoonotic disease. It has a high fatal rate (%5–30). Tokat is one of the cities having the most reported CCHF cases, in Turkey. Clinical presentation of the disease varies widely among patients. Thymic peptides are small molecules synthesized by thymic epithelial cells. They play role in the immune response, as well as anti-inflammatory process.

Fourty patients referring to the hospital with tick-contact history and/or presenting clinical manifestations consistent withCCHF and with positive PCR results for CCHF virus in blood samples were included to the study. The WBC and platelet values at application and before the patients were discharged were recorded. The healthy control group consisted of age and gender matched healthy volunteer adults free of any chronic disease. Thymosin alpha1 (Tα1), thymuline and thymosin beta4 (Tβ4) were studied by the ELISA method in this study. Biochemical parameters were also analysed.

AST and ALT values were significantly higher ($p < 0.01$) and PLT and WBC levels were significantly lower in the CCHF group ($p < 0.05$). Levels of TF, Tα1 and Tβ4 were found to be significantly higher in CCHF ($p < 0.01$). There was no mortality during the study period. Duration of hospitalization was 6.73 ± 2.74 days. Levels of Tβ4 were significantly correlated with duration of hospitalization ($r = -0.351$, $p = 0.036$). ALT levels were significantly correlated with TF levels ($r = 0.413$, $p = 0.010$). 17 patients received FFP and apheresis for the supportive treatment, while 5 patients received only FFP and 2 patients got only apheresis. 16 patients did not get any of these blood products. There was not a statistically significant differences in thymus peptides among these treatment groups ($p > 0.05$).

We report 40 survived CCHF patients with elevated thymic peptides. Pathogenesis of CCHF has many points to be highlighted. Thymic peptides may play role in the clinical situation of the patients with the disease.

P-02.02.2-041
The effect of methocarbamol on the peroxiadse activity of human erythrocyte hemoglobin

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Hemoglobin is released to blood circulation, after red blood cells lysis. It is carried in circulation by binding to haptoglobin. In normal persons, no free hemoglobin is observed in the blood, because most of hemoglobin is in the form of haptoglobin complex. In some diseases that are accompanied by hemolysis, the amount of released hemoglobin is higher than its complementary haptoglobin. As a result, free hemoglobin appears in the blood, which is a toxic compound for these patients. Free hemoglobin has been showed to have peroxidase activity and considered a pseudoenzyme. In this research, the effect of methocarbamol on the peroxidase activity of human hemoglobin was studied. Our results showed that the drug inhibited the pseudoenzyme by un-competitive inhibition. Both K_m and V_{max} decreased by increasing the drug concentration. K_i and IC_{50} values were determined as 6 and 10 mM, respectively. Molecular docking results showed that methocarbamol did not attach to heme group directly. A hydrogen bond connected NH_2 of carbamate group of methocarbamol to the carboxyl group of Asp126 side chain. Two other hydrogen bonds could be also observed between hydroxyl group of the drug and Ser102 and Ser133 residues of the pseudoenzyme.

P-02.02.2-042
DCA reduces viability and down regulates MAPK protein activations in human malignant mesothelioma cells

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Malignant Mesothelioma (MM) is a cancer resulting from mesothelial cells on the serosal surfaces of pleura, peritoneum

and pericardium. Microarray analyze results performed in MM patients revealed that one of the most prominent changes is upregulation of many genes involved in glycolysis and the Krebs cycle. Dichloroacetate (DCA) is an inhibitor of pyruvate dehydrogenase kinase (PDK) that enhances the oxidative activity of cells by activating pyruvate dehydrogenase (PDH) in mitochondria. DCA has shown as a promising anti-neoplastic agent that re-sensitizes cancer cells to apoptosis. The aim of this study is to elucidate the coupling between PDK inhibition and MM cell proliferation and cell cycle.

Human malignant mesothelioma (SPC212) cell line was used as a model for DCA treatments. Cell viability was measured by MTS assay; MAPK protein activations and expressions were assessed by western blotting; cell cycle profile was analyzed by flow cytometry. Statistical analysis was performed by utilizing one-way ANOVA test.

Results showed that DCA reduced viability of SPC212 cells in a concentration and time dependent manner. Protein analysis indicated that MAPK pathway was down regulated at concentrations greater than 50 mM. Moreover, primary cell cycle analysis has indicated arrestment at G2/M phase in 24 hours.

Our findings corroborate with recent reports where DCA treatments resulted in reduction of viability and G0/G1 and G2/M arrest in other cell lines. Abnormalities in MAPK signalling play a critical role in the progression of cancer. Here, we showed for the first time that DCA decreased MAPK activation in 24 h.

Our results suggest that DCA is an anti-prolifertive agents for MM cells *in vitro*. However, it requres extra analysis with other mesothelial cells. Future study will focus on investigating relation between MAPK and mitochondrial apoptosis.

P-02.02.2-043
Adrenomedullin affects hypoxia inducible factor 1 alpha in rats under hypoxic conditions

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Adrenomedullin (AdM) is a vasodilator peptide consisting of 52 amino acids. AdM is synthesized in many tissues. and is a biologically active peptide that has various effects including vasodilatation, the regulation of vascular endothelial function and adjusting adipogenesis. Hypoxia inducible factor 1 alpha (HIF 1-α) is a subunit of a heterodimeric transcription factor hypoxia inducible factor 1. It is the master transcriptional regulator of cellular and developmental response to hypoxia. The dysregulation and overexpression of *HIF1A* by either hypoxia or genetic alternations have been heavily implicated in cancer biology as well as a number of other pathophysiologies.

In our research, the AdM and HIF 1-α levels in heart, kidney and lung tissues of rats were investigated in control, hypoxia, control+AdM and hypoxia+AdM groups. Rats in hypoxia groups were provided hypoxic environment containing of 10–12% oxygen and 88–90% nitrogen for 1 week. Rats in AdM groups were injected intraperitoneally in a dose of 1.25 nmol/kg for four days before the collection of the tissues. The control group was oxygenated with normal air. The control and treatment groups were formed from 7–8 animals and AdM, HIF 1-α levels were measured in taken tissues with immunoassay method.

The aim of this study was to investigate the reaction of the organism when exposed to hypoxic conditions and the effect of AdM over HIF 1-α level. AdM levels and HIF 1-α in heart tissue were found decreased in hypoxia group, and AdM levels increased in hipoxia+AdM group. HIF 1-α levels decreased in hypoxi+AdM group. AdM levels in liver tissue were found decreased in hipoxia and control+AdM groups than control group. HIF 1-α levels were higher in control+AdM group.

AdM has a role in angiogenic process, and our experiment showed that AdM reacts earlier than HIF 1-α, and affects its synthesis. Organism increases its vascularization as a reaction to hypoxic condition, and AdM treatment may provide a rapid adjustment.

P-02.02.2-044
Covalent conjugation and characterization of immunogenic protein of *Toxoplasma gondii* and polyacrylic acid as vaccine candidate

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Toxoplasmosis is a major medical and veterinary disease caused by *Toxoplasma gondii* which infect approximately half of the world's population. This infectious disease especially gains importance in pregnant women and immunodeficient individuals. Also *T. gondii* infection has economic importance. However, there are only one attenuated-live *T. gondii* vaccine for veterinary uses and no vaccine against *T. gondii* is available for humans. Therefore development of an effective vaccine would be extremely valuable for preventing disease in human and veterinary medicine.

Subunit vaccines are very attractive vaccine candidates but there is low antigenicity problem when they are used alone. Polymers themselves don't stimulate immune response while they used with antigenic structure of various infective agents enhance immune response Because when proteins are covalently conjugated with hydrophilic polymers, (1) their circulatory-lives and stability (in different pH and temperature values) enhance (2) binding to proteases and clearance by the reticuloendothelial-system decreases.

In this study, immunogenic protein of *T.gondii* and polyacrylic acid with immune stimulant properties was covalently conjugated and conjugation was demonstrated by Size-Exclusion Chromatography (SEC) and Fluorescence spectroscopy.

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P-02.02.2-045
Role of apoptotic markers for determination of postmortem interval (PMI)

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It is significant to detect time of death in case of a sudden death for medical and legal concerns. There is no known method that can be used for post mortem time detection. Based on this deficiency pmi detection in narrow time frame is a big problem.

In this study, we aimed to investigate and determine time-dependent expressional changes of apoptotic markers by western blot technique. 14 postmortem skeletal muscle were analyzed 12-hour periods in first 36-hour after death. 2nd and 3rd 12-hour periods were statistically significant ($p < 0.005$).

Keywords: post mortem interval, time of death, apoptosis.

P-02.02.2-046
An examination of Hyaluronidase enzyme activity in four different sheep breeds

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Hyaluronidases are excessively found in nature and involved in numerous biological functions. Hyaluronidases primarily degrade hyaluronic acid (HA) and have significant role in fertilization during acrosomal reactions. Therefore, the measurement of hyaluronidase enzyme activity may provide valuable information about acrosomal function and the fertilizing ability of the sperm. The aim of this study was to investigate the semen hyaluronidase enzyme activity changes among four different sheep breeds (Akkaraman, Suffolk, Merino, and Kıvırcık).

In this research, ten ram testis tissues from each sheep breed, a total of 40, were cut and collected on ice. Ovine testicular hyaluronidase of four different sheep breeds was purified from a crude ammonium sulfate-precipitated fraction of an extract of ram testis. The semen hyaluronidase enzyme activity differences between the sheep breeds were examined by spectrophotometrically monitoring the appearance of HA at 232 nm. Analysis of variance test was used to examine the possible mean differences among the four different sheep breeds.

The observed mean differences in enzyme units for Kıvırcık, Suffolk, Akkaraman, and Merino were as follows 891.60, 817.60, 729.40, and 681.60, respectively. The observed mean differences in absorbance values for Kıvırcık, Suffolk, Akkaraman, and Merino were as follows 0.4455, 0.4088, 0.3648, and 0.3408, respectively.

The results showed that the observed mean differences in enzyme units and absorbance values among the four different sheep breeds were not statistically significant. Despite that, in average Kıvırcık had higher values for the activity of each sample and yet it had the smallest values for standard deviation. Therefore, in order to achieve higher enzyme activity and more homogenous samples Kıvırcık breed should be preferred.

P-02.02.2-047
What is extra to learn from protein drying measurements?

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Hydrations of soluble proteins are crucial for their functionality. Therefore elucidating the details of protein hydration is still of interest in the proteins' action mechanisms. This is the motivation of the present study.

In order to study protein hydration, changing concentrations of the well-studied serum albumin protein was measured with the spectroscopic techniques like UV-vis and FT-IR spectroscopy. Spectral data is analysed and calculations were performed on the data to extract the relevant changes in the protein.

Experimental parameters' variation in association with the spectral changes implies the involvement of protein structure and hydrogen bonding in the drying process.

The protein's reactions may not be merely a feature of the protein structure in the common sense but it could be related directly to the protein hydration states as well. This is understandable since it is already known that enzymatic proteins lose their functionality when they are dried while this drying may or may not involve dramatic structural changes. On the other hand, here it is claimed that the role of water in gaining the functionality that was lost in the dried state is not just about enhanced diffusion processes and the dynamicity but could be related to the functionality of water in the energy transfer processes as well.

P-02.02.2-049
Investigating the cellular effects of the aldo-keto reductases AKR1B1 and AKR1B10 in HCT-116 colon cancer cells

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Aldo-keto reductases (AKR) are NAD(P)H dependent oxidoreductases are best characterized as glucose reducing agents, and have been implicated in diabetic pathophysiology. Increased expression of AKR has been associated with tumors of lung, breast, prostate, cervix, ovarian and colon. Two members of the AKR superfamily that have been associated with different cancer types are AKR1B1; aldo-keto reductase family 1, member B1, and AKR1B10; aldo-keto reductase family 1, member B10. Both are 36-kDa cytosolic reductases that are similar in both amino acid sequence identity (71%) and tertiary structure with the (α/β) 8 barrel topology.

While HCT-116, a colorectal cancer cell line, cells expresses AKR1B1 robustly, there is no expression of AKR1B10. In this study, we have stably knocked down AKR1B1 through shRNA technology and overexpressed AKR1B10 in HCT-116 cells. Comparisons were made with a known AKR inhibitor sorbinil. With the knock down of AKR1B1, we have observed reduced cellular proliferation, enhanced apoptosis, delay in cell cycle progression, reduced expression of mitogenic proteins and a decrease in activation of the inflammatory transcription factor nuclear factor kappa B (NF-kappaB). Interestingly, although AKR1B10 overexpression did not affect cell proliferation, apoptosis or cell cycle, some effect was observed with NF-kB signaling.

Our data indicate that, although closely related, AKR1B1 and AKR1B10 have very different contributions towards signaling pathways in colorectal cancer.

P-02.02.2-050
Comparison of different nisin quantification methods and optimization of nisin production by *Lactococcus lactis*

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Nisin, which is produced by certain strains of *Lactococcus lactis*, is the only bacteriocin approved by World Health Organization (WHO) as a food additive. It prevents the growth of foodborne bacteria which cause food spoilage. Nisin research and applications necessitates developing an accurate and reproducible method for its quantification. The agar diffusion bioassay is the most widely used method for quantifying nisin, although it has limitations especially diffusion-related difficulties of the active substance.

In the present study, "agar diffusion bioassay", "enumeration of colony forming units", "colorimetric assay" and "flow cytometry" methods were compared with each other to determine antibacterial activity of nisin on *Micrococcus luteus*.

Moreover, this study also covers the results about the effect of different cultural conditions to optimize nisin production by *L. lactis*. Galactose, lactose and their combination in M17 medium (pH 7) boosted nisin production at 25 °C, as the addition of 1.5 μg/ml hemin into the fermentation broth.

To our knowledge, this is the first study showing the usage of "flow cytometry" method to determine nisin activity of fermentation broth filtrates.

P-02.02.2-051
Coronaviral nucleocapsid protein is an anti-viral target for drug development

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Between 2003 and 2004, the severe acute respiratory syndrome (SARS)-CoV caused a worldwide epidemic and had a significant economic impact in the countries affected by the outbreak. Recently, the Middle East Respiratory Syndrome human coronavirus (MERS-CoV) was found in patients with severe acute respiratory tract infections in the Middle East and South Korea. As is true for all coronavirus infections, there are no efficacious therapies currently available against coronaviral diseases, making the development of anti-coronavirus compounds a priority. The CoV genome consists of positive-sense, single-stranded RNA approximately 30 kb, and it contains several genes encoding several structural and non-structural proteins that are required for progeny virion production with a conserved order. The N proteins exist in the center of the viral particle and represent a helical structure complex. Nucleocapsid protein is most abundant structural protein of CoVs, binds the viral RNA genome to form the virion core, leading to the formation of a ribonucleoprotein (RNP) complex or to a long helical nucleocapsid structure, that is important for maintaining the RNA in an ordered conformation for replication and transcription. The CoV N protein is also involved in the regulation of cellular processes, such as gene transcription, interferon inhibition, actin reorganization, host cell cycle progression, and apoptosis. Two strategies to inhibit oligomeric N protein function have been reported. The first strategy is to discover antiviral agents that target the RNA-binding site. The second one is to impair normal N protein function by interfering with monomer–oligomer equilibrium. Our recent studies suggest that N proteins in infections caused by coronaviruses will be useful antiviral drug targets because they serve many critical functions during the viral life cycle.

P-02.02.2-052
Post-translational modification of vascular endothelial growth factor (VEGF) in colon cancer cells

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Vascular Endothelial Growth Factor A (VEGF-A), commonly referred as VEGF, is a potent secreted mitogen crucial for tumor initiation and progression. The gene for VEGF is translated into a number of splice isoforms that lead to 121, 165, 189 and 206 amino acid proteins, with different receptor-binding and matrix-binding properties.

In the present study, we discuss the functional significance of post-translational modification/processing of $VEGF_{165}$ isoform in HCT-116 colon cancer cells. We also focus on the role of calcium in the post-translational modification of $VEGF_{165}$.

We show that $VEGF_{165}$ undergoes N-linked glycosylation in HCT-116 cells. Perturbation of cellular calcium may affect VEGF driven malignant phenotypes.

P-02.02.2-053
Novel methods for modulating the activity of Bcl2 family proteins in apoptosis

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Apoptosis, also known as programmed cell death, is an essential cellular process, but is implicated in several human diseases, including diabetes and cancer, when it is up- or down-regulated respectively. Bcl-2 family proteins are major players in the control of intrinsic, or mitochondrial apoptosis; they respond to intracellular stress signals, function through protein-protein interactions and converge on the mitochondrial outer membrane to cause membrane permeabilisation, release of cytotoxic molecules, and initiation of an apoptotic cascade that leads to cellular demise.

Our work aims to identify molecules able to bind and modulate the activity of several key players in the Bcl-2 family, including the pro-survival members Bcl-2, Bcl_{XL} and Mcl1, and the death promoting family member Bax. Adhirons, novel non-antibody peptide display scaffolds developed at the University of Leeds, have been used to construct a phage display library containing over 10^{10} clones, and form a key part of the strategy to identify such molecular modulators. Adhirons able to selectively bind individual Bcl-2 family members have been identified, *in vitro* assays carried out to test for modulatory activity, and X-ray crystallography used to elucidate details of how they interact with their target proteins. More recently, studies have been carried out to identify adhirons able to target multiple Bcl-2 family members, with the aim of selectively inhibiting defined groups of proteins in cells.

This work provides opportunities to differentiate the activities carried out by different Bcl-2 family proteins in apoptosis, enabling us to better understand how their dysregulation contributes to human disease.

P-02.02.2-054
Biophysical and evolutionary study of the structural flexibility of ADP-dependent sugar kinases from mesophilic and psychrophilic archaea

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The capability of extremophiles microorganisms to live at low temperatures is mainly attributed to the high structural flexibility of its enzymes. Several sequence and structure features have been associated to a high structural flexibility that enables metabolic processes to occur at low temperatures. During evolution, the general mechanism adopted by these enzymes has been to reduce the free energy of the transition state rather than the Michaelis constant, K_m. Increased structural flexibility and decreased affinity for its substrates in psychrophilic enzymes is compensated by an increase in the catalytic rate, k_{cat}. Few psychrophilic enzymes have been reported to performance the optimization of their catalytic efficiency (k_{cat}/K_m) by decreasing K_m values.

We use the ADP-dependent kinase sugar family of archaea as a model, to identify particular structure and sequence features of a psychrophilic enzyme that would make this enzyme more flexible than their thermostable homologues.

We characterize the bifunctional psychrophilic enzyme phosphofructokinase/glucokinase from *Methanococcoides burtonii*

(*Mb*PFK-GK) and the bifunctional mesophilic enzyme phosphofructokinase/glucokinase from *Methanococcus maripaludis* (*Mm*PFK-GK) by spectroscopic, biophysical and computational techniques. The comparison showed that the absence of two ion pairs is primarily responsible for the increased structural flexibility accounted in the psychrophilic model. This increase in structural flexibility is reflected in the exponential increase in the K_m values with temperature. Additionally, we reconstruct the sequences of all ancestral enzymes between the current enzymes and their last common ancestor, which was used to trace the occurrence of these electrostatic interactions during evolution in the ADP-dependent sugar kinase family.

Our results suggest that electrostatic interactions are a dominant feature in the transition from psychrophilic to thermophilic environments. Fondecyt 1150460.

P-02.02.2-055
Elucidating the domain swapping mechanism of the forkhead domain of human FoxP1

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Fox transcription factors control gene transcription during key processes, such as embryogenesis and immunity, and feature a conserved DNA-binding domain known as forkhead. While most forkhead domains are monomeric, solved structures of members from the P subfamily (FoxP) show that they can oligomerize by domain swapping (DS), a mechanism where protein segments are exchanged between subunits leading to an intertwined dimer.

The biological relevance of DS in FoxP has been stressed by disease-causing mutations that impair this process. However, for many proteins DS takes days to occur and requires global protein unfolding. Thus, the current mechanism impedes a conciliation of the occurrence of DS of FoxP1 in a biological context.

Here, we elucidate the biological feasibility of this process by biophysically characterizing the DS mechanism of the forkhead domain of FoxP1 using size exclusion chromatography (SEC), circular dichroism, and hydrogen-deuterium exchange mass spectrometry (HDXMS). Our results show that DS of FoxP1 occurs at micromolar protein concentrations, within hours and is energetically favored. Remarkably, dimeric FoxP1 follows a three-state N_2 « 2I « 2U folding mechanism, where dimer dissociation into a monomeric intermediate (I) precedes protein unfolding, in contrast to other DS proteins.

Using SEC and HDXMS, we show that the I state of FoxP1 largely resembles the native state, but has a larger hydrodynamic radius and a higher deuterium uptake in regions that maintain the compact monomer, suggesting that the I state is an 'open' conformation *en route* of DS. Finally, we compared the local flexibility of the dimer and monomer of FoxP1, showing that only the hinge region connecting DS segments exhibits different deuteration rates.

Our results show that DS in FoxP1 follows an unusual three-state folding mechanism that proceeds through transient structural changes rather than needing protein unfolding as in most DS proteins. (Fondecyt 1130510, 11140601).

P-02.02.2-057
The sustained release of growth factor proteins following their implantation in tissue engineering

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Bone tissue engineering has become a promising approach for bone regeneration. However, insufficient vascularization during bone regeneration, particularly with large bone defects, results in poor and unsustainable bone formation due to central necrosis. Therefore, vascularization following implantation *in vivo* is essential to the successful formation of new bone tissue. We evaluated the release profile of VEGF from BGs using a novel fluorescence-based retention assay, which revealed that VEGF loaded on BGs can be released in a sustained manner without an initial burst (near zero-order cumulative release) with a controlled release rate of 13.6% per week for up to 7 weeks. In contrast, an ELISA-based release assay showed VEGF to have an early burst-release profile for the first week. However, the biological activity of VEGF released from the BGs was preserved over the 7-week release period, which is consistent with the sustained-release profile observed in the fluorescence-based retention assay. We developed a novel fluorescence-based retention assay to evaluate the release of VEGF from BGs. This fluorescence-based retention assay, which detects the VEGF that remains on BGs, reveals that VEGF loaded on BGs can be released in a sustained manner, with a minimal initial burst, for up to 7 weeks. These results indicate that the sustained biological activity of the VEGF released from BGs over the full 7-week period promotes bone regeneration, and suggest its potential use for bone tissue engineering. Research Grant Sponsor: Korea Health Technology R&D Project [Korea Health Industry Development Institute (KHIDI)]. Grant Number: HI14C0522.

P-02.02.2-058
Serum irisin levels in patients with morbid obesity

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Irisin is a recently discovered myokine which regulates energy metabolism and is associated overweight. We aimed to evaluate serum irisin levels in the patients with morbid obesity.

A total of sixty patients with morbidly obese and thirty healthy control subjects were included in this study. All participants were measured body weight and height, the lipid profile, and plasma glucose, HbA1c, insulin and irisin levels. Irisin levels were measured by ELISA method.

Serum irisin levels were significantly lower in morbidly obese patients than healthy controls ($p < 0.05$). There was no statistically significant difference in terms of age or gender. Serum irisin was negatively correlated with BMI, insulin levels, and HOMA-IR ($p = 0.006$, $p = 0.046$, $p = 0.048$, respectively).

Our study revealed lower irisin levels in morbidly obese patients with respect to control subjects. The lower irisin levels observed in morbidly obese patients might suggest a loss of browning of subcutaneous adipose tissues.

P-02.02.2-059
PKA inhibition restores adenosine uptake in renal tubular epithelial cells under high D-glucose conditions

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Introduction: Diabetic nephropathy (DN) is the leading a cause of end-stage renal failure whose pathogenesis must to be elucidated. The progression of DN has been associated with elevated levels of adenosine. Extracellular adenosine availability is regulated by the activity of the equilibrative nucleoside transporters (ENTs). Due to the ENTs have putative sites of phosphorylation our objective was to evaluate the role of PKA and CK2 kinases on ENTs activity.
Methods: Adenosine uptake (10 μM adenosine, 60s, 22°C) was assayed in HK2 cells preincubated with 5 mM or 25 mM D-glucose for 24 h and exposed to 10 μM 8-Br cAMP, 10 μMH89 or 100μM TBB for 1 h. Plasma membrane and intracellular proteins were fractionated by the biotinylation method and ENT1 and ENT2 contents were quantified by western blot.
Results: High D-glucose in HK2 cells inhibited the uptake of adenosine. This effect was reversed using a PKA inhibitor (H89) through an increased ENT2 uptake activity. We noticed this PKA inhibitor did not regulate the plasma membrane localization of ENT1 or ENT2 under normal D-glucose (5 mM) or high D-glucose conditions (25 mM). Also, we saw that TBB (CK2 inhibitor) decreased the activity of ENT1 and ENT2 under normal glucose conditions, decreasing the localization of ENT1 at cell surface, while the membrane localization of ENT2 decreases under the effect of TBB and high D-glucose conditions.
Conclusions: PKA inhibition reversed the effect of high D-glucose, increasing the uptake of adenosine mediated by ENT2. This could be a new target for the restoration of adenosine levels in DN.

P-02.02.2-060
Relation between serum lipo (a), plasma fibrinogen, red cell distribution width and mean platelet volume in healthy adult men

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The aim of this study was to investigate the relationship between the serum lipo (a) and plasma fibrinogen, Red cell Distribution Width (RDW) and Mean platelet volume (MPV) in healthy adult men.

For this purpose, 37 healthy adult men have normal physical examination and laboratory findings and not use any drug were included to the study. Serum lipo (a) levels and hematologic parameters (fibrinogen, RDW-SD and MPV) were measured by autoanalyzer and commercial kits.

The mean of the age of the persons was 27.2; body mass index was 24.2; serum lipo (a) level was 0.21 and plasma fibrinogen level was 1.61. There was significant positive correlation between the serum lipo (a) and plasma fibrinogen levels ($r = 0.246$; $p = 0.025$), significant positive correlation between the serum lipo (a) and RDW-SD ($r = 0.267$; $p = 0.004$) and significant negative correlation between lipo (a) and MPV ($r = -0.205$; $p = 0.027$).

The plasma fibrinogen and the serum lipo (a) levels have been known as the risk factors for CAD (Coronary Artery Disease) increase together in healthy adult men. Similar findings have been reported in CAD patients. It has reported that elevated RDW is associated with intracoronary thrombotic burden and may be associated with the severity and instability of acute myocardial infarction. In addition, MPV is predictor of severe atherosclerosis and may be used for the prediction and identification of cardiac risks in CAD patients. Our findings show that elevated RDW and decreased MPV may predict to increased risk of CAD in the future, in healthy adult men.

P-02.02.2-061
The role of follicular fluid and serum kisspeptin in infertile women with polycystic ovary syndrome and poor responders undergoing IVF/ICSI

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Follicular fluid is rich in peptides, which significantly influence the growing oocyte. Due to existence of a link between kisspeptin (metastin) cells and gonadal steroids kisspeptin might manipulate the gonadotropin axis and folliculogenesis. In this context, the study was planned to investigate for the first time that the follicular fluid (FF) and serum concentration of kisspeptin in high and poor responders undergoing in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI). Biological samples were collected from twenty infertile women with polycystic ovary syndrome (PCOS) and 20 poor responder participants undergoing controlled ovarian stimulation (COS) with gonadotropin-releasing hormone (GnRH) antagonist protocol for IVF/ICSI treatment. Kisspeptin concentrations were measured in serum and follicular fluid by using ELISA, whereas FSH and LH levels were detected by routine laboratory methods. It was found that kisspeptin levels were significantly lower in serum and follicular fluid of infertile women with PCOS. Kisspeptin levels were correlated with FSH and LH level in infertile women with PCOS. It can be concluded that low level of kisspeptin might inhibit GnRH release that might cause to the inhibition of FSH and LH release and might disrupt folliculogenesis. Decline in serum and FF levels of kisspeptin might be possible cause of anovulation and subfertility in PCOS subjects.

P-02.02.2-062
Immobilization of *Cryptococcus albidus* D24 lipase for enzymatic esterification

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Cryptococcus albidus D24 is a newly identified yeast isolates from petroleum area in İzmir as a Lipase producer. The molecular weight of the enzyme is 36.31 kDa as found. Optimum temperature was 40 °C and half-life times were 180, 27, 9 and 0.59 min at 30, 40, 50 and 60 °C, respectively. Optimum pH value was 8.0. However, it shows significant pH stability at pH values 4.0 and lower. The existence of acetone in the solution as a solvent enhanced lipase activity. *Cryptococcus albidus* D24 lipase was

The FEBS Journal **283** (Suppl. 1) (2016) 129–417 DOI: 10.1111/febs.13808

able to catalyze the esterification reaction between fructose and palmitic acid to produce fructose palmitate using acetone as the solvent. Due to its stability in organic solvents, we propose that in order to increase the yield of fructose palmitate, we could immobilize D24 lipase.

Therefore, the effect of immobilization on kinetic parameters of D24 lipase was investigated. Different immobilization materials and methods were used to find efficient support materials for D24 lipase immobilization. Additionally, fructose palmitate production processes will be optimized with immobilized lipase.

P-02.02.2-063
The evaluation of serum CTRP-1, CTRP-3 and kallistatin levels in patients with knee osteoarthritis

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Introduction: The diagnosis of osteoarthritis (OA) is based on clinical symptoms and radiographic findings. It is known that the pathologic changes at the molecular level in the joint cartilage tissue start before symptoms appear in OA. C1q Tumor Necrosis Factor-Related Protein 1 (CTRP-1), C1q Tumor Necrosis Factor-Related Protein 3 (CTRP-3) and kallistatin are related to many different cellular processes including bone and cartilage tissue metabolism. The aim of this study was to investigate any probable association between the serum CTRP-1, CTRP-3 and kallistatin levels and diagnosis and radiologic staging of knee OA patients.

Materials and Methods: This study included 60 patients with knee OA and 30 healthy individuals for control purposes. The patient group was divided into four stages radiologically. CTRP-1, CTRP-3 and kallistatin levels were measured in serum samples of patient and control groups with ELISA method, and the differences between the groups were analyzed with statistical methods.

Results: The levels of serum CTRP-1 in the patient group were significantly higher than in the control group ($P = 0.001$), serum CTRP-3 and kallistatin levels were not statistically different (in order of $P = 0.251$, $P = 0.160$). In the patient group, there was not a significant difference between serum CTRP-1, CTRP-3 and kallistatin levels and radiologic stages (respectively $P = 0.811$, $P = 0.715$, $P = 0.202$). There was a significant positive correlation between the radiologic stage and patient's age, body mass index, Western Ontario and McMaster Universities Arthritis Index and Visual Analogue Scale values (respectively $P = 0.001$, $r = 0.510$; $P = 0.010$, $r = 0.331$; $P = 0.001$, $r = 0.683$; $P = 0.001$, $r = 775$).

Discussion and Conclusion: Serum CTRP-1 levels were detected significantly increased in patients with knee OA, but there was no significant difference in CTRP-3 and kallistatin levels. There was not a significant association between the radiologic stage and levels of CTRP-1, CTRP-3 and kallistatin.

P-02.02.2-064
A research on thermophilic bacteria and their enzyme profile in different geothermal regions of Turkey

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Enzymes in microorganisms, especially termophilic bacteria are more attractive in biotechnology and molecular biology due to the higher catalytic activity. Turkey is rich in geothermal resources and it is important to determine unknown microbial content of geothermal sources.

In this study, water and sludge samples were taken from Ayder, Kizilcahamam and Gonen hotsprings. Firstly, pH, temperature, salt concentration, Gram reaction, mobility, endospore formation, oxidase and catalase tests were carried out as conventional characterization. Molecular characterizations of isolates were achieved by FAMEs, rep-PCR and 16S rRNA sequencing. Finally, test isolates were evaluated according to enzyme production capability by petri dish.

As result of conventional tests, isolates were determined as Gram positive, mobile- rod shaped, aerobic, oxidase, catalase and endospore positive. The growth range for pH and temperature of the isolates were determined as 5–9 and 50–65 °C. In consequence of the salt test, the test isolates were grown at 2–10% NaCl. 19 of thermophilic isolates were selected by rep-PCR and according to 16S rRNA sequencing analysis test isolates were belonging to *Bacillus, Geobacillus, Anoxybacillus* and *Brevibacillus* genus at a range of 98–99%. Enzyme tests showed that, some of the isolates were able to produce protease (F17, F31, F76, F6, F99 and F19), amylase (F41, F76, F42, F98 and F10), cellulase (F17, F41, F31, F6, F99 and F19), xylanase (F17, F31, F76, F99 and F19) and lipase (F17, F31, F6, F99 and F19).

It can be concluded that, geothermal regions are rich in *Bacillus* and related genera. FAME analysis was particularly insufficient for diagnosis of thermophilic microorganisms, but rep-PCR was successful in separation of organisms at species and even subspecies levels. Most of our bacterial isolates have industrially important enzyme production capacities. It is a pioneer result to use bacteria for industrial applications which need higher temperatures.

P-02.02.2-065
Warburg effect was investigated by studying various metabolic molecules and assays in mammalian cell lines

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Majority of the different cancer cells switch their metabolism from oxidative phosphorylation pathway to glycolytic pathway; in order to meet excessive energy requirement, which is also called Warburg effect. Acetylation is one of the most crucial post-translational modifications playing key roles in metabolic reprograming. In this study, the relationship between acetylation dependent changes in energy metabolism and apoptotic pathways were investigated in PNT1A, DU145, HELA, HEP3B, HEK293T, SHSY5Y.

Immunoblotting experiments were applied by using antibodies against acetylated-Lysine to examine the changes in overall

acetylome. Candidate proteins displaying elevated acetylation was identified with mass spectrometry based-proteomic analysis. Glucose transporter 4 (GLUT4) was used to detect insulin-stimulated glucose transport, total oxphos rodent antibody cocktail to identify variations in complexes which are responsible for most of the ATP production. Caspases (Casp-3, -9) to unreveal different activation levels of apoptotic pathway among the cell lines. Mitochondrial membrane potential was measured by using Rhodamine123 by employing confocal microscopy.

The expression level of respiratory chain complex IV subunit MTCO1 and Casp-3 was higher in HEK293T compared to other cell lines. Casp-9 was upregulated in cancer cell lines, mostly in HEP3B. GLUT-4 levels were downregulated in cancer cell lines in contrast to healthy cell lines.

Findings imply that these proteins might have significant roles leading to variable metabolic and apoptotic activity of each cell line during energy production. Due to the results, MTCO1 might be important in adaptation of different cell lines to regulate the overall respiratory chain complex activity. Reduction in GLUT4 level demonstrates insulin desensitization in cancer cell lines, which might lead to metabolic defects in these cells. Besides, since p53 has a repressive effect on GLUT4, it also can lead us to study about p53 levels.

P-02.02.2-066
The effect of inhibition of PI3K/Akt/mTOR signaling pathway on receptor tyrosine kinase expression in breast cancer cells

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The increased expression and activation of receptor tyrosine kinases (RTK) (EGFR, HER2, HER3) play important roles in breast cancer pathogenesis. HER2/HER3 interaction is the most potent heterodimer and it causes oncogenic PI3K/Akt activation. Inhibitors of PI3K/Akt pathway (Akt inhibitor and PI3K/mTOR dual inhibitors) lead to increase in RTK levels and activities while blocking signaling pathway. In this study, the time dependent effect of dual PI3K/Akt inhibitor PI-103 on receptor tyrosine kinase expressions' in breast cancer cells was investigated.

Two breast cancer cell lines, MDA-MB-468 cells (which has EGFR overexpression and PTEN deficiency) and SKBR-3 cells (which has HER2 overexpression) were evaluated for the effect of dual inhibitor. These cells were treated with dual PI3K/Akt inhibitor PI-103 for different time periods (1–24 h). EGFR, HER2 HER3 total RTKs expression and PI3K/Akt pathway inhibition (p-Akt and p-p70S6K expression) were evaluated by Western Blot.

In MDA-MB-468 cells, there were significant decrease in p-Akt and p-p70S6K proteins' expression during the first 3 h. This inhibition was followed by reactivation of the signaling pathway after 6 h. In SKBR-3 cells, p-Akt and p-p70S6K proteins' expression were significantly decreased during the first 6 h. The PI3K/Akt signaling pathway in these cells were reactivated after 12 h. Basal expression of EGFR and HER3 in MDA-MB-468 cells and basal expression of HER2 and HER3 in SKBR-3 cells were found to be very high. Transient inhibition of Akt and mTOR protein kinase activation in tumor cells followed by reactivation of signaling pathway did not result in a time-dependent difference on EGFR, HER2 and HER3 expression levels. These results suggest that dual PI3K/mTOR inhibiton by PI-103 may trigger receptor tyrosine kinase reactivation due to the signal distruption without affecting total protein expression level.

P-02.02.2-067
Fluorescence-based chemical tools: bioorthogonal targeting of proteins for live cell imaging

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Site-specific bioorthogonal reactions are one of the significant tools for discovering different aspects of biological systems in live cells. The reactions should be highly stable and rapid in physiological conditions. Various chemical tools can be used in bioorthogonal reactions to monitor biological systems, therapeutics, microscopy and diagnostic applications in live cells. Synthetic covalent chemistry in the study of biological systems has been used to label biomolecules selectively in their native environment. For example, small synthetic fluorophores can be added to biomolecules without disturbing other molecular biological pathways.

Aldehydes or ketone-based functional handles can be attached onto protein at specific sites via chemoenzymatic reactions. Labeling of carboxy terminus of α-tubulin has been successfully studied in our previous studies by replacement of wild type tyrosine with unnatural amino acid 3-formyltyrosine in the presence of tubulin tyrosine ligase enzyme (TTL)- as its role can suggest whether certain cancer cells might grow more aggressively than others.

In this work, we highlight the synthesis and spectroscopic properties of azacoumarin chemical probes to study tubulin-tubulin tyrosine ligase (TTL) system in live cells. Significant increase in fluorescence quantum yield or a red shift on absorption and emission maxima is observed when the conjugated product is formed. Bioorthogonal fluorescent labeling is such a favorable reaction to perform rapid kinetics, localization and high site-specificity in cell environment. Newly synthesized azacoumarin fluorophores should therefore not only be useful for studying TTL-based biological systems, but also would enable broad range of high-yielding and fast diagnostics for future biolabeling applications in biochemistry, cell biology and beyond.

P-02.02.2-068
RNA-seq analysis of A549 cells treated with bacterial ribonuclease binase demonstrating biological activities

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Binase is an extracellular ribonuclease from *Bacillus pumilus* which shows antiviral and antitumor activities in cell cultures. However, the action of binase on intracellular functions and processes has not yet been identified. Here, for the first time we report the whole transcriptome analysis of binase-treated human lung adenocarcinoma epithelial A549 cells.

A plasmid-based reverse genetics system and colorimetric cell viability assay were used to identify the binase internalization and binase cytotoxicity towards A549 cell line, respectively. For the whole cell transcriptome analysis A549 cells were treated with 100 μg/ml of binase for 24 h followed by mRNA extraction and library preparation. Sequencing was performed on SOLiD 5500xl Wildfire next-generation sequencer.

We found that binase internalized into A549 cells after 2 h of incubation. The binase at 100 μg/ml was absolutely non-cytotoxic towards A549 cell line and was active in the cell culture medium during 48 h incubation. The analysis of RNA-Seq data showed

The FEBS Journal **283** (Suppl. 1) (2016) 129–417 DOI: 10.1111/febs.13808

that among 13 thousands of protein coding transcripts 791 transcripts were up and down regulated by binase, among them 79 transcripts were induced and repressed.

Binase repressed the production of S100A16 and TNXB which act cancer biomarkers, SCN8A and DRD4 which play a crucial role in cancer metastasis and responsible for pediatric tumors, respectively. The induction of transmembrane protein transcript ABCB11 by binase can help binase to internalize into the cell as ABC transporters are often account for transporting drugs across the cellular membrane. Binase induced the production of NLRP3, RASGRP1and ALPK2 transcripts which can activate apoptosis, cytokine or T cell activation in cancer cells.

Thus, binase exerts different effects in cancer cells. The RNA-seq data obtained will help to understand the mechanism of binase anticancer action.

P-02.02.2-069
Cloning and expression of *Pantoea* sp. 3.5.1 phytase gene (agpP) in *Escherichia coli*

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Phytases (EC 3.1.3.8, EC 3.1.3.26 and EC 3.1.3.72) – is a specific group of phosphatases capable of hydrolyzing *myo*-inositol 1,2,3,4,5,6-hexakisphosphate (phytate) with the formation of less phosphorylated inositol derivatives (from mono- to pentaphosphate). Three major types of phytases are recognized on the basis of the first phosphate group hydrolyzed by the enzymes: 3-phytase, 4/6-phytase, and 5-phytase. Due to the stereospecific way of phosphate release from the phytate molecule by the action of phytases, these enzymes by themselves and their composition may serve as a potential alternative for production of myo-inositol phosphate isomers with therapeutic properties. Chemical synthesis of these compounds is inefficient and costly.

Pantoea sp. strain 3.5.1 showing high phytase activity was isolated from the forest soil sample of the Republic of Tatarstan, Russia. In this study the main objective was the cloning and expression of *Pantoea* sp. 3.5.1 phytase gene in *E. coli*. First, we amplified the phytase gene (*agpP*) from the genomic DNA of the bacteria using specific primers PhMH_dir and PhMH_rev. Size of phytase gene corresponded to 1729 base pairs. During the optimization of amplification conditions it was found that the optimum temperature for primer annealing was 66 °C. This temperature increases the specificity and efficiency of annealing. Then the PCR-product of *agpP* gene was cloned into the pBAD MYC/HIS vector first. On the next step we carried out subcloning of the *agpP* into a pET28a expression vector. Multicopy plasmid pET28a/agpP contained the sequence of the phytase gene of *Pantoea* sp. 3.5.1 under T7 promoter. The corresponding recombinant protein was expressed in *E. coli* as a fusion with a 6 His-tag and was detected by Western blotting. Recombinant phytase was purified via affine chromotography on the Ni-NTA column and displayed high phosphomonoesterase and phytase activities.

P-02.02.2-070
Bag-1 induces cell survival in MDA-MB-231 breast cancer cell lines

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BAG-1 (Bcl-2 associated athanogene) is a multifunctional protein that interacts with diverse array of cellular targets and modulates a wide range of cellular processes, including proliferation, cell survival, transcription, apoptosis, metastasis and motility. In human cells BAG-1 exists as three major isoforms (BAG-1S, BAG-1M and BAG-1L) derived by alternative translation initiation from a single mRNA, which allows interactions with various molecular targets such as Hsp70/Hsc70 molecular chaperones, components of the ubiquitylation/proteasome machinery, Bcl-2, Raf-1 kinase, nuclear hormone receptors and DNA. Our work aims to investigate how altered Bag-1 expression levels affect cell survival in MDA-MB-231 (ER, PR and HER2/Neu negative) breast cancer cell lines. We first cloned Bag-1L gene to a cloning vector, later we transfected MDA-MB-231 cells for overexpression of Bag-1. We also used Bag-1 siRNA to silence *Bag-1* gene. Western blot analysis was applied to demonstrate relative expression levels of Bag-1, its interacting partners and certain proteins which are important for apoptosis pathway. We performed XTT cell viability assay for Bag-1 overexpressed cells to checkBag-1's impact on cell survival, and observed enhanced survival rates on cells compared to that of the untreated cells with Bag-1 overexpression. In addition, our study revealed that once BAG-1 forms a complex with C-Raf/B-Raf/Hsp70/Akt/Bcl-2, modulation of cell survival was observed. We believe that once the exact localization and involved molecular mechanisms of Bag-1 and its isoforms are found, the role of each Bag-1 isoform in cell survival can be understood better. This can further provide routes to study tumor development.

P-02.02.2-071
The encapsulation of recombinant incretin hormone

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The aim of this study is testing the recombinant GLP1 encapsulation into a biocompatible material. We also tested if it can be a therapeutic candidate drug for type 2 diabetes. The incretin hormones, which are also named as endogenic peptide hormones have become a more attractive therapy for type 2 diabetes because of different physiological effects. In circulation, GLP1 is cleaved by DDP4 in a very short time. If GLP1 can be protected from cleaving, the effective time of GLP1 would be increased and by this way it can replace the therapy of insulin. Chemical synthesis methods of peptides are limited because of low efficiency and high cost. The production of peptides by recombinant *E. coli* is an alternative way because of effective production, simplicity and low cost. However, the major disadvantage derived from the recombinant *E. coli* is the frequent formation of inclusion body. For that reason, extra methods are needed for obtaining soluble recombinant peptides. Glutathione S-transferase (GST) tag is commonly used as affinity and solubility tag to improve the solubility of recombinant peptides. In this study, we cloned and heterologously produced GLP1 using the GST fusion system in *E. coli*. Affinity purification of recombinant protein was achieved by using glutathione immobilized columns. Characterization of the GST-tagged GLP1 was performed by SDS-PAGE. The purity of fusion protein was found to be 65%, as confirmed by GLP1 ELISA kit. Then, the fusion protein was encapsulated in a chitosan coated polygalacturonic acid. The different pH stability and in vitro release tests also in different pHs was studied.

P-02.02.2-072
Novel metalloprotease from *Morganella morganii*

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Morganella morganii is an opportunistic pathogen capable of causing a wide range of clinical infections. It is known that microbial metalloproteases play an important role in the development of bacterial infections. Thus, investigation of *M. morganii* metalloproteases has a particular interest.

Bacteria were grown in LB medium at 37 °C. As a bioinformatics tool BLAST was used. For molecular biological experiments, Thermo Scientific Kits and SibEnzyme enzymes were used. pBAD/Myc-His plasmid was used as an expression vector. Bacterial transformation was carried out by heat shock method. Bacterial cells were disrupted by sonication. Gene expression products were analyzed by western blotting. To analyze the actinolytic activity of bacterial extracts SDS-PAGE electrophoresis was used.

The putative metalloprotease gene (AN CP004345.1) has been found in the genome of annotated strain of *M. morganii* KT. Its amino acid sequence has partial homology (37%) with actin specific metalloproteases grimelysin from *S. grimesii* and protealysin from *S. proteamaculans*. Using specific primers the gene with 99% homology was identified in the genome of clinical isolate of *M. morganii* 4. RT-PCR analysis showed that this gene had the maximum expression at 48 h of growth. In addition, the cellular extract of *M. morganii* 4 had small actinolytic activity. Cloning of the gene into *E. coli* DH5α cells led to the synthesis of the 35 kDa protein. It is known that the highest expression of *Serratia* proteases is observed at 48 h of growth, and the molecular weight of the mature proteins is 32 kDa. It was shown that metalloprotease gene of *M. morganii* 4 expressed at the same time of growth. Moreover, the recombinant *E. coli* cells synthesized protein with the similar weight (35 kDa) which perhaps is a mature form of the metalloprotease from *M. morganii*.

As a result, in the genome of *M. morganii* 4 the metalloprotease with similar properties to grimelysin and protealysin proteases was identified.

P-02.02.2-073
The preliminary characterization of P-II like protein GlnK from *Lactobacillus brevis*

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The P-II proteins in bacteria, archaea and plants regulate the activity of a variety of proteins in response to specific metabolic signals which affect their structural state and interaction ability. Among various bacteria belonging to *Lactobacillus* only some species have genes encoding PII protein in the genome. Here we report the preliminary characterization of PII-like protein LbrGlnK from *Lactobacillus brevis*. While the amino acid sequence alignment revealed only 50–70% homology of LbrGlnK with other well studied PII proteins, LbrGlnK also has the ATP binding motive GDGK. Trimeric structure of LbrGlnK was confirmed *in vitro* by size exclusion chromatography, suggesting possible similarities of LbrGlnK properties with PII proteins. The isothermal titration calorimetry revealed a preferential binding of ADP (Kd = 50 μM) over ATP (Kd = 357 μM) suggesting that they compete for binding to LbrGlnK. Neither 2-oxoglutaric acid nor other nucleotides were interacting with LbrGlnK in ITC measurements. The mutation Gly91Ala in the ATP binding motive completely abolished the interaction with both ADP and ATP. The pull down of LbrGlnK with *L. brevis* cell extract allowed identification of chaperonin GroL, transketolase and GlnR-like transcriptional regulator from MerR family as most probable partner proteins for interactions with LbrGlnK.

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P-02.02.2-074
A modified FXa as a TFPI trap: structure-function relationships

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Background: Hemophilia is a bleeding disorder due to the deficiency in coagulation factors VIII (hemophilia A) or IX (hemophilia B). Hemophilia patients are essentially treated with intravenous replacement of the missing or dysfunctional factors FVIII or FIX by recombinant proteins. These therapies often induce the generation of acquired antibodies, and thus, novel approaches are needed. Most recent hemophilia strategies target the Tissue Factor Pathway Inhibitor (TFPI), which is the major inhibitor of the coagulation cascade, particularly of the extrinsic tenase complex. Anti-TFPI agents have been empirically developed such as aptamers, peptides, monoclonal antibodies. We have followed a structure-based strategy, to design a mutated FXa that would show more affinity for TFPI, and thus trap this inhibitor. TFPI exists as two isoforms are folded as multi-Kunitz domains related by linkers. The second Kunitz type domain of TFPI (TFPI-K2) is known to bind the catalytic site of FXa.
Methods: The molecular complex of TFPI-K2-FXa was modeled and submitted to molecular dynamics (MD), allowing the identification of low-spots interaction. Modified FXa with theoretically stronger interaction with TFPI-K2 were predicted using MD. The mutants and wild type proteins were expressed in HEK cells, and their processing status was checked. They were tested by Western Blotting, by chromogenic activity using a specific substrate of FXa, by thrombin generation assay in FVIII depleted plasma. Finally, their binding to a TFPI-K2 peptide array was compared.
Results: The mutants showed better efficiency to restore thrombin generation in plasmas from hemophiliacs and displayed stronger binding to TFPI-K2 than the wild type FXa.
Conclusions: The proof of concept of the synergistic approaches of MD and mutagenesis was obtained and an efficient TFPI trap was designed. The mutated FXa is a candidate for a new hemophilia therapy.

P-02.02.2-075
Efficient biodegradation of diisopropylfluorophosphate and chlorpyrifos using displayed organophosphorus acid anhydrolase on bacterial cell surface

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Organophosphorus acid anhydride (OP) nerve agents are potent inhibitors which disrupt the mechanisms of neural transmission. Organophosphorus acid anhydrolase (OPAA; E.C.3.1.8.2) is a

The FEBS Journal **283** (Suppl. 1) (2016) 129–417 DOI: 10.1111/febs.13808

class of enzyme that potentially acts on phosphorus anhydride bonds, reported intracellularly in diverse organisms, albeit notably the enzyme belongs to *Alteromonas* species are more extensively studied. Whereas mass-transfer problem is a major issue in native whole cell biocatalysis, new anchor system derived from the N-terminal domain of ice-nucleation protein from *Pseudomonas syringe* InaV (InaV-N) was used for the first time to display OPAA onto the cell surface. Tracing of the recombinant protein and its activity assay showed a successful presentation of OPAA and its significant ability for biodegradation of organophosphorus compounds. Further studies on bacterial fractions confirmed that OPAA is remarkably located on the outer membrane. The specific activity of recombinant bacteria to degrade diisopropylfluorophosphate (DFP) was measured at 260 U/mg of cell wet weight, which almost all was observed in the outer membrane fraction. Recombinant cells could also degrade chlorpyrifos (Cp) compound in 195.6 U/mg activity. It can be concluded that InaV-N anchor is efficient for targeting OPAA on the cell surface and can effectively eliminate the mass-transfer problem in native whole cell bioconversion system.

P-02.02.2-076
Modulation of cell signaling via interfering specific protein-protein interactions of a hub scaffold protein

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Proper spatial and temporal organization of proteins involved in cell signal transduction is crucial for the specific and efficient information transfer. Scaffold proteins coordinate the action of signaling molecules by their physical binding and organization in multiprotein complex assemblies. Multiple protein binding is often mediated through intrinsically disordered regions of the scaffold, where the interaction epitopes are defined by linear peptide motifs.

Using a hub scaffolding protein Axin as a paradigm, we have employed peptide microarray technique to identify the binding epitopes for Axin interaction partners at high resolution. This enabled us to design Axin-derived peptides corresponding to the respective binding epitopes that compete for the interaction *in vitro*. By transfection of chemically stabilized competitive peptides directly into the cells, we have shown the effect of specific interaction blocking on Axin-mediated signaling *in vivo*.

Our data demonstrate a proof of concept for a rational design of inhibitors of protein-protein interactions that allow specific intervention with single function of the targeted protein (i.e. recruitment to the Axin complex). Contrary to the inhibitors that completely disrupt the protein function (e.g. inhibition of a kinase catalytic site), this approach provides a tool for investigating specific action within the Axin complex, while the other cellular functions of the targeted protein remain preserved.

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P-02.02.2-077
Role light chain constant domain switch in structural organization of organophosphate metabolizing antibody

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De novo design of an artificial biocatalyst using immunoglobulin template became rather routine procedure due to the achievements of molecular biology and crystallography. Recently the 'reactibody' approach was developed based on the chemical selection of catalytic repertoires from immunoglobulin library followed by expression of these biocatalysts in eukaryotic system. In this study we structurally characterize the A5 antibody, its kappa and lambda variants, in order to understand the difference on the active site between A5 and A17 which although there are two antibodies sharing very high homology and sequence identity their active residues are located in a different region of the antibody. The structures of the A17 antibody kappa and lambda variants have been already determined, there was no structural information though about the A5 antibody. The structural analysis revealed dramatically different angle in position of nucleophilic residue Tyr33 and area of solvent accessible surface. The structural difference of active center reflects on kinetics of the A5 organophosphate modification. Both variants of antibody bind with organophosphate throw induce-fit mechanism, but rate of the step of induce fitting is different (K_{obs} are 35 s^{-1} and 16 s^{-1} for A5kappa and A5lambda respectively). This observation may hint at novel means of regulation of velocity and specificity of artificial biocatalysts.

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P-02.02.2-078
Structural and functional features of translation elongation factor 1Bα

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Translation elongation factor 1Bα (eEF1Bα) is a component of a heavy form of translation elongation factor 1 (eEF1H). It functions as a catalyst of GDP/GTP exchange in translation elongation factor 1A (eEF1A) restoring its active conformation appropriate for aminoacylated tRNA binding. eEF1Bα forms a tight complex with translation elongation factor 1Bγ (eEF1Bγ) via the N-terminal domain, while its C-terminal domain executes the catalytic activity. eEF1Bγ has been shown to enhance the attributed to the C-terminal domain catalytic activity of eEF1Bα. This suggests that the eEF1Bα N-terminal domain may influence the guanine nucleotide exchange process. To test this hypothesis we prepared a set of N-terminal truncated forms of human eEF1Bα and checked their activity in the guanine nucleotide exchange assay on both isoforms of eEF1A, eEF1A1 and eEF1A2.

We showed that recombinant eEF1Bα is a non-globular monomeric protein in solution with an elongated shape by analytical ultracentrifugation approach. The truncation of the dispensable for the catalytic activity N-terminal domain of eEF1Bα resulted in significant acceleration of the rate of guanine nucleotide exchange in eEF1A2 comparing to full-length eEF1Bα. Similar effect on the catalytic activity of eEF1Bα was observed after its interaction with eEF1Bγ. In contrast, the effect of full-length

eEF1Bα and its truncated forms on the rate of guanine nucleotide exchange in eEF1A1 was similar but relatively modest compared to eEF1A2. This can be explained by higher rate of spontaneous GDP dissociation from eEF1A1 comparing to eEF1A2 and lower affinity of eEF1A1 to eEF1Bα.

Thus, we propose that the N-terminal domain of eEF1Bα via flexible linker region may interfere with eEF1A binding to the C-terminal catalytic domain that results in a decrease of the overall rate of guanine nucleotide exchange reaction. The formation of a tight complex between eEF1Bγ and eEF1Bα N-terminal domains abolishes this inhibitory effect.

P-02.02.2-079
Assessment of quantitative proteomics results in large-scale data-independent with fragmentation spectra reproducibility measure reduces variation and allows to use low-intensity signals

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Organisms with reduced genomes that lack the vast majority of transcriptional or translational regulation systems tend to adapt to changing environment with a variety of subtle changes in protein abundances. As soon as relative changes for most proteins fall below 50%, the power of traditional label-free proteomic analysis rapidly becomes insufficient for robust profiling of hundreds of samples. Intoduction of frament-by-fragment and sample-by-sample signal quality assesment in MRM and DIA experiments helps to increase accuracy of methods and at the same time reintroduce cases which could have been excluded during bulk quality assessment due to lower signal-to-noise ratio for several fragments.

240 samples of Mycoplasma gallisepticum were acquired in data-independent manner on Sciex TripleTOF 5600+ mass-spectrometer (SWATH acquisition) during the year. The samples were produced from Mycoplasma gallisepticum culture cultivated at different temperatures. The signals for each fragment were extracted with vendor-supplied software with the theoretical fragment ions for each peptides instead of spectral library. The results were used for relative protein quantitation in two manners – the first conventional method included direct use of peptides with top 3 most intense signals. The second included selection of peptides and ions for quantification for each pair of samples based on the reproducibility of fragmentation patterns after computing the areas of chromatographic peaks for each ion. Spectral angle was used as a distance measure for fragmentation patterns for clustering. Further, a base set of detected ions was selected for each peptide and a subset for comparison of each pair of runs.

The first method resulted in quantitation of 354 proteins across all samples with variation across LC-MS replicates was 21% on average, and the second approach led to quantitation of 515 proteins in total, 390 of them across 75% of samples, all with the variation about 11% on average.

P-02.02.2-080
Interaction of plasminogen fragments K 1-3 and K 5 with fibrin fragment DD

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Introduction: Plasminogen interaction with specific binding sites in C-terminal D-domains of fibrin molecule initiates the activation process of proenzyme and subsequent fibrin clot lysis. The sites are exposed under fibrin polymerization. Plasminogen kringle domains ensure the proenzyme interactions with fibrin clot. In this study, we investigated the binding of human plasminogen kringle fragments K 1-3 and K 5 with human fibrin fragment DD and their effect on Glu-plasminogen interaction with DD.
Results: Kringle-containing fragments K 1-3 and K 5 reduce plasminogen activation by tissue-type activator on fibrin fragment DD. The level of Glu-plasminogen binding to DD is decreased by 50–60% in the presence of K 1-3 and K 5. Fragments K 1-3 and K 5 have high affinity to fibrin fragment DD (dissociation constant is 0.02 μM for K 1-3 and 0.054 μM for K 5). Analysis of K 1-3 and K 5 binding to fibrin fragment DD with reduced disulfide bonds showed the interaction of both plasminogen fragments with γ-γ-chains of fragment DD.

K 1-3 interacts with complex of fragment DD-immobilized K 5 as well as K 5 with complex of fragment DD-immobilized K 1-3. The plasminogen fragments do not displace each other from binding sites located in fibrin fragment DD, but can compete for the interaction. Analysis of K 1-3 and K 5 binding to fibrin fragment DD with reduced disulfide bonds showed the interaction of both plasminogen fragments with α- and γ-chains of fragment DD.
Conclusions: Widely known specific plasminogen-binding site located in Aα148-160 region of fibrin molecule is not a single binding sequence of fibrin peripheral domains or plasminogen-binding site is not linear and contains amino acid residues from other polypeptide chains of fibrin D-domains. Fibrin fragment DD contains different binding sites for plasminogen kringle fragments K 1-3 and K 5, which can be located close to each other. Possible plasminogen kringle-binding sites are located in α- and γ-chains.

P-02.02.2-081
Implementation of budded baculovirus particles for characterization of ligand binding to G protein-coupled receptors

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G protein-coupled receptors (GPCRs) constitute the largest class of membrane receptors involved in regulation of signal transduction into the cell in response to various extracellular stimuli. For that reason, GPCRs have become important targets for variety of drugs. As these receptors are present in native tissues at very low concentrations, efficient recombinant expression systems are needed to produce sufficient amounts of protein. We have shown that budded baculovirus particles, which display GPCRs on their surfaces can be used as a source of receptors for the investigation of ligand-receptor interactions. This expression system can be used for radioligand binding assay as well as for fluorescence anisotropy-based assay (FA).

We have validated the system with budded baculovirus particles produced in *Spodoptera frugiperda* (Sf9) cells expressing human dopamine D_1 receptors using [^{3}H]SCH-23390 and

The FEBS Journal **283** (Suppl. 1) (2016) 129–417 DOI: 10.1111/febs.13808

Bodipy-FL-SKF-83566 as reporter ligands for corresponding assays. This system has many advantages, for example good signal to noise ratio, homogeneity of the receptor, high expression levels and long-term stability of the receptor preparation. FA method allowed real-time monitoring of reporter ligand binding in the absence and presence of different dopaminergic ligands, giving information about their kinetic properties. Association, as well as dissociation of the Bodipy-FL-SKF-83566 itself were rapid with an apparent half-life of $t_{1/2}$ = 38.5 ± 0.3 s for association (2 nM) and $t_{1/2}$ = 73.4 ± 3.8 s for dissociation. We determined the pharmacological profiles of different dopaminergic ligands in displacement binding assays with membranes of Sf9 cells or budded baculovirus particles. The data were in good agreement for both membrane preparations tested in radioligand binding as well as in FA assay. Obtained results indicate that budded baculovirus particles can be proposed as a source of GPCRs for performing fluorescence anisotropy as well as radioligand binding assays.

P-02.02.2-082
A preliminary study of plasma Gas6 levels in gastrointestinal cancer patients

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Gastrointestinal (GI) cancer includes a variety of cancer types affecting the structures and functions of the GI system, encompassing the GI tract and the accessory organs of digestion, from the esophagus, stomach, biliary system and pancreas to the intestine, rectum and anus. Despite the significant advances however, much remains to be learned in the spectrum of GI cancer. Several investigators have shown that both Gas6 and its receptors, Axl, Sky, and Mer are expressed in various types of cancers. However, the expression level of Gas6 in GI cancer remains unclear. The aim of the study was to determine and compare plasma Gas6 levels in GI cancer patients.

15 female and 27 male patients were included in the study (n = 42): 21 colorectal, 8 gastric, 4 pancreatic, 3 liver, 2 ampullary, 2 gall bladder and 2 esophageal. From all GI cancer patients, 2 ml venous blood was collected in citrate tubes before surgery. Blood samples were centrifuged at 3000 ***g*** for 10 min, and plasma samples were carefully removed and stored in −80 °C prior to use. The level of plasma Gas6 was measured using a commercial developmental ELISA kit (R&D Systems, Minneapolis, MN) which is validated by our laboratory.

Plasma Gas6 levels in cancer patients were determined as follows: 1.84 ± 1.1 ng/mL in colorectal; 1.16 ± 1.1 ng/mL in gastric; 1.63 ± 0.61 ng/mL in pancreatic; 3.91 ± 0.89 ng/mL in liver; 2.15 ± 0.26 ng/mL in ampullary; 1.34 ± 0.46 ng/mL in gall bladder and 2.32 ± 1.7 ng/mL in esophageal cancer.

Preliminary findings indicate that there is a relation between GI cancers and plasma Gas6 levels. Taken together, these results suggest that Gas6 may be a candidate biomarker for diagnostic use in GI cancer. Inhibition of Gas6 would be an attractive therapeutic target for slow down the progression of GI cancer.

Monday 5 September 12:30–14:30

Computational biology

P-03.03.2-001
Computational approaches as an assay for β-lactam hydrolysis in class A β-lactamases

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β-lactam hydrolysing enzymes, in particular carbapenem-hydrolysing enzymes, are an increasing clinical threat. Herein we show that Molecular dynamics (MD) and combined quantum mechanics/molecular mechanics (QM/MM) approaches are a predictive tool of carbepenemase activity in class A β-lactamases. β-lactam drugs are the most prescribed class of antibiotics worldwide, especially in the treatment of Gram-negative pathogens such as *Klebsiella pneumoniae* and *Escherichia coli*. These organisms produce β-lactamases, enzymes which hydrolyse the β-lactam ring, a key resistance mechanism. Class A β-lactamases have the ability to hydrolyse carbapenems, termed 'last resort' antibiotics. In particular, the KPC (Klebsiella pneumoniae carbapenemase) family are the most clinically important, and recently identified natural KPC variants show increased hydrolytic activity against ceftazidime, a third generation cephalosporin.

Here we use computational simulations of β-lactam hydrolysis by β-lactamases. In particular, molecular dynamics (MD) combined with QM/MM approaches have been used to model the deacylation of the carbapenem meropenem across 8 class A β-lactamases. This method has been extended to model cephalosporin hydrolysis across class A β-lactamases, including KPC variants.

These approaches calculated the free energy barriers and correctly distinguished carbapenemases from carbapenem-inhibited enzymes. Preliminary results suggest this protocol is also a predictive tool for ceftazidime hydrolysis. Further, MD simulations of 5 KPC variants (single and double amino acid changes) were analysed to identify structural changes in the active site, highlighting that variants differ in the size of the active site opening, corresponding with experimentally derived Kcat values.

These computational assays provide a predictive tool of β-lactam hydrolysis and has potential to provide insights into important mechanistic differences both across class A β-lactamases and within the same families.

P-03.03.2-002
Computational design of a novel poly–glutamic dendrimer-based platform as an anticancer therapeutic approach

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Computational techniques are useful to predict interaction models and molecular properties for the design of therapeutic agents and specific drug delivery systems, such as dendrimers. Dendrimers are hyperbranched macromolecules with repetitive building blocks and defined architecture and functionality. This work constitutes a rational innovative approach using multifunctional

poly (glutamic acid) (PG)-dendrimer as potential nanocarriers for cancer therapies, to specifically deliver tumor associated antigens (TAA) – mannosamine and MelanA – to target cells and to modulate cancer antigen intracellular trafficking within the cytoplasm to promote an efficient and selective antitumor immunotherapeutic effect.

The theoretical structures were obtained using X-PLOR software. The molecular dynamics simulation of PG-G4-dendrimer and TAAs was performed using Desmond. The electronic properties of the structures were determined by semi-empirical methods using MOPAC. Docking studies of TAA to PG-G4-dendrimer to mannose receptor (MR1) were performed using HEX 8.0.0 software. TAA LUMO atoms were conjugated to HOMO atoms of PG-G4 dendrimer using Maestro software.

Results showed that PG-G4-dendrimer displays 64 carboxylic end groups available for covalent interaction with TAAs. The HOMO molecular orbitals of the dendrimer was located on the α-carbon of the carboxylic acid groups from backbone chain and it preferentially interacts with LUMO molecular orbitals of amine group from TAAs. No differences in the gap energy of HOMO/LUMO of all PG-G4-conjugates. TAAs bind preferentially to α-carbon of COOH of backbone chain instead of COOH from side chain. Docking results showed that majority of TAA conjugated PG-G4-dendrimer binds to the core of the MR1 receptor. Increasing of the number of mannosamine conjugated to PG-G4-dendrimer more close and stable is the conjugated to the receptor.

This system shows promising results as a novel functionalized PG-dendrimers for cancer therapy.

P-03.03.2-003
Molecular modeling, structural analysis and identification of ligand binding sites of 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR) of *Theileria parva*: a potential target for antitheilerial

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Theileria parva is one of the the economically important protozoan of the Theileria genus belong to apicomplexa phylum which include *plasmodium spp.* and *Toxoplasma gondii,* causative agents of malaria and toxoplasmosis respectively. This parasite is the disease agent of tick-borne East Coast Fever (ECF) ranks first among the tick-borne diseases of cattle in sub-Saharan Africa. The disease caused by the parasite affects a large proportion of domestic and wild animals and leads serious economic losses in the world. Major problems in dealing with this illness are the high cost of drugs, development of resistance, and absence of effective vaccines. Thus, it is important to develop an efficient and affordable antitheilerial agent. For this aim, 1-Deoxy-D-Xylulose-5 Phosphate Reductoisomerase (DXR) which subjected to identify novel drug aganist malaria and toxoplasmosis, of *Theileria parva* was selected as potential target for improving novel inhibitors aganist ECF.

A computational molecular modeling approach was conducted to determine the 3D structure of *Tp*DXR by Phyre2. Energy minimisation and root mean square deviation (RMSD) was performed by 3Drefine and SuperPose servers. To ensure the quality of modelling, stereochemistry, energy profile and residue environment of modelled structure were checked by different servers and possible ligand binding pockets were identified by MetaPocket 2.0 server

A reliable 3D model for DXR from *T. parva* was modeled by using 3AU9 as a template. The Cα RMSD and the backbone RMSD deviations for the model and the template crystal structure were found to be 0.85 and 0.86 Å, respectively. The Ramachandran plot for the predicted model by RAMPAGE reveals that model shows an acceptable stereochemistry. Top three considered possible binding pockets have been identified. These results have important implications for future screens aimed at finding new and safe molecular entities active against *Tp*DXR through docking studies.

P-03.03.2-004
Molecular binding profile of protoberberine alkoloids on amyloid precursor protein-cleaving enzyme 1 (BACE1) as a drug candidate for Alzheimer's diseases

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Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder that leads to dementia and nowadays over 46 million people live with dementia worldwide. Because of the prevalence and economic burden of the disease, drug development studies have picked up speed and scientists especially focused on natural products.

AD is basically characterized with tau hyperphosphorylation and accumulation of amyloid β (Aβ) proteins. Aβ proteins are generated from sequential cleavages of amyloid precursor protein (APP) by β and γ secretases, and β-site APP cleaving enzyme 1 (BACE1) is a β secretase essential for Aβ production.

The alkaloids represent a very extensive group of secondary metabolites, with diverse structures, distribution in nature and important pharmacological activities. Protoberberine alkaloids, which belongs to isoquinoline alkaloid class, are widely arranged in many species of the Berberidaceae, Annonaceae, Fumariaceae, Papaveraceae, and other plant families. Recent searches showed that some of the protoberberine alkaloids such as berberine, palmatine, jatrorrhizine, columbamine, magnoflorine prevents the progress of neurodegenerative disorder. However, the mechanisms of them are not absolutely clear.

Therefore, we have aimed to elucidate the binding and affect mechanism of these alkaloids on the BACE1 open and closed forms in here. For this purpose, molecular docking studies were applied for these natural products to the both forms of BACE1 by using AutoDock Vina and it was subjected to explicit solvent simulations by AMBER molecular dynamic package.

Our preliminary studies indicate that GLY34, THR72, GLN73, PHE108, TYR198, LYS224, THR232, ARG235, THR329 residues of binding pocket have affiliations with all of the mentioned alkaloids and the binding of them generates alterations on closed form of BACE1.

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P-03.03.2-005
Regression analysis as quantitative method for determination of DST parameters of cow milk using its fat and protein contents

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The complexity of animal milk needs to apply numerous approaches and methods for its investigations. An understanding of the processes occurring in the milk can be used, for example, for quality control of the products. Fat and protein are main components of milk which have a significant influence at its colloid properties, such as dynamic surface tension (DST). The application of regression-correlation analysis to milk data enables to develop a reliable quantitative model.

The aim of our investigation was to perform the regression analysis to establish the relationship between above-mentioned parameters. For this purpose, we used a statistical software packages *R* version 3.1.2. DST was determined by BPA – 1P tensiometer. Milk fat (*F*) and protein (*P*) contents were measured by analyzer Bentley 150. This work was supported by the Russian Scientific Foundation (grant 14-16-00046).

Obtained formulas characterized the degree of influence of fat and protein contents of a milk sample for each of the DST parameters (σ_0, σ_1, σ_2, σ_3, λ_0, λ_1):

$$\sigma_0 = 61.1538 + 0.8908 * P - 1.2953 * F$$
$$\sigma_1 = 63.3297 + 0.5658 * P - 1.4132 * F$$
$$\sigma_2 = 55.4274 + 0.4585 * P - 1.0143 * F$$
$$\sigma_3 = 45.6265 + 0.34634 * P + 0.05197 * F$$
$$\lambda_0 = 6.0986 + 0.1543 * P + 0.1351 * F$$
$$\lambda_1 = 10.7832 - 0.1470 * P - 1.0302 * F$$

These formulas show that the maximum total effect of fat and protein contents influences at σ_0 and σ_1. A significant coefficient (> 1) before the fat is observed in the formula, which describes the value of the tilt of final part of the tensiogram (λ_1).

The resulting regression equations have fundamental importance. With their help it is possible to calculate the DST parameters without their experimental determination, positioning fat and protein contents data. Obtained DST parameters promote more complete characterization of the properties of the milk that may be used for dairy products.

P-03.03.2-006
Molecular studies of scorpion toxin and its mutants interactions with voltage-gated potassium channels

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The voltage-gated potassium Kv1.3 channel is mostly expressed in neurons and immune cells. Its blockage has a high therapeutic potential, for example, specific inhibitor ShK toxin is undergoing clinical trials on psoriasis.

Goal of the current study was an interface analysis in complexes of hybrid channel KcsA-Kv1.3 with peptide blockers agitoxin and its mutant forms. 3D structure was generated by homology modeling method using complex of mutated KcsA channel with charybdotoxin (pdb-code 2A9H) as a template and equilibrated by molecular dynamic simulation in Gromacs software. Analysis of hydrophobic and stacking interactions, hydrogen and ionic bonds of the toxin and potassium channels was performed for representative frames with optimal toxin orientations using program Platinum and APBS software package.

We performed contacts energy characteristics estimation to predict key toxin residues for binding process and possible mutation sites for changing selectivity against Kv1.x channels. The results of investigation are in good agreement with the experimental values of binding constants, obtained by competitive binding assays. Results of the conducted investigation may find an application in fundamental science and drug design.

The research was supported by the Russian Science Foundation grant No. 14-14-00239. Simulations were performed using the Supercomputing Center of Lomonosov Moscow State University.

P-03.03.2-007
Homology modeling and molecular docking study of the paraoxonase-1 and its polymorphic variants Q/R 192 and M/L 55 for non-statin lipid lowering drugs

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Paraxonase-1 (PON1) enzyme is an HDL associated ester hydrolase exhibiting paraoxonase, arylesterase and lactonase activity, and reduces the formation of atherosclerosis blocking the LDL oxidation and reducing levels of oxidized lipids. In this study, molecular docking approach and molecular dynamics simulation were applied for finding the affinity of non-statin lipid-lowering drugs to PON1 and its polymorphic structures PON1 Q/R 192 and M/L 55.

Fibrates (Bezafibrate, ciprofibrate, clofibrate, fenofibrate, gemfibrozil), phytosterols (Beta-sitosterol, brassicasterol, Campesterol, stigmasterol) and other lipid lowering drugs (ezetimibe, niacin, orlistat, probucol, and sibutramine) was obtained from pubchem database. X-ray crystallographic structure of human PON1 and its polymorphic variants PON1 Q/R 192 and M/L 55 was generated via 'MODELLER', homology modelling software, from human-rabbit hybrid X-ray crystal structure of PON1 (PDB code: 3SRE). 10 ns molecular dynamic simulation analysis was performed using GROMACS 4.5.5. Affinity of lipid lowering drugs to PON1 and its polymorphic variants was predicted by molecular docking approach using Autodock 4.2 suite.

Unlike other lipid lowering drugs that they have negative ΔG values for affinity, probucol, orlistat and betasterol was calculated by positive ΔG values (18.7, 12.3 and 1.4 kcal/mol). These values suggest that they may have no affinity to PON1 Q/R 192 polymorphic structure. In all drug groups, brassicasterol and stigmasterol to PON1-M/L 55 and sibutramine to PON1 Q/R 192 were calculated as the highest affinity. In generally, phytosterols predicted by high affinity to PON1 and M/L 55 polymorphic structures in comparison to other lipid lowering drugs.

Our study demonstrated that phytosterols predicted as high affinity compounds on PON1 structures may reduce the activity of antioxidant PON1 enzyme. This study need to be supported by *in vitro* and *in vivo* detailed studies.

P-03.03.2-008
Comparative analysis of mammalian prolaction receptor genes

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Prolactin and its cognate receptor, prolactin receptor (PRLR), are involved in over 300 distinct functions in mammalians. The mammalian PRLR gene consists of 10–13 exons and several 5′ and 3′ regulatory sequences. In this study, gaps and annotation errors in the rat PRLR gene were corrected by comparing the genomes of mammals and rodents and new putative exons were identified.

The rat PRLR gene sequences from two different sources (Rnor_6.0, NC_005101.4 and Rn_Celera, AC_000070.1) were used and primary analysis showed that both sequences contain several gaps (varying from 0.35 to 4 Kbp), corresponding to about 5.6% (10–11 Kbp) of the gene. Using the rat known PRLR mRNA exon sequences, it was found that the Rnor_6.0 PRLR gene has two exon-10 (one is about 2 Kbp long and the other immediately after this). Comparisons of mammalian and rodent PRLR gene structures showed that the 2 Kbp stretch is an assembly artifact. By comparing both gene sequences (and also other available rodent PRLR genes), the gaps in the rat PRLR gene were reduced from 5.6% to 3.8% (from 11 Kbp to 7 Kbp). Functional annotation of the gene revealed that *R. norvegicus* PRLR gene could have two more additional exons, exon-12 and -13, similar to *Mus musculus* PRLR gene. In mammals, PRLR mRNAs contain non-protein coding exons in the 5′ UTR (exon-1 and -2). In rats, there are 5 exon-1 variants, resulting from alternative promotor usages. Studies on the rat and mouse PRLR genes revealed that both rodents share 4 common non-protein coding exon-1 variants.

In conclusion, it is found that the Rnor_6.0 version of the PRLR gene has the highest number of unidentified base pairs (corresponding to 5.6% of the gene) and the second exon-10 is the assembly artifact. The rat PRLR genes in both databases have several gaps and our corrected version is the best available and characterized form of the rat PRLR gene.

P-03.03.2-009
In silico affinities of some statins to paraoxonase-1 enzyme

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Paraoxonase 1 (PON1) enzyme is an ester hydrolase associated with high density lipoprotein (HDL), having paraoxonase, arylesterase and lactonase activities, and has protective effects against cardiovascular diseases. Statins are cholesterol lowering drugs. The aim of this study was to examine the *in silico* affinities of statins on PON1 enzyme by considering polymorphic structures [PON1 (M/L) 55, (Q/R) 192].

The structure of the statins (atorvastatin, fluvastatin, lovastatin, mevastatin, pitavastatin, pravastatin, rosuvastatin and simvastatin) was obtained from Pub Chem database, and X-ray crystal structure of PON1 (PDB ID:3SRE) from Protein Data Bank. MODELLER software was used for homology modeling of PON1 and its polymorphic variants that's called as PON1 Q/R 192 and M/L 55. Amino acid sequence of human serum PON1 (uniprot: P27169) was used as the MODELLER template. All molecular dynamics simulations were carried out with GROMACS 4.5.5 software. Molecular docking calculations on each of the polymorphic structure of the PON1 was performed with Auto Dock4.2. suite.

For each substrate, Y71 residue showed open conformation in PON1 and M/L 55 polymorphic structures while Q/R 192 polymorphic structure showed closed conformation. In comparison between structures of PON1 variants, in most cases statins had lower affinity to Q/R 192 polymorphic structure than to the other variant. In this study, among statins, atorvastatin showed lowest but simvastatin highest affinities to PON1.

By considering that the high affinity drugs can have reducing effect of PON1 activities, it may be more appropriate to use the low affinity statins in hyperlipidemia treatment. However, these findings need to be supported with *in vivo* and *in vitro* studies.

P-03.03.2-010
Self-assembly of lipidoids for siRNA uptake and release mechanisms studied by molecular dynamics simulations

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Small interference RNA (siRNA) has the ability to bind a specific mRNA which provides silencing of selected genes. Nanocarriers made out of self-assembled lipidoids encapsulate siRNA and deliver them into target cells effectively.

In this study, a library of lipidoid structures is constructed and studied by molecular dynamics (MD) simulations in different solvents, including sodium acetate, to ferret out their self-assembling mechanisms. The effect of the protonation state of the head group of lipidoids on the final shape of the self-assembled carrier is also studied. We further examine the role of the size of hydrocarbon tails in the packing. We study the final topology and the geometry of the self-assembled lipidoids both in the presence and in the absence of siRNA.

We find that stable clusters form with as few as 20 chains. For lipidoids having neutral head groups, clusters are in the form of dense bundles, while those with charged head groups form spherical capsids which are depleted of the salt on the inside and having a salt rich phase on the outside. In the self-assembled structure, lipidoids are arranged so as to expose the nitrogen and oxygen atoms to the solvent. While partial capsids with these properties also form at lower lipidoid numbers, 200 chains are necessary to form a fully closed capsid. In the presence of the siRNA, the capsid assembles around the nucleotide.

The free energy to remove the siRNA from the assembly is calculated via repeated steered MD calculations utilizing Jarzynski's equality relating it to the irreversible work along and ensemble of trajectories. We therefore determine an optimal tail length for the most stable nanostructure, paving the way for designing nanocarriers with high efficacy.

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P-03.03.2-011
Correlation between the dynamic surface tension and parameters of cattle milk

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Milk is one of the most valuable products for humans and attracts a lot of interest of researchers in various fields such as biochemistry, biology, food science and technology. The methods of milk study are quite varied. We chose the combination of the ultrasonic and dynamic surface tension (DST) measurements with the possible correlations among the obtained parameters. The aim of this work is to study the correlation between the parameters of milk, such as a content of fat, protein, lactose, minerals, dry milk solids and DST parameters. For this purpose we used milk analyzer 'Klever–1M' and tensiometer 'BPA-1P'.

Three groups of animals were formed from clinically healthy Holstein cows at the age of 4–5 years according to the fat content in the milk sample. Group I – 5 cows (milk fat content 4.01 ± 0.41%), group II – 6 cows (milk fat content 3.32 ± 0.14%), group III – 11 cows (milk fat content 2.73 ± 0.20%). This work was supported by the Russian Scientific Foundation (grant 14-16-00046). The biochemical parameters of the milk samples of all three groups are in the range of the 'normal' values for healthy Holstein cows: protein content varies from 3.0% to 4.2%, lactose and mineral content varies from 4.6% to 0.7%, respectively.

The DST parameters ($\sigma 1$, $\sigma 2$ and $\lambda 0$) for the group I have strong positive correlations with the fat content in the studied milk samples. At the same time for the groups II and III the fat content in the milk indicates only medium positive and weak positive correlations with the $\sigma 1$, $\sigma 2$ and $\lambda 0$. Obtained absolute values of the DST parameters of the milk samples showed some differences between all three groups. Thus, the DST parameters are changing in direct proportion to fat content in the milk sample that can be explain by the primarily role of the milk lipids in the formation of the water/fat surfaces (such as fat globules, lipid-protein particles, etc.).

P-03.03.2-012
Exploration of allosteric paths in caspase molecules using energy dissipation

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Caspases are highly regulated aspartate-specific cysteine proteases that have major roles in programmed cell death; apoptosis. Effector caspases are at the terminal step of the pathway, hence they are considered as death switches. With the discovery of the presence of allosteric sites, these molecules attracted the attention of the pharmaceutical studies and became drug targets. As a result of the binding of small molecules to the dimeric interface, active site loops are shifted to an unfavorable position. This is associated with a network between distal allosteric sites and the active site loop.

An energy dissipation model was applied in order to analyze this matter in further detail. Perturbation of specific residues enable us to determine a possible signaling network in proteins using external energy as an input, while focusing on the dispersion of this energy between residues throughout the structure. Molecular dynamics simulations were performed with and without energy perturbation using NAMD software with CHARMM27 force field. Energy perturbation was applied by increasing the velocity of a chosen residue at the desired time step of the initial MD simulation. Energy change of each residue was calculated upon the application of perturbation.

As a result, residue response times, corresponding to the time of the response of a residue after the perturbation of another chosen residue, are obtained. Combining reponse time data with a residue interaction network, it is possible to construct a final network that shows the communication started by perturbation within the molecule.

It is shown that perturbation of allosteric sites result in the disruption of the catalytic sites given in literature. Our findings support this and also gives a little detail of the possible communication between distal allosteric site and the active site loops.

This finding enables the usage of this methodology for similar structures where the exact allosteric mechanism is yet not known.

P-03.03.2-013
Effect of complex mammalian membrane models with multiple membrane components on Ras protein nanoclustering

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Ras proteins are essential for the cellular signal transduction that regulates cell proliferation and differentiation and act as binary switches between GDP and GTP forms. A wide range of human tumors are associated with defective Ras protein signaling. The production of permanently activated Ras proteins is correlated with mutations in Ras genes. Experiments and computer simulations have shown that membrane-bound Ras proteins form non-overlapping dynamic nanosized subdomains (nanoclusters) in activation state-/isoform-dependent manner.

We performed coarse-grained molecular dynamics simulations to investigate the effect of complex mammalian membrane models on formation and evolution of Ras nanoclusters. A fundamental part of the plasma membrane is the phospholipids bilayer, which contains phosphatidyl-choline (PC), phosphatidyl-ethanolamine (PE), phosphatidyl-serine (PS), sphingomyelin (SM) and cholesterol (CHOL). The nature of lipid-lipid and protein-lipid interactions was studied in binary (PC:CHOL) and quinary mixtures (PC:PE:PS:SM:CHOL). Because the polar lipids are not uniformly distributed between the two leaflets of the membrane, the construction of the plasma membrane with five-component lipid mixtures took into account the asymmetry between the outer and inner mono-layers. The phospholipids chain saturation (combined with the presence of cholesterol) constitute the dominant factor in phase separation and was, therefore, modeled in different lipid tail combinations for various headgroups.

Using microsecond timescale simulations of membrane-embedded Ras proteins, we have shown that the nanoclusters are spontaneously forming dynamic structures whose behavioral characteristics is modulated not only by the Ras isoform, but also by the complexity of the membrane model. Furthermore, we showed that variations in the plasma membrane lipid composition have important implications in the localization of Ras protein nanoclusters.

P-03.03.2-014
Knowledge-based prediction model for characterization of microbial rhodopsins for optogenetics

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Optogenetics comprises biological methods to achieve gain or loss of function of well-defined events in specific cells of living tissue by means of targetable control tools that respond to light and deliver the effector function. Microbial rhodopsins (MRs) have been established as powerful light-sensitive tools for optogenetics. Acting as ion pumps or channels, MRs are used to induce cell (de)polarization to control neuronal activity in a wide range of living organisms. MRs are membrane proteins found in a large clade of organisms, including Eukaryotes, Bacteria, and Archaea. They share a common architecture of 7 transmembrane α-helices and a covalently linked retinal, which is employed to absorb photons for energy conversion or the initiation of cellular signaling. Major efforts are put into screening of natural and generating of synthetic MRs with desirable properties for optogenetics, e.g. ion selectivity. However, experimental study of MRs is difficult and resource consuming owing to, among other factors, low expression levels and protein stability. Thus, there is a need in developing of computational tools for identification of MRs with desirable properties.

We used non-redundant atomic structures of MRs taken from Protein Data Bank to develop a set of numerical descriptors that reflects functional properties of MRs. Then, we calculated the descriptors for non-redundant sequences of MRs with known function taken from the UniProt database, resulting in the feature matrix. We applied the support vector machine and the 5-fold cross-validation procedure, using the feature matrix as the training set.

As a result, we obtained the classifier that discriminates MRs in terms of the ion selectivity, e.g. Na^+, H^+, or Cl^- pumps, with high precision. Finally, we used the derived classifier on a test set of proteins and identified MRs for the further experiment *in vivo*. This work was supported by RSF 16-15-00242.

P-03.03.2-015
Peptide-based inhibitor of RecA protein activity

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Rational design of peptides with required stability and functional activity properties becomes a real instrument for the new generation drug development. The RecA bacterial protein (and human Rad51 homolog) is considered to be the central catalyst of homologous recombination, a mechanism essential for the accurate repair of double-strand DNA breaks. DNA repair via homologous recombination requires RecA nucleoprotein filaments assembly.

Using SeqOPT (http://mml.spbstu.ru/seqopt/), a novel method for α-helix sequence optimization we present the successful design of peptide sequences capable to maintain a very stable α-helix structure and to inhibit RecA activity.

Novel α-helical 18 amino acids peptide is constructed based on RecA-DNA complex structure. We observed *in vitro* inhibition of RecA ATP hydrolysis, DNA strand exchange reaction and RecA filament formation. Also, we observed lower *E. Coli* resistance to UV and SOS-response suppression *in vivo*.

P-03.03.2-016
Computational identification of promiscous enzyme activity for the Morita-Baylis-Hillman reaction

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Enzyme promiscuity attracts considerable attention in terms of enzyme evolution, protein engineering and biocatalysis. Especially, development of highly efficient novel biocatalysts starting from promiscuous enzymes that have the catalytic machinery to perform desired chemistry is an intense area of research in recent years.

In this work, we computationally explored the catalytic promiscuity of natural enzymes for the synthesis of Morita-Baylis-Hillman (MBH) adducts, which display antitumoral activity against human cervical cancer cells, by mining structural protein databases using quantum mechanically optimized theoretical active site models (theozymes). Catalytic interactions in the active sites of selected hit proteins with potential MBH activity were evaluated in solvated dynamic environment using molecular dynamics simulations.

Computational screening of the protein data bank for the quantum mechanically determined optimal arrangement of catalytic functional groups for the target MBH reaction successfully identified an enzyme with experimentally determined promiscuous MBH activity.

P-03.03.2-017
Mammalian membrane model behavior in the presence of Ras protein nanoclusters

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Ras proteins mediate a wide variety of signal transduction pathways that regulate cell growth, proliferation and differentiation. These proteins are small GTPases that act as binary switches between GDP-bound 'off' and GTP-bound 'on' states. Oncogenic point mutations of Ras are associated with ~ 15% of all cancers and up to 90% in specific tumors and many developmental disorders. Both experimental and *in silico* results showed that the membrane-bound Ras proteins form non-overlapping *dynamic* nanosized subdomains called *nanoclusters* in an activation state-/isoform-dependent manner. We performed coarse-grained molecular dynamics simulations in order to investigate the formation and evolution of Ras nanoclusters in mammalian model membranes. Ras proteins were inserted into the cytoplasmic side of the plasma membrane model (di-C16:0-phosphatidyl-choline: di-18:2-phosphatidyl-choline: cholesterol 5:3:2) where they formed highly dynamic nanoclusters, both in size and in composition. Furhermore, we found that the presence of Ras protein nanoclusters has a significant impact on the model membrane behavior. Properties such as phase behavior, diffusion coefficient, surface tension and lipid tails order parameter are also influenced by the temperature variation of the model membrane.

P-03.03.2-018
Dynamics in protein crystals: insights from MD simulations complement new solid-state NMR and X-ray data

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We have investigated dynamics in three different crystal forms of ubiquitin, as well as ubiquitin in solution, with particular emphasis on (i) conformational exchange between β turn type I and II in the region 51–54 and (ii) rocking dynamics where protein molecules as a whole undergo subtle reorientational motion within the confines of the crystal lattice. Experimentally, both motional processes have been probed using relaxation dispersion techniques, including recently developed near-rotary-resonance dipolar relaxation dispersion experiments. Thereby it has been determined that rocking motion in one of the crystal forms (PDB ID 3N30) occurs on the time scale of tens of microseconds, whereas the conformational exchange has characteristic time constant of ca. 100 μs. Using Molecular Dynamics simulations, we have shown that the similarity of motional time scales is not accidental: βI↔βII exchange and rocking motion appear to be coupled. We have investigated the mechanisms of this coupling and predicted a number of point mutations that are expected to abrogate (or enhance) rocking. The crystals of ubiquitin containing these mutations have been modeled *in silico*. We have also investigated the interactions (in particular, crystal contacts) that control the balance between βI and βII conformations in different crystal forms. Finally, we have used MD simulations as a basis for chemical shift calculations and illustrated how relaxation dispersion effects can emerge as a function of βI↔βII exchange in conjunction with the rocking motion. The MD simulation study was supported by RSF grant 15-14-20038.

P-03.03.2-019
Molecular evaluation of the polypharmacological effect of quercetin on protein kinases

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Serine/threonine kinases are attractive targets in targeted cancer therapy due to their overexpression in several forms of cancer. Flavonoids are highly bioactive plant secondary metabolites that are important in human health due to their antioxidant property. Quercetin, a natural flavonoid derivative, has been shown to regulate several signal transduction pathways and is in phase I clinical trial as an anticancer drug. This study explored the inhibitory potential of quercetin and its derivatives using *in silico* methods like molecular docking and molecular dynamics simulations. Quercetin and its derivatives were observed to bind to several serine/threonine kinase family proteins with binding energy significantly better than other known inhibitors and commercially available drugs. This study thus sheds light on the atomic level interactions that define the polypharmacological nature of quercetin and its ability to interfere with a number of cancer pathways.

P-03.03.2-021
A new biosensor for fetal RHD detection from circulating cell-free fetal DNA in maternal plasma

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Introduction: Noninvasive prenatal diagnosis (NIPD) of the fetal RhD status by RHD genotyping of the maternal plasma was initially applied in alloimmunized pregnant women. Fetal rhesus D status detection for management of RhD incompatibility using circulating cell-free fetal DNA from maternal plasma or serum is now accepted by many obstetricians in Europe as reliable and useful. The aim of the study was to detect fetal RHD specific antibodies in maternal plasma using a nanopolimer based electrochemical biosensor.
Materials and Methods: A three-electrode system, consisting of a gold electrode, an Ag/AgCl reference electrode and a Pt counter electrode, was accommodated in a 10-mL electrochemical cell. Anilin and jelatin were used for immobilization of RHD antibody. The polimerization was occured at 319 nm UV light. Antibodies of RhD antigen were detected using differential pulse method at between 0.4 and 0.6 V potentials by observing the differentiations in the current values.
Results: The RhD status of the fetus was predicted in 20 RhD-negative pregnant women (8–36th week of pregnancy). RHD antibody were detected in maternal bood using biosensor in 15 of the fetuses. The results were confirmed with real-time PCR. The fetuses found RhD (+) for exon 5 and 7 of RHD gene by multiplex real-time PCR.
Discussion and Conclusion: Biosensors based studies might be useful, because they allow to monitor the molecular interactions in real-time providing qualitative and quantitative information, through kinetics, affinity and concentration analyses. We found that more advantages in comparison to other methods reported in the literature so far; it was determined that the method is sensitive, specific, economic, practical and less time-consuming. Fetal RHD detection at low concentrations and in the early week of pregnancy is possible with this method.

P-03.03.2-022
Investigation of phylogeography of *Cricotopus sylvestris* (Diptera: Chironomidae) using mitochondrial and nuclear molecular markers

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The family Chironomidae is one of the most widely distributed insect families of Diptera, and this family is distributed in all continents and all habitats from the tropics to the Arctic in lakes, streams and puddles. In this study, we aimed to determine the dispersal of *C. sylvestris* using molecular phylogenetic markers not only in Turkey but in the world and to reveal from where this species may have entered to Turkey in the past.

C. sylvestris larvae were collected from 8 lakes across Turkey. After total genomic DNA extraction from body of larvae, fragments of two mitochondrial genes, *cytochrome c oxidase subunit I* (*COI*) and *cytochrome b* (*cytb*), and one nuclear gene, *carbomyl phosphate synthase* domain (*CPS*) of *CAD*, were amplified and sequenced. In addition, several sequences of these three genes of *C. sylvestris* from different countries of different continents such

as South Corea, Japan, Canada, Denmark, and Sweden were obtained from GenBank. All sequences were aligned using MEGA 6 and BioEdit version 7.0.9.0 and were used for phylogenetic analyses. Neighbour-joining (NJ) tree was created in MEGA 6 and PAUP 4.0b10 with 1000 bootstrap replicates. Maximum likelihood (ML) analysis was performed in RAXMLGUI 1.0 using GTRGAMMA model with 1000 bootstrap replicates. BEAST v1.8.0 was used for Bayesian analysis.

Our phylogenetic analyses indicated that the Japanese, South Corean and American *C. sylvestris* were different from European and Turkish members. Turkish members of *C. sylvestris* were closely related to European ones according to our Bayesian, NJ and ML analyses. In Turkish members, *C. sylvestris* collected from Hazar and Çıldır Lake was more ancient than those from Marmara, Sapanca, Çıldır, Aygır, Beyşehir, Eğirdir and Sıhke Lakes.

In conclusion, our results clearly suggest that several transoceanic dispersal events among the continents may have occurred and that the entrance of Turkish *C. sylvestris* to Turkey may have been from southeast and northeast of the country.

P-03.03.2-023
Metagenomic analysis of microbial community in fresh water lake

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Metagenomics is providing great help to explore world of unculturable microorganisms in the natural samples to enhance our knowledge about microbial diversity. Here, we have performed metagenomic analysis of fresh water lake bacterial community using 454 pyrosequencing techniques. We have observed a wide array of bacteria from phylum proteobacteria and family Enterobacteriaceae as well as very few viruses from Podoviridae, Siphoviridae and unclassified phages. We have conducted a metagenomics analysis with the primary focus on the examination of the community of bacteria in a fresh water lake ecosystem. Roche GS FLX Software gave us total 156 253 reads (with an average read length of 795.274 bp). There were 15 226 contigs having > 100 bp sequence length whereas 10 481 contigs with > 500 bp sequence length. For further analysis we have taken contigs with > 500 bp only. Further, we have analyzed the microbial community composition using BLASTN/BLASTX against NT/NR databases with *E*-value cutoff of 10^{-5}. ≥70% of total contigs were mapped to the reference with ≥60% contig match coverage. The community analysis revealed that domain bacteria is predominantly present (99.8%) in the water sample, followed by Eukaryota (0.02%), viruses (0.08%) and other sequences (0.02%). Most abundant phyla was proteobacteria (99.8%) and the most dominant family was Enterobacteriaceae (89%) followed by Xanthomonadaceae (10%), Vibrionaceae (0.4%), Pasteurellaceae (0.1%), Shewanellaceae (0.07%). We performed functional analysis of all 5974 contigs using Rapid Annotation using Subsystems Technology (RAST)[4] which detected 15 319 coding sequences and 197 RNAs in 619 subsystems. Among the classified CDS from RAST showed major CDS hits for enzymes involved in the subsystems amino acids and derivatives and the carbohydrate metabolism. The great diversity of microorganisms present in the Lake may reflect the human activity in the area.

P-03.03.2-024
Characterization of unknown proteins: Additional information available from MALDI-TOF mass spectrometry data

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MALDI-TOF mass spectrometry is a ubiquitous and widespread tool for protein identification. Once the protein sequence is unavailable, unambiguous identification cannot be performed, and predictability is limited by the presence of sequenced homologous proteins. We present a statistical approach to predict a number of structural, localization and functional properties of unknown proteins by direct analysis of mass distribution shapes of their post-cleavage fragments obtained from MALDI-TOF mass spectrometry data. Secondary structure of proteins is best predicted by their specific cleavage at the inertial hydropathy group amino acid residues (FILMV), with Thermolysin (AFILMV) being the closest commercially available reagent, leading to distinguishing between proteins with presumably α-helixes or β-sheets with 90% accuracy. Cellular localization of proteins is best predicted by their specific cleavage at the external hydropathy group amino acid residues (DEHKNQR), exemplified by GluC(phosphate)+LysC(DEK) cleavage. Protein location in the cell membrane and its localization character (monotopic/transmembrane, single-pass/multi-pass transmembrane) are predictable with ~ 75% accuracy by this single cleavage, with optimal combination of 3–4 cleavages improving the accuracy to ~ 80%. Functional prediction of proteins is the best among membrane-associated proteins with characteristic structural conformations. We attribute the differences in the mass distribution shapes to the characteristic clustering of amino acids residues with respective hydropathy properties that are involved in the formation of 3d structural conformations of proteins. The suggested approach allows for a non-parametric statistical prediction of uncharacterized proteins from their MALDI-TOF mass spectrometry data without knowledge or reconstruction of their primary sequence. Potential applications include proteomic studies of organisms with unavailable genomic sequences and highly variable proteins analysis.

P-03.03.2-025
CrossHub update 2016: identification of functionally important transcription factor targets based on the mutational analysis and associations with clinical characteristics

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The Cancer Genome Atlas (TCGA) represents a comprehensive database of genomic, transcriptomic and epigenetic alterations across more than 20 tumor types. Earlier we developed CrossHub tool aimed at multi-way analysis of TCGA data in the context of gene expression regulation. In the present work, the software was updated with new features that are described below.

CrossHub is a Python-based application. One of the features of CrossHub is the combining TCGA RNA-Seq co-expression analysis to ENCODE ChIP-Seq data in order to reveal most possible transcription factor (TF) targets and coupling miRNA-

mRNA co-expression to several algorithms of miRNA target prediction in order to enhance its efficacy.

The key feature of the updated CrossHub version is the analysis of the associations between expression ratio of TF to its targets and TF mutation status. This allows identification of TFs that are functionally (in)activated with driver mutations in a particular cancer type. The second novel feature of CrossHub is the analysis of associations between 'TF-to-targets' expression ratio and tumor characteristics (TNM classification, pathological stage), patient follow-up, *etc*. In turn, this analysis may result in the identification of 'TF-targets' functional relations that are important for disease progression, tumor invasion, response to chemotherapy.

Thus, CrossHub was supplemented with new features that can be useful for comprehensive TCGA data analysis. The updated version of CrossHub is freely available at http://sourceforge.net/projects/crosshub/. This work was financially supported by the Russian Foundation for Basic Research (grants 15-04-08731, 16-16-00114 and 15-34-70055) and RAS Presidium Program 'Molecular and Cellular Biology'.

P-03.03.2-026
Mutations leading to increased RNase production and streptomycin resistance in *Bacillus pumilus*

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Bacillus pumilus strain 3-19 which was derived from soil-isolated *B. pumilus* 7P using chemical mutagenesis is characterized by resistance to streptomycin (Str, up to 500 μg/ml) and ability to produce extracellular enzymes in quantities almost 10-fold higher than the parent strain. These features make the 3-19 strain suitable for industrial production of RNase (binase) which is known for its antitumour and antiviral properties and can be used as an RNA-degrading tool in molecular biology. The whole genomes of both mutant and wild-type *B. pumilus* strains were sequenced recently.

To reveal the exact genetic features responsible for RNase overproduction and Str resistance we have fulfilled comparative genome analysis of *B. pumilus* 7P and 3-19 strains. Facilities of RAST server, EDGAR platform and additional bioinformatics tools (Plasmid finder, Prophinder, bl2seq) were used.

It is found that both *B. pumilus* genomes under study contain an intact prophage, while only wild-type strain bears a 6 kb cryptic plasmid. None of the systems is inactivated in mutant strain according to the results of metabolic reconstruction. 3.4% of total CDSs differ in 3-19 strain in comparison to 7P one, 36% of them are hypothetical. The altered genes are involved in membrane transport, cell wall composition, chemotaxis, spore formation, carbohydrate metabolism, DNA metabolism, translation and transcription regulation. Mutation (K56N) leading to Str resistance is identified in 30S ribosomal protein S12p. Regulatory and coding regions of binase gene have no modifications. Candidate genes which can account for binase overproduction are selected.

Mutation K56N is classical in Str resistance and leads to enhancement of decoding accuracy while decreasing elongation speed. RNase overproduction is brought about by non-specialized mechanism since other hydrolases are also overproduced in mutant strain. Genes encoding extracellular serine protease, sporulation initiation phosphotransferase F, GNAT-family acetyltransferase and cell wall modifying enzymes are reported previously to increase production of degradative enzymes. The action of encoded by them proteins lead to increase of stability and release of secreted proteins to environment and to derepression of their transcription from negative regulators

P-03.03.2-028
Genomic characterization of two *Bacillus pumilus* strains

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Bacillus pumilus strain 7P has been identified on its ability to produce ribonuclease and different extracellular proteases. In order to increase inherent biosynthesis of proteases the 7P strain was screened on culture medium supplemented by streptomycin. Derivative *B. pumilus* strain 3-19 gains the resistance to streptomycin and also shows the increased ribonuclease activity. We used genomes of both these strains to explore streptomycin susceptibility and increased activity of hydrolytic enzymes.

Whole-genome shotgun sequencing was performed using a combination of pyrosequencing and ion semiconductor sequencing, which provided 23x (7P) and 30x (3-19) overall genome coverage. Assembled genome sequences of 7P and 3-19 strains included 9 and 8 scaffolds > 500 bp with a calculated genome size of 3 577 758 bp and 3 572 739 bp, respectively. The GC content was 42%. Both draft genomes have been deposited at GenBank (JOJX00000000.2 for 7P and JHUD00000000.2 for 3-19).

Detailed comparative genomic analyses of strains have been performed. We calculated average nucleotide identity (ANI) values between the genomes of our strains and 9 completed *B. pumilus* genomes deposited in NCBI database. Two *B. pumilus* strains (SH-B9 and SAFR-032) revealed the max. ANI value (95.17% and 94.65%, respectively). *B. pumilus* SH-B9 strain has been used as a reference for SNP calling in strains 7P and 3-19. 3828 SNPs for the 7P strain and 862 for the 3-19 strain were classified as nonsynonymous variants. 20 radical nucleotide substitutions from the 3-19 genome were not found in 7P genome. Among them, the mutation in the 56 codon of *rpsL* gene (coding 30S ribosomal protein S12) is probably associated with resistance to streptomycin. Also, two mutations in *rpoB* and *nusA* genes (coding RNA polymerase and transcription termination factor Rho, respectively) may be related to increased enzymes activity. Both our strains contain 143 protease-coding genes. Twelve of them are encoding extracellular proteases.

P-03.03.2-030
Directed evolution of functional properties of antibody, derived from combinatorial library of immunoglobulin genes

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Here we propose an algorithm that can predict an antibodies mutant forms with desired specificity. This algorithm allows to determine the position and type of amino acid residue for mutagenesis. Approach is based on a hybrid method of quantum and molecular mechanics (QM/MM) that allows to understand the reaction mechanism and the role of active center amino acids. Catalytic antibody A17, that is able to hydrolyze pesticide paraoxon, was selected as a model. However, the hydrolysis efficiency of paraoxon by A17 antibody is only 9 M^{-1} min^{-1}, that is insufficient for using this antibody as antidote. The main fundamental goal of our study is to determine the necessary conditions for

improving the binding reaction of paraoxon by catalytic antibody A17.

The hybrid QM/MM method allows to study the reaction mechanism of interaction of A17 with paraoxon. It was shown that the reaction proceeds via the classical S_N2 mechanism. The key step of the reaction is the proton transfer from the catalytic residue Tyr-37 to paraoxon. QM/MM approach determines position for mutagenesis – Leu-47 in light chain. For one of the mutant in this position – Leu47Arg – were predicted (i) increased probability of formation of a hydrogen bond between the catalytic moiety and paraoxon compared to the wild type antibody and (ii) smaller value of the diffusion coefficient, which reflects the best positioning of paraoxon in the active center. Steady-state kinetic analysis shows that Leu47Arg exhibits a 70-fold increase in k_2/K_D compared to A17 (90 M^{-1} min^{-1} vs. 1.35 M^{-1} min^{-1}). Double mutant Leu47Arg/Ser35Ala also has improved constants of interaction with paraoxon in comparison with the wild type antibody, however, a single mutant Leu47Arg still binds paraoxon three times better, that may be due to the fact that the serine in 35 position increase the nucleophilicity of Tyr37. Thus, our results are in line with our computed predictions.

This work was supported by RFMEFI60414X0069.

P-03.03.2-031
Immunohistochemistry based detection of offal tissues in meat products by image analysis

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Due to high prices of meat and meat products, low quality raw materials like offal tissues are commonly used in Turkey. In the retrospect of the studies for evaluating and detection of unwanted tissues in the sample is basic histological examination. The light microscopy techniques are very strong method if a researcher qualification is enough. A new researcher-independent method must be developed. Therefore, different tissues and organs constitute of unique mRNA and protein. Our method is based on this event, so the antigenic sites of the tissues can be detected by selected antibodies. The first set of the antibodies are for detecting muscle and adipose, consist on Muscle Actin and Adipose Triglyceride Lipase. This set is used for calibration on standard meat sample. The second set of the antibodies are detecting of offal tissues, consist on TRRAP and Casein. Anti-Casein antibody is selected because the mammary gland usage in grinded-meat is very common. Immuno-staining started with HIER (Heat mediated epitope retrieval), then classical IHC method applied to slides with DAB-chromogen. After all process completed the slides were photographed by LAS (Leica Application Suite) on microscope. The capture settings were remained same on both sets. Image capture size is 1392x1040 pixels and field of view (FOV) is 449 × 335 μm. All the image files were converted to binary for threshold operation. The threshold values of first set and second set were calculated and their ratios were compared. The formula is based on the distribution (DST) of pixel intensity (INT) over threshold (THRS) values on all FOV (axis: X, Y) as DST(x, y) = {1 |If INT(x, y) > THRS <<>> otherwise 0}. The results are good enough to detect the unwanted micro-structures on 80% raw meat and 20% offal tissue. Calculations proofed with ImageJ®. Future application of this method and OpenCV-based software algorithm is to port the source code to a single board computer (SBC) with a digital microscope connected.

Monday 5 September 12:30–14:30

Mechanisms of pro-inflammatory diseases

P-04.02.2-001
The effects of RAAS inhibition in rate limiting step by aliskiren on testicular torsion injury in rats

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Testis torsion is a urological emergency condition that results in necrosis of the testis if the condition is not treated. Unfortunately treatment of testis torsion is not fully understood, therefore clinical and experimental studies are performed continuously. Renin-angiotensin-aldosterone system (RAAS) contributed to pathophysiology of several diseases. Aliskiren (ALS) inhibits the renin on the first step of this system. Our aim is to investigate the protective effect of aliskiren on unilateral testis damage caused by experimental testis torsion and detorsion.

The forty-eight rats were separated into eight groups of six animals: SHAM, SHAM+ALS 200 mg/kg (oral) group, Torsion group (TOR), Torsion/Detorsion group (TOR/DET), TOR+ALS 100 mg/kg (oral) group, TOR+ALS 200 mg/kg (oral) group, TOR/DET+ALS 100 mg/kg (oral) group, TOR/DET+ALS 200 mg/kg (oral) group. In the TOR and TOR/DET groups, the left testes were rotated 720° clockwise together with the spermatic cord and tunica vaginalis in the scrotal space. The left testes of the rats were subjected to torsion and detorsion during 2 h. After experimental procedures, testicular tissues were examined by histopathologic and molecular analyses.

The IL-1B and iNOS mRNA expressions were increased in TOR and TOR/DET groups when compared with SHAM group. Both doses of Aliskiren administration decreased these expressions in TOR/DET groups. The stereological results revealed that Aliskiren administration promote the numerical density of mature spermatids in TOR and TOR/DET groups. The numerical densities of TOR/DET+ALS100 and TOR/DET+ALS200 groups were similar and these two groups have significant difference when compared to the TOR and TOR/DET groups. The administration of ALS may be useful for preventing ischemic damage on unilateral testes injury in rats.

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P-04.02.2-002
Vitamin D3 in ameliorating diabetes-induced liver failure associated with impaired VDR signaling and inflammation

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Introduction: Vitamin D_3 (D_3) has recently been recognized as a potent immunomodulator which acts through regulation of gene expression involved in immunity response thus affecting various inflammatory and autoimmune diseases. The study was aimed at investigating hepatoprotective role of D_3 in VDR-mediated regulation of pro-inflammatory factors in diabetic liver.
Materials and Methods: Type 1 diabetes was induced in male C57BL/J6 mice by i.p. injection of high-dose STZ (150 mg/kg b.w.). After 2 weeks of STZ-induced diabetes animals were treated with/without D_3 (15 IU/mouse per os) for 8 weeks. Blood serum $25OHD_3$ was assessed by ELISA. Rel-A, VPF, iNOS and VDR expression was measured by qRT-PCR and/or Western-blot.
Results and Discussion: Diabetes caused two-fold reduction of serum $25OHD_3$ level, indicative of D_3 deficiency. Significant alterations in D_3-endocrine system were found as is evident from reduced expression of CYP27A1, CYP2R1, VDBP and VDR at transcriptional and translational levels. These changes were accompanied by a marked increase in mRNA and protein levels of inflammation markers Rel-A, VPF and iNOS in hepatic tissue of diabetic mice. Diabetes also led to structural lesions in liver tissue. Complete restoration of $25OHD_3$ content and partial normalization of liver tissue structure were achieved after D_3 treatment. D_3 administration partially normalized expression of cytochromes involved in D_3 metabolism and hepatic pro-inflammatory factors. D_3 treatment prevented overexpression of Rel-A and phosphorylated p65/Rel-A translocation to hepatocellular nuclei that is most likely mediated through $1,25(OH)_2D_3$ and VDR.
Conclusion: Study confirmed that diabetes-induced liver abnormalities are associated with chronic inflammation that can be linked to impaired D_3 metabolism and deficiency. Our findings demonstrate protective VDR-mediated effect of vitamin D_3 against diabetes-induced liver injury.

P-04.02.2-003
Lavandula stoechas extract increased glucose uptake and protein levels of key signaling molecules in insulin resistant C2C12 muscle cells

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Introduction: The aim of this is to identify remedial effects of Lavandula stoechas, Anatolian traditional medicine, against metabolic disorders developed on the ground of insulin resistance. Ethyl acetate extract (EAE) of *L. stoechas* was investigated in C2C12 myotubes which were made insulin resistant by free fatty acid (FFA) treatment, for its effects on glucose uptake and as well as on the activation of AKT-1 (by pAKT/AKT ratio) molecule which plays a central role in insulin signaling through Serine (473) phosphorylation. In addition, the protein level of lipoprotein lipase (LPL) enzyme was also evaluated.
Material and Methods: C2C12 cells were made insulin resistant by palmitic acid (FFA) and effects of EAE on p-AKT (Ser473)/AKT ratio and LPL level were determined by SDS-PAGE/Western Blot. The effect of EAE on glucose uptake in insulin resistant cells were determined by the 2-deoxyglucose uptake assay.
Results: EAE at 25 and 50 μg/mL significantly increased the glucose uptake 120 and 182% compared to insulin resistant control cells. Metformin at 2 mM increased this parameter up to 132%. EAE increased pAKT Ser473/AKT level 43–37% and LPL expression 50–92% for 25 and 50 μg/mL, in insulin resistant myotubes, respectively ($P < 0.05$).
Discussion: EAE of *L. stoechas* improved impaired insulin sensitivity through both enhancing glucose uptake and activation of AKT1 molecule through Ser473 phosphorylation. In addition, it also considerably increased LPL level which has very important function in lipid metabolism.
Conclusion: Overall, results demonstrated that *L. stoechas* contain phytochemicals which can be effective for the prevention and also treatment of insulin resistance and associated conditions. Our research group is on the way for the identification of these 'bioactive' molecules with bioassay guided fractionation studies. TUBITAK (ProjectID: 112T442) supports this study.

P-04.02.2-004
Analgetic effect of docosahexaenoic acid is mediated by the neuron-astrocyte interactions in the spinal cord dorsal horn in a rat model of neuropathic pain

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Achievement of complete pain control is very difficult task, which requires a search for new molecular targets during the analgesic substances development. Considering the importance of glial cells and their signaling molecules, development of new gliotropic therapeutic methods is a promising direction in pain treatment. Polyunsaturated fatty acids, including docosahexaenoic acid demonstrating anti-inflammatory and antioxidant activity are of considerable interest.

Docosahexaenoic acid (DHA, 22:6 $n-3$) analgesic activity was studied using a chronic constriction injury (CCI) rat model. Animals were subcutaneously injected with DHA emulsion at a dose of 4.5 mg/kg (125 mM/kg) daily during 2 weeks after surgery. Collection of material for subsequent immunohistochemistry investigation was performed on day 28.

We clearly demonstrated that the activation of neurokinin neurotransmission and nNOS synthesis are coincided with the astroglial activation in the spinal cord dorsal horn (SCDH) superficial lamina during neuropathic pain development.

However, the detailed mechanisms of interaction between substance P (SP)-, NO-ergic systems and astrocytes in the spinal cord remain to be elucidated. Systemic administration of DHA to CCI animals reduced neurogenic pain intensity and duration, leading to an earlier stabilization of paw weight distribution and preventing the development of degenerative changes in denervated limb. This drug treatment reduced the level of the SP- and NO-ergic neurotransmission and decreased astrocytosis in the SCDH superficial lamina.

Thus, the ability of DHA to affect nociception is a promising and safe alternative to current pharmaceutical therapeutics. Immunohistochemistry studies carried out with the Russian Science Foundation financial support (agreement No. 14-50-00034), obtaining DHA and all manipulations with animals of the material was funded by RFBR according to the research project No. 16-34-00023 mol_a.

P-04.02.2-005
Circulating endothelial-derived apoptotic microparticles and AOPPs are related to high-sensitive troponin T in patients with chronic hepatitis C infection

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The aim of this study was to evaluate non-standard risk factors for cardiovascular events, such as endothelial dysfunction assessed by endothelial-derived microparticles (EMPs) ($CD144^+/CD31^+$), advanced oxidation protein products (AOPPs), and low-grade inflammation, that are potentially associated with elevated levels of high-sensitivity troponin T (hs-TnT) and N-terminal pro-brain natriuretic peptide (NT-proBNP) in patients with chronic hepatitis C (CHC).

Methods and Results: Eighty-six CHC patients and 60 healthy control subjects were enrolled in the study. Circulating levels of hs-TnT, NT-proBNP, AOPPs-albumin (the ratio of AOPPs to albumin content), EMPs ($CD144^+/CD31^+$), hs-CRP, and TNF-α were assessed. Compared with CHC patients, the CHC patients with diabetes mellitus (DM) had higher levels of EMPs ($CD144^+/CD31^+$) and AOPPs-Alb, which were associated with elevated hs-TnT levels (≥13.3 pg/mL). NT-proBNP positively correlated with TNF-α level in all CHC patients and this correlation was stronger in diabetic patients. In multivariate logistic regression analysis, the independent factors associated with the presence of elevated hs-TnT levels were the presence of DM ($P < 0.001$) as well as high levels of AOPPs-Alb, apoptotic EMPs ($CD144^+/CD31^+/AN\text{-}V^+$), and NT-proBNP ($P = 0.04$, $P = 0.03$, $P = 0.04$ respectively).

Conclusion: The prevalence of elevated hs-TnT were increased significantly in the diabetic patients with chronic hepatitis C. hs-TnT was related to non-standard risk factors for cardiovascular events, and circulating endothelial-derived apoptotic microparticles ($CD144^+/CD31^+/AN\text{-}V^+$) level was an independent predictor for elevated hs-TnT levels, potentially indicating some abnormalities in the myocardium.

P-04.02.2-006
The role of vitamin D is relationship with fibromyalgia and obstructive sleep apnea syndrome

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Objective: Comparison of serum Vitamin D levels of the patients who have Fibromyalgia Syndrome and Obstructive Sleep Apnea together; the patients who have only Obstructive Sleep Apnea; and healthy individuals; and assessing the connection between the pain and the dimension of the sleep disorder.

Material and Methods: 60 patients who were diagnosed with Obstructive Sleep Apnea and 40 healthy individuals who were similar in terms of age and gender were included in this study. The patients, who were diagnosed with Obstructive Sleep Apnea with the examination and sleep tests, were assessed according to the 1990 American College of Rheumatology (ACR) criteria in terms of FMS. Serum D vitamin level was measured by using the Ultra Performance Liquid Chromatography Method.

Findings: When the Fibromyalgia Syndrome and Obstructive Sleep Apnea and Pure Obstructive Sleep Apnea patient groups are compared with the Control Group, the vitamin D level was found to be low at a significant level ($P = 0.038$, $P = 0.001$, respectively). No significant difference was found between the Vitamin D levels in Fibromyalgia Syndrome, Obstructive Sleep Apnea and Pure Obstructive Sleep Apnea patient groups. A negative correlation was found between the number of the sensitive points and Vitamin D levels in Fibromyalgia Syndrome patients ($P = 0.013$).

Results: It has been concluded that the Obstructive Sleep Apnea and Fibromyalgia Syndrome Patients have low Vitamin D levels, and this situation must be considered in treatment modalities. On the other hand, the results obtained in the study make us consider that Vitamin D metabolism is not influential in the pathogenesis of the Fibromyalgia Syndrome and Obstructive Sleep Apnea togetherness.

P-04.02.2-007
Decreased chitotriosidase activity and levels in familial Mediterranean fever

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Introduction: Different studies have demonstrated that the change of chitotriosidase (ChT) activity and levels in multiple diseases. However, the change of ChT activity and levels has not been evaluated concurrently in patients with Familial Mediterranean Fever (FMF). In this study we determined ChT enzyme activity and levels in patients with FMF.

Material and Methods: The study included a total of 80 patients with FMF and 80 healthy controls. ChT enzyme activity and levels were measured and then compared between groups.

Results: We found decreased levels of ChT activity and levels in patients with respect to healthy controls. Additionally, we found no association between clinical disease activity and the activity and levels of ChT.

Discussion: Familial Mediterranean fever is an inflammatory disease. Several cytokines and inflammatory mediators are playing role on pathogenesis of the disease. Although ıt has been demonstrated that the increased concentrations of ChT in patients with FMF. We found lower ChT activity and concentrations in patients with FMF.
Conclusion: Serum ChT enzyme activity and concentrations may not be considered as a biomarker in FMF patients taking colchicine. New studies are needed to evaluate the changes of the enzyme activity, concentration and the role of ChT in patients with colchicines negative patients.

P-04.02.2-008
Inflammation-based scores in diabetes mellitus type 2 patients

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Chronic hyperglycemic state leads to an increase in subclinical systemic inflammatory response in Diabetes mellitus type 2 (DMT2) patients. Inflammation-based scores, neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR) and red blood cell distribution width to platelet ratio (RPR) are biomarkers able to quantify systemic inflammation. The aim of the study was to investigate association of the inflammation-based scores with short- and long-term glycemic control markers, and whether they could be used as indicators of glucoregulation in DMT2 patients.

The cross-sectional study included 92 DMT2 patients, treated at the Primary Health Care Centre Zenica from December 2015 to April 2016, distributed into groups according to glycated hemoglobin (HbA1c) values: A ($n = 59$, HbA1c $\leq$7.0%) and B ($n = 33$, HbA1c > 7.0%). Complete blood cell count, fasting blood glucose (FBG) and HbA1c measurements were determined at the Primary Health Care Centre Zenica and at the Department of Laboratory Diagnostics, Cantonal Hospital Zenica by standard laboratory methods. All statistical tests were performed using SPSS 19.0. *P* values

Fasting blood glucose and HbA1c were significantly higher in the group B compared to the group A ($P < 0.0005$). There was no significant difference of NLR, PLR and RPR between the groups ($P = 0.50$; $P = 0.220$; $P = 0.525$, respectively). Significant correlation of inflammation-based scores with FBG and HbA1c was found only between PLR and HbA1c in the group A of DMT2 patients ($r = 0.328$, $P = 0.011$).

Inflammation-based scores could gather meaningful clinical information, either diagnostic or prognostic, on a variety of hyperglycemic, inflammatory, cardiovascular and thrombotic disorders. Since there was no statistically significant difference of NLR, PLR and RPR between DMT2 patients with good and poor glycemic control, we conclude that these scores could not be used as indicators of glucoregulation in DMT2 patients.

P-04.02.2-009
Neutrophil-lymphocyte ratio and platelet-lymphocyte ratio are useful predictive markers in prediabetes and diabetes

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Chronic inflamation plays a central role in the development and progression of diabetes and in the pathogenesis of its comlications. The neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR) are indicators of subclinical inflamation. Mean platelet volume (MPV) is one of the platelet function indices that reflects the platelet production rate and stimulation. We investigated the association of NLR, PLR and MPV with prediabetes and type 2 diabetes mellitus (T2DM) and determine whether or not these are reliable markers for diagnosis.

We evalueted 76 people's results who were carried out oral glucose tolerance test (OGTT). Acording to 2-h values of plasma glucose in the OGTT; 1. group (normal glucose tolerance: NGT): under 140 mg/dL ($n = 42$), 2. group (prediabetic: impared glucose tolerance (IGT)): ranging from 140 mg/dL to 199 mg/dL ($n = 25$), 3. group (firstly diagnosed diabetic by OGTT): above 200 mg/dL ($n = 9$). 4. group is clear diabetic without complication (taking treatment) group ($n = 34$). We compered NLR, PLR, MPV and some biochemical markers between four groups.

There are significantly differences between all groups in NLR ($P = 0.004$) and PLR ($P = 0.021$) values. NLR values are significantly higher in prediabetic (1.60 ± 0.85), firstly diagnosed diabetic (1.58 ± 0.78) and clear diabetic (2.07 ± 0.95) groups compered to normal group (1.37 ± 0.69)(mean, SD). PLR values are significatly lower in prediabetic (90.35 ± 44.34) and firstly diagnosed diabetic (86.38 ± 45.24) groups compered to normal group (100.55 ± 48.14) but significatly higher in clear diabetic group (122.45 ± 37.43)(mean, SD). There is no significantly differences between all groups in MPV values.

Inflammation markers NLR significantly increases in prediabetic and diabetic patients. PLR significantly decreases in prediabetes and early diabetes but increases in late stage diabetes. NLR and PLR values may be reliable predictive markers in prediabetes and T2DM.

P-04.02.2-010
Diagnostic relevance of inflammatory markers in female patients with type 2 diabetes mellitus

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It is recognized that a chronic low-grade inflammation and an activation of the immune system are involved in the pathogenesis of insulin resistance and type 2 diabetes mellitus (T2D). This study aimed to analyze the long-term impact of altered metabolism in female T2D patients at the level of mediators of inflammatory response.

This study included 65 femaleT2D patients and 107 control subjects, which were recruited at the Clinical Center University of Sarajevo and the General Hospital Tesanj. In this study the effects of glycemic control on markers of the inflammatory response CRP, fibrinogen, leukocytes, sedimentation, and cytokine IL-6, were analyzed. All subjects included in this study were free of evidence infections, surgery, thyroid disease, polycystic ovarian syndrome, active liver and kidney damage. All biochemical analyses were performed by employing standard IFCC protocols.

Results from this study demonstrated significant increase of fibrinogen ($P = 0.0001$), CRP ($P = 0.001$), IL-6 ($P = 0.013$),

leukocytes ($P = 0.0001$) and sedimentation rate ($P = 0.008$) in female T2D population compared to control subjects. Interestingly, a significant correlation was shown between CRP and HbA1c ($P = 0.035$), IL-6 and glucose (0.032), IL-6 and BMI (0.007).

In our study, female T2D compared to the healthy population had significantly higher levels of fibrinogen, leukocytes, IL6, CRP and sedimentation. Other studies conducted in female population associated elevated levels of IL-6 and CRP with T2D independent of other risk factors for diabetes. CRP being most robust predictor of diabetes. Studies have shown that CRP is an important predictor of T2D for the female but not the male population.

Thus, our data suggest that inflammation play an important role in the pathogenesis in female diabetic population. A more detailed study on a far larger number of subjects should point out fact if they can effectively be used as biomarkers in the primary prevention of T2D in this population.

P-04.02.2-011
The relationship between vitamin D status and graft function in renal transplant recipients

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Objectives: Bone and mineral metabolism disorders hold an important place among the complications after renal transplantation. The purpose of this study was to demonstrate the relationship between vitamin D, calcium, phosphorus metabolism with graft function and to measure $1,25(OH)_2D_3$ levels with LC-MS/MS in renal transplant recipients.

Design and Methods: This study included 30 renal transplant recipients (10 female, 20 male; mean age: 40.30 ± 12.86) from living related donors were transplanted. Blood samples were collected immediately before and after transplantation at month 6. Serum creatinine, BUN, calcium, phosphorus, alkaline phosphatase, glucose, albumin, PTH, 25(OH)D and $1,25(OH)_2D_3$ levels were measured. GFR values were estimated by CKD-EPI. Plasma $1,25(OH)_2D_3$ levels were determined in a LCMS-8040 triple quadrupole tandem mass spectrometer (Shimadzu Corporation, Japan) by MRM. SPSS 20.0 software was used for statistical analysis.

Results: Although plasma $1,25(OH)_2D_3$ levels significantly increased ($P = 0.0001$), we did not find any significant differences for serum 25(OH)D levels after transplantation. When posttransplant levels of serum phosphorus, PTH, creatinin, BUN and ALP levels were found to be significantly decreased ($P = 0.0001$, $P = 0.011$ for ALP), we observed significantly higher calcium and GFR values ($P = 0.0001$). Vitamin D insufficiency was present 13.3%, deficiency 36.7%, severe deficiency 50% before transplantation, insufficiency was also seen 26.7%, deficiency 50%, severe deficiency 23.3% after transplantation at month 6.

Conclusions: In our study, all patients were found to vitamin D deficiency and insufficiency. Determination of vitamin D deficiency and consequently treatment with vitamin D supplements could lead to better graft surveys.

P-04.02.2-012
Association of free fatty acids with leptin in newly diagnosed type 2 diabetes

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Free fatty acids (FFA) represent important link between obesity, insulin resistance, Type 2 diabetes (T2D), and dyslipidemia. Increased adiposity, as approximated by body mass index (BMI), correlates well with increased serum levels of leptin-adipocyte derived hormone implicated in the regulation of adipose mass and alterations in insulin action and secretion. The main objective of the present study was to investigate the potential association of serum FFAs with leptin levels in healthy and newly diagnosed Type 2 diabetic subjects.

This study involved 13 newly diagnosed Type 2 diabetics and 13 healthy subjects. All participants in the study were free of evidence of hepatitis, viral infection or active liver and kidney injury. For biochemical analyses of glucose, glycosylated hemoglobin (HbA1c), and lipid profile, standard IFCC protocols were used. Analysis of free fatty acids (FFAs) was done by gas chromatography, while serum leptin levels were determined by the ELISA kit.

In addition to the expected differences in glucose, HbA1c, and BMI, our results also showed significant differences in leptin, myristoleic, palmitic, linolenic, arachidic, and arachidonic acids between T2D and control subjects. In healthy subjects, a significant correlation was demonstrated between glucose and lauric, arachidic, arachidonic acid levels, body weight, and BMI. Newly diagnosed diabetics showed significant association between glucose and lauric, myristoleic and linolenic acid levels; with leptin being associated with myristic and palmitoleic acid levels. Interestingly, in all participants, significant association was found between glucose and HbA1c, glucose and leptin, myristoleic, arachidic, and BMI as well as between leptin, arachidic acid, and BMI.

Thus, our data point out association of different types of FFAs with leptin levels in newly diagnosed Type 2 diabetics. However, further studies should be done in larger number of patients to confirm our results.

P-04.02.2-013
Evaluation of nitric oxide and angiogenic factors in rheumatoid arthritis and ankylosing spondylitis patients

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Rheumatoid arthritis (RA) and ankylosing spondylitis (AS) are chronic inflammatory diseases with distinct clinical manifestations in many ways. The aim of this study is to evaluate the serum levels of molecules which may be used as markers for angiogenesis and vascular leakage in the processes of two clinically different pictures, RA and AS.

30 RA patients, 30 AS patients and 30 healthy volunteers with mean age of 30–50 were included in the study. Serum levels of

The FEBS Journal **283** (Suppl. 1) (2016) 129–417 DOI: 10.1111/febs.13808

VEGF, Angiopoetin-2 and Tie-2 were measured by enzyme-linked immuno-sorbentassay (ELISA) using a commercially available kit. Serum nitricoxide levels were evaluated by the Griessreaction.

Serum VEGF, Ang-2 and NO levels were significantly higher in the AS group (VEGF: 316.4 ± 97.1 pg/ml; Ang-2: 321.1 ± 89.7 pg/ml; NO: 305.8 ± 77.0 pg/ml) and RA group (VEGF: 299.4 ± 52.4 pg/ml; Ang-2: 306.3 ± 80.8 pg/ml; NO: 308.1 ± 61.2 pg/ml), compared to the control group (VEGF: 185.7 ± 83.9 pg/ml; Ang-2: 168.1 ± 25.6 pg/ml; NO: 253.1 ± 71.2 pg/ml, $P < 0.001$; $P < 0.001$; $P < 0.05$). No differences were found between AS and RA for Tie-2 ($P > 0.05$). VEGF, Ang-2, Tie-2 and NO levels were positively correlated in both AS and RA patients ($P < 0.001$), but no correlation was detected between clinically activation index DAS-28, BASDAİ scores and laboratory measurements such as sedimentation, CRP and anti-CCP ($P > 0.05$). When the diagnostic performance of the parameters were evaluated with the ROC analysisonly the performance of the Ang-2 in AS patients was sufficient (AUC (95% Cl): 0.718, $P < 0.05$).

Elevation of angiogenic factors in the serums of AS and RA patients supports the role of angiogenesis in the etiopathogenesis of these diseases. However, lack of relationship between disease activity leads to not to use these factors as a marker for clinical follow-up. Only Ang-2 measurements may be useful for the differantial diagnosis.

P-04.02.2-014
The evaluation of ischemia modified albumin as an early biomarker of acute myocardial infarction

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Introduction: Acute myocardial infarction (AMI), remains a leading cause of morbidity and mortality worldwide. Early diagnosis of AMI is very important because early treatment may reduce the extent of injury to the myocardium. Currently, biomarkers of myocardial necrosis such as myoglobin, CK-MB and troponins are highly sensitive and exhibit good specificity. However, these biomarkers increase after tissue injury, approximately 4–6 h after the cardiac event and detect only the consequences of prolonged ischemia. Recently, Ischemia Modified Albumin (IMA) has been assessed and found to be very useful for the diagnosis of myocardial ischemia and it is considered as a serum biomarker. The aim of the present study was to evaluate the serum level of IMA and determine the relation between patients with AMI and control group, in order to verify its potential as a novel marker for early detection of MI.
Materials and Methods: The study was performed with 25 patients and 37 healthy controls. Blood samples from all subjects were collected by venipuncture in plain tubes, and immediately centrifuged at 4000 ***g*** for 10 min at 4 °C. The serum samples were stored at −20 °C until analysis. The serum levels of IMA were determined using the Cusabio Biotech Human Ischemia Modified Albumin, ELISA Kit according to manufacturer's instructions. The results are given as International units/milliliter (IU/mL).
Results: Our findings revealed that IMA showed no significant difference between the groups.
Conclusion: Our results suggest that IMA assay is not a sensitive marker for early detection of ischemic hearth disease and cannot be used alone for the diagnosis of AMI. Prospective studies are needed to identify IMA's potential as a biomarker for AMI.

P-04.02.2-015
Neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio in polycystic ovary syndrome

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Polycystic ovary syndrome is a complex and multifactorial disease with metabolic dysfunction and the etiopathogenesis is not well established. Emerging data suggest that adiposity and chronic low-grade inflammation are involved in the development of the metabolic dysfunction. Neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) have recently been investigated as two new inflammatory markers used in the assessment of systemic inflammation in many diseases. The purpose of the study was to investigate their relation with PCOS patients.

The study population consisted of 44 patients with polycystic ovary syndrome and 23 healthy women controls. NLR and PLR obtained by dividing absolute neutrophil to absolute lymphocyte count and absolute platelet count to absolute lymphocyte count, respectively.

The neutrophil count (4.41 ± 1.6 vs. 3.77 ± 1.23, $P < 0.05$) and platelet count (320.34 ± 55.11 vs. 283.96 ± 52.17, $P < 0.05$) were higher in patients with PCOS compared to the control group. Lymphocyte count was 2.08 ± 0.43 in PCOS patient and 2.30 ± 0.57 in control group. The NLR and PLR of PCOS patients were significantly higher compared to those of the controls (2.18 ± 0.92, 1.69 ± 0.57 $P < 0.05$, 158.31 ± 32.85, 128.59 ± 31.94 $P < 0.05$, respectively).

In this study we found NLR and PLR were significantly increased in patients with PCOS compared to healty control. NLR and PLR were two useful inflammatory markers for assessment of patients with PCOS.

P-04.02.2-016
Effect of transforming growth factor β-1 on the expression of selected synaptic proteins in a murine model of acute liver failure

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Imbalance in neurotransmission in conjunction with neuroinflammation contribute to neurological dysfunction observed during acute liver failure (ALF). Own observations indicate that ALF in a mouse model is associated with altered expression and/or intracellular distribution of synaptic proteins. Since neutralization of TGF-β1 appears to improve the neurological score in ALF mice, we examined the possibility that increased levels of TGF-β1, caused by liver damage, may affect the expression of selected synaptic proteins.

Expression and/or cytoplasmic vs. membrane distribution of a number of functionally critical synaptic proteins in cerebral cortex and blood TGF-β1 were measured in C57Bl6 mice with ALF induced by single i.p. injection of AOM (100 mg/kg of b.w.) and after neutralization of TGF-β1 induced by single i.p. injection of ab-TGF-β1 (1 mg/kg) 2 h before AOM injection.

In ALF mice, blood TGF-β1 was increased, and the expression of presynaptic proteins: synaptophysin and synaptotagmin was increased in the cytosolic (S2) fraction by ~ 45% and ~ 30%, respectively, but was slightly depressed in the membrane (P2) fraction by ~ 20% and ~ 15%. AOM induced an increase of postsynaptic proteins: PSD-95 and nNOS by ~ 40% in P2 fraction. TGF-β1 neutralization resulted in a reduction in the expression of presynaptic proteins by ~ 30% in S2 fraction and ~ 20% in P2 fraction, in control animals and normalized their amount in the cytosolic fraction after AOM injection, but was ineffective with regard to PSD-95 and nNOS.

The results indicate that in ALF mouse, neutralization of cytokine TGF-β1 normalizes synaptophysin and synaptotagmin expression in the synaptoplasm, without affecting their synaptic membrane content. Effect of TGF-β1neutralization appear to be confined to the presynapse.

P-04.02.2-017
Protective effects of new selective COX-2 inhibitors on a rat model of severe acute pancreatitis

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25% of acute pancreatitis (AP) patients develop severe acute pancreatitis (SAP), which is is resulted in multiple organ dysfunction syndrome. An extensive inflammatory response occurs due to inflammatory mediators synthesized and secreted during SAP. Since preventing the inflammation in SAP is important in the prognosis of the disease, new drug candidates having strong anti-inflammatory effects will provide a new concept for therapeutic strategies against acute pancreatitis. Non-steroidal anti-inflammatory drugs (NSAIDs) show their effects by inhibiting cyclooxygenases (COX-1 and COX-2) and they play an important role in the pathogenesis of acute pancreatitis. Since conventional NSAIDs inhibit both COX-1 and COX-2, they have serious side effects on gastrointestinal system. Therefore, new highly selective COX-2 inhibitors having fewer side effects are needed.

In the present study, selective COX-2 inhibitory activities and cytotoxic effects of new series of 2-benzoxazolinone and thiazolo [3,2-b]-1,2,4-triazole derivatives previously synthesized as specific COX-2 inhibitors with no side effects on gastrointestinal system were investigated. Permeability of the compounds was tested by PAMPA using Caco-2 cells. Compounds were found highly selective, non-toxic and permeable. AP was induced in rats via retrograde injection of STC into the pancreatic duct system. Rats were pre-treated with saline or celecoxib or the new compounds before STC injection and sacrificed 24 h later.

The severity of AP was evaluated using biochemical and histopathological analyses. Edema, inflammation, hemorrhage and acinar cell necrosis were detected in the pancreatic tissue of SAP group. SAP was remarkably increased serum lactate dehydrogenase, AST, ALT, lipase and amylase activities and serum TNF-α, IL-1β, IL-2, IL-6 and IL-8 levels. Tissue myeloperoxidse activity was also increased. Pretreatment with the novel compounds reserved all these biochemical and histopathological parameters.

P-04.02.2-018
Role of YKL-40 in alopecia areata

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Alopecia areata (AA) is an inflammatory disease which is affects hair follicles, and sometimes nails. It is suggested that cytokine – mediated immunity plays an important role in etiopathogenesis of AA. This study was planned to evaluate the serum YKL-40 and TGF-β1 levels of patients with AA.

40 patients with AA and 40 healthy volunteers were recruited into the study. Fasting venous blood samples were collected from the participants and serum was obtained by centrifugation. Serum YKL-40 and TGF-β1 levels were measured by enzyme linked immunosorbent assay (ELISA).

Serum TGF-β1 levels in the patient group were significantly lower compared to the control group whereas serum YKL-40 levels were significantly higher in patient group. TGF-β1 levels of men and women with AA were found to be significantly lower than that of controls. While serum YKL-40 level of male control group is higher than the male patients, there were no significant differences between women groups.

The increased serum YKL-40 levels in AA patients suggests that YKL-40 plays a crucial role in the pathogenesis of AA.

P-04.02.2-019
Recent onset psoriatic arthritis induces early proatherogenic inflammatory signs

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Arterial immune mediated inflammation participates centrally in all stages of the development of atherosclerosis, from the initial lesion to the end-stage thrombotic complications. Although emerging evidence supports augmented cardiovascular morbidity

and mortality in cutaneous psoriasis (PsC) and psoriatic arthritis (PsA) as compared to the general population its underlying mechanism is poorly understood. Here we analyzed the inflammatory burden in recent onset of PsA patients without traditional cardiovascular risk factors (CVRF) in a transversal study measuring carotid intima media thickness (cIMT) (measured with ecodoppler), and proatherogenic inflammatory molecular markers like C-reactive protein (CRP), Interleukin 6 (IL-6), and soluble intercellular adhesion molecule-1 (sICAM-1) in comparison with control patients. cIMT values are similar in PsA (0, 59 ± 0.045*) and PsC (0, 62 ± 0.10) patients. However, both of them were significant increase compared with control (0.436 ± 0, 05). Regarding inflammatory markers IL-6 serum levels in patients with APs was higher than PCs (18 ± 2.9) and healthy controls (16, 3 ± 2.5) but the difference did not achieve statistical significance (*$P > 0.05$). On other hand mean of sICAM-1, value from patients with recent onset of PsA is significant higher than controls. PsC remain without significant changes compared to control (*$P > 0.05$). In addition mean value from patients with recent onset of PsA is significantly higher than in controls (*$P < 0.05$) and PsC group. Overall, preliminary findings suggest for the first time that patients with early PsA, without evident traditional CVRF have significant increased values of cIMT, sICAM-1 CRP against the general population control group. This data strongly supports that early CV molecular markers are increased after the first symptoms and signs of this disease even in the absence of traditional cardiovascular risk factors. Furthermore, this give new windows for a proper treatment.

P-04.02.2-020
Protective effect of TRAIL against proinflammatory cytokines on pancreatic beta cells correlated with decrease in DR5 and increase in DcR1 expressions

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Introduction: Proinflammatory cytokines are known to have destructive effects on beta cells, which contribute to Type 1 Diabetes (T1D) development. The combinatory effects of three of these cytokines in particular, namely TNF-alpha (TNF-α), IFN-gamma (IFN-γ), and IL-1beta (IL-1β), are claimed to render beta cells prone to T cell-mediated destruction. The recently identified anti-inflammatory feature of TNF-Related Apoptosis-Inducing Ligand (TRAIL), its possible protective role in this process. In this study, the effects of applications of TRAIL with TNF-α, IFN-ϒ, and IL-1β on beta cell viability and correlation of these effects with TRAIL receptor expression patterns were investigated.

Methods: Glucose-responsive insulin-secreting NIT-1 mouse beta cell lines were treated with TNF-α, IFN-ϒ, IL-1β, and soluble TRAIL (sTRAIL) individually and in various combinations. Cell viabilities were determined at 24 and 48 h by MTT assay. TRAIL ligand and receptor expression profiles on NIT-1 cells, and alterations in receptor expression levels following cytokine applications were determined by Western blotting analysis.

Results: TRAIL treatment did not have any cytotoxic effects on NIT-1 beta cells at 48 h, while increasing cell viability following IL-1/IFN/TRAIL and IL-1/TNF/TRAIL combined applications. Substantial levels of Death Receptor 5 (DR5) expression were detected on NIT-1 cells before applications, yet it displayed decreased levels at 48 h of TRAIL treatment. Lower levels of Decoy Receptor 1 (DcR1) expression detected prior to treatments increased significantly in contrast.

Discussion: The fact that TRAIL co-treatment with TNF-α, IFN-ϒ and IL-1β increased cell viability in NIT-1 beta cell lines along with reduction in DR5 death receptor expression and an increase in the decoy receptor DcR1 expression, points out to a possible protective effect of TRAIL in insulitis, and strengthens its potential as a putative therapeutic molecule in prevention of beta cell loss.

P-04.02.2-021
Whole-genome expression analysis in Turkish patients with Behçet's syndrome

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Behçet's syndrome (BS) is a multisystemic inflammatory disorder with a strong and complex genetic background. Being a prevalent disorder both in Turkey and also in the ancient trade road 'Silk Road' countries, BS is an important cause of impairment and disability owing to its chronic and relapsing nature. Besides, BS is reported to be an important cause of mortality among the young male patients. While the epidemiology of BS is substantially well documented, currently, the etiology, the molecular mechanisms underlying its pathogenesis, and the classification of the disorder remain to be elucidated.

Our aim was to disclose the disease mechanisms at molecular level in Turkish BS patients by obtaining, comparing, and analysing the transcriptome data of BS patients with age and gender matched healthy controls. For this purpose, by using the Affymetrix HG U133 Plus 2.0 microarrays, peripheral blood cell mRNA profiles of 30 BS patients (B) and 15 matched healthy controls (C) were obtained. Following bioinformatics, gene ontology, and pathway analysis, validation experiments of the identified prominent mRNAs were performed by QRT-PCR methodology.

The comparison of B vs. C yielded differentially expressed gene numbers of 30 and 625 for the chosen fold changes of 2.0 and 1.5 respectively ($P \leq 0.05$ for both). During gene ontology and pathway analysis, immune system process, immune system diseases, systemic lupus erythematosus, arthritis, and intestinal immune network for IgA production categories/pathways were significantly enriched. Clustering analysis revealed a molecular signature which accurately distinguished B and C samples, while the QRT-PCR analysis successfully validated the chosen mRNA transcripts.

This study documented differential expression of a large number of immune system and immune disease related genes in BS patients. The uncovering of the molecular disease mechanisms of BS will point to novel candidate molecules to be targeted for the treatment of the disorder.

P-04.02.2-022
Asymmetric dimethylarginine as risk marker of endothelial dysfunction in obese children

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Obesity is a public health problem in developed countries and worldwide with increasing prevalence through a relationship

primarily with atherosclerotic cardiovascular diseases as well as several metabolic disturbances such as increased insulin resistance and diabetes. Although several studies identified obesity as an independent risk factor for atherosclerotic cardiovascular diseases, the mechanism underlying the increased cardiovascular risk in obese patients has not been clearly delineated. ADMA, NO, Endothelin-1 and homocysteine are an indicator of endothel disfunction that plays an important role in the pathophysiology of atherosclerosis.

In our study, obese children and the control group were compared in terms of ADMA, NO, Endothelin-1 and homocysteine, we also investigated whether there is a correlation between these parameters. 58 obese and 30 healthy children, participated in the study.

When the obese group was compared to the healthy controls, the ADMA level of the obese group were significantly higher than those of the control group but there was no statistically significant difference in NO, Endothelin-1 and homocysteine.

Increased ADMA level might trigger the pathogenesis of atherosclerosis starting from the childhood years onward. That is why controlling obesity in this age group with diet and other treatment modalities will prevent the mortality and morbidities that will be seen in adult years.

P-04.02.2-024
Measurement of fetuin A, lipid peroxydation and thiol groups on people with C1 esterase inhibitor deficiency

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Introduction: The presence of C1 esterase inhibitor (C1İNH) in the complement system prevents the activation of C1 and other classical complement systems. The lack of this protein in the classical complement systems causes uncontrolled activations. C1İNH deficiency leads to the formation of bradykinin causing to dilation of blood vessels. Furthermore, the study conducted by Shagdarsuren, on the damage done by C1-esterase, demonsrates that the complement system and triglyceride levels are affected. We investigated lipid oxidation and fetuin A levels in patients with C1İNH deficiency.
Materials and Methods: 44 people with C1İNH and 44 people without any illnesses were taken into the study. Fetuin A was studied using an ELİSA kit from Raybio (USA). Ferrous ion oxidation-xylenol orange test was used to find LOOH serum levels. SH (Free thiol groups) test was studied with regards to Ellmans method modified by Hu. İBM SPSS 20.0 was used for statistical results.
Results: In assessments made between the healthy and the illness groups, there was significant differences in the levels of fetuin ($P = 0.035$), LOOH ($P = 0.000$) and SH ($P = 0.047$). When Pearson correlation analysis was performed, we detected a significant positive correlation between fetuin A and LOOH levels (r: +0.326)
Discussion and Conclusion: In these patients, lipids is secreted from the adipose tissue. In response, anti-atherosclerotic Fetuin A levels were risen. Patients also possessed increased lipid peroxidation, this increase shows positive correlation with fetuin A levels. In conclusion, we identified that SH with antioxidant properties have increased levels.

P-04.02.2-025
May caffeic acid phenethyl ester have a protective effect in high fructose induced fatty liver?

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Aim: High fructose corn syrups are found in soft drinks, juice beverages, breakfast cereals, most of the processed foods. It has been shown that high dose of fructose intake may lead to a reduction in the number of hepatocytes, deterioration of liver function, increasing reactive oxygen species and liver steatosis. The aim of this study was to explore whether Caffeic Acid Phenethyl Ester (CAPE) has any potential protective effect on high fructose diet-induced fatty liver model.
Materials and Method: Totally fifty rats were divided into five groups. Control group, %10 fructose administered group, CAPE group, %10 fructose + CAPE administered group and ethanol group. After 6 weeks, liver oxidant and antioxidant status, and blood TNF alpha, IL-6, and IL-8, tissue NFkB levels were quantified. Protein levels were investigated against, NFkB and p-NFkB antibodies and normalized and analyzed against β-Actin antibody by western blotting.
Results: Serum TNF-alpha, IL-6, IL-8 levels were found to be increased in fructose group compared with the control group ($P < 0.05$). In liver tissue of 10% fructose administered group, MDA, protein carbonyls and NO levels were higher than control group. However SOD activity did not show any difference among the groups. In the fructose administered group, caspase 3 showed liver apoptosis and was considered as positive. Acquired data revealed that NFkB protein level was decreased in the presence of CAPE while increment in NFkB protein level was observed in the fructose administered group compared with control group. In case of pNFkB antibody, increment observed in fructose only and both CAPE and fructose administered groups, respectively. In CAPE only administered group, there was a decrement in the level of pNFkB protein.
Conclusion: Depending on further analysis, experimental findings are expected to implicate the role of CAPE as a protective agent on high fructose diet-induced fatty liver model in relation of iNOS, NFkB and p-NFkB pathways.

P-04.02.2-026
The investigating association of hepcidin levels with iron homeostasis and inflammation variables in pregnant women with intrauterine growth restriction

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This study was designed to investigate hepcidin levels and their associations with iron homeostasis and inflammation variables in pregnant women with intrauterine growth restriction (IUGR). A total of 88 pregnant women were included in this study. Pregnant volunteers were divided into two groups (30 healthy pregnant

The FEBS Journal **283** (Suppl. 1) (2016) 129–417 DOI: 10.1111/febs.13808

women and 58 pregnant women with IUGR). Serum hepcidin, total free iron, ferritin, transferrin, transferrin receptor and interleukin-6 (IL-6) levels were measured by ELISA. Also, hemoglobin (Hb) and C-reactive protein (CRP) levels were determined in serum samples from the healthy pregnant women and the pregnant women with IUGR. There were significant differences in hepcidin, ferritin, transferrin receptor, CRP and IL-6 levels between healthy pregnant women and pregnant women with IUGR. Hepcidin, ferritin, CRP and IL-6 levels in pregnant women with IUGR were significantly higher than healthy pregnant women (p).

P-04.02.2-027
The mediators of systemic inflammation in lipopolysaccharide-induced neonatal sepsis rat model

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Sepsis is an excessive inflammatory response that causes shock, multi-organ failure and high mortality. Foreign bacterias and lipopolysaccharides lead to stimulation of endothelial cells to produce biologically active mediators such as proinflammatory cytokines and chemokines, cell adhesion molecules, and growth factors. Then these mediators could be act on targets, which were involved in the initiation of systemic inflammation in neonatal sepsis. Our aim was to indicate a protective role of Thalidomide and Etanercept, which have anti-TNF-α activity on systemic inflammatory response in lipopolysaccharide (LPS)-induced neonatal sepsis rat samples.

Thirty 7-day-old Wistar rats were randomly divided into five groups: a control group that received normal saline, a sepsis group that received LPS, Thalidomide, Etanercept and both Thalidomide and Etanercept treatment group that were administered with therapeutic agents 6 hrs after LPS injection. The rats were sacrificed at 24 hrs after LPS or normal saline injection ($n = 6$). Hepatic tissue TNF-α, IL-6, ICAM-1 and PDGF levels were determined by Enzyme-Linked Immuno Sorbent Assay (ELISA) method in all groups.

In sepsis group, tissue TNF-α, IL-6, ICAM-1 and PDGF levels were statistically significantly higher than in controls ($P < 0.001$). At same time, pretreatment with both Thalidomide and Etanercept were found statistically dramatically decreases the levels of TNF-α, IL-6, and PDGF when compared to sepsis group ($P < 0.001$). There were no significant differences in the ICAM-1 levels between the all treatment groups and the sepsis group.

Higher liver tissue TNF-α, IL-6, ICAM-1 and PDGF levels are associated with severe bacterial infection. These proinflammatory cytokines and angiogenic factors may be important in the endothelial dysregulation seen in sepsis. Therapeutic agents used in the present study can be help to avoid devastating effects of neonatal sepsis.

P-04.02.2-029
Neuroprotective effect of N-stearoylethanolamine under development of insulin resistance

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N-stearoylethanolamine (NSE) – is saturated minor compound of natural origin that represents the large family of signaling lipids N-acylethanolamines, which belong to endocannabinoid system. Considering the crosstalk between obesity-induced inflammatory response and its key role in synaptic dysfunction and neurodegeneration, our current study aimed to investigate the biological effect of NSE on brain tissue under high fat diet-induced insulin resistance.

Previously we found that NSE administration to insulin resistant rats caused normalization of liver and pancreas lipid composition followed by the improvement of glucose tolerance and insulin sensitivity (decline in serum insulin level and HOMA-IR value). Moreover, this effect of NSE correlated with inhibition of NF-kB translocation into the nucleus of peritoneal macrophages and decreased pool of serum TNFα level in obesity-induced insulin resistant rats.

Further experiments showed that fat overload triggered significant reduction in the level of main phospholipids (phosphatidylethanolamine, phosphatidylcholine and sphingomyelin), while there were no changes in cholesterol content. NSE at a dose of 50 mg/kg during 2 weeks of administration to insulin resistant rats showed a tendency to restore the phospholipid level that was accompanied by increased neural cell survival (91%) compared to rats without treatment (84%).

Neuroinflammation accompanied by intensive reactive oxygen species (ROS) production impairs neurotransmission in a wide range of neurodegenerative pathologies. Flow cytometry analysis detected that high fat diet triggered enhanced formation of ROS (O_2^-) in neural cells, while NSE treatment normalized O_2^- production to control values.

The present study indicates neuroprotective effect of NSE in metabolic pathologies (insulin resistance and diabetes type 2) related to the development of systemic inflammation.

Monday 5 September 12:30–14:30

Epigenetics and cancer

P-05.02.2-001
Quenching of cellular autofluorescence is necessary for specific detection of DNA methylation by flow cytometry compared to microscopy-based analysis

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Flow cytometry is used for quantitative analysis of global DNA methylation, but fluorescence microscopy is mostly preferred to qualitatively reveal intranuclear localisation of DNA methylation and its copattern with other markers. Both methods use a similar immunostaining protocol.

In this study, we aimed to compare these methods concerning the detection of the global amount of DNA methylation. For this, mouse embryonic fibroblasts were cultured either with or without phenol red and then stained for DNA methylation or positive controls (histone, betaactin, phosphoAkt) by specific antibodies, or nonspecific control antibodies. Some cells were incubated with trypan blue before or after the addition of antibodies. Fluorescence intensities were measured by the green fluorescence channel (530/30 nm). Autofluorescence spectrum of cells was analysed, and fluorescence channel used for DNA methylation detection was changed to red (650 nm LP).

A poor discrimination between signal and noise was detected due to cellular autofluorescence interfering with specific detection of DNA methylation by flow cytometry but not by microscopy. It was also the case for the other markers examined. Conventional advances to reduce autofluorescence such using phenol red free culture media or trypan blue quenching were not effective, but using the red channel regarding autofluorescence spectra allows detecting specific staining of DNA methylation by flow cytometry. But, green channel did work well for microscopy analysis.

Findings show that flow cytometry detection of DNA methylation requires much attention to quench cellular autofluorescence compared to detection by fluorescence microscopy. One reason could be that flow cytometry detects all cellular content, but manual image-based analysis can exclude cytosolic components.

These results suggest the usability of flow cytometry and microscopy as complimentary methods for DNA methylation detection, but optimisation to reduce autofluorescence is crucial for flow cytometry.

P-05.02.2-002
Anti-cancer efficacy of deguelin against lung cancer cells with and without docetaxel and cisplatine

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Objectives: Lung cancers are divided in two main groups as Small Cell Lung Cancer (SCLC) and Non-Small Cell Lung Cancer (NSCLC). Docetaxel (DTX) and Cisplatine are chemotherapeutic that has an anti-tumor activity against various solid tumors. The growing resistance against DTX and Cisplatine (Cis) still continues to be the biggest obstacle for the treatment success of NSCLC patients. Deguelin (Deg.) is a natural plant derivative and has an encouraging activity against a lot of human cancers. The comparison of the treatment activity of the separate and combined usage of Deg., which is a potential chemotherapeutic agent, and DTX, Cis which are used in standard treatment, is aimed in this study.

Material-Method: The IC50 doses of DTX, Cis and Deg. on the A549 and H1299 NSCLCcell lines were determined via the cell vitality tests in our study. The active concentrations determined were applied to NSCLC cell lines as Deg., DTX, Cis and their combinations. The impacts of the medicine are studied by applying flow cytometric analyzes (apoptosis, cell cycle), glutathione and reducted glutathione, colony formation, migration and angiogenesis analyzes on the treated cells and measuring the Oxidative Stress Index (OSI). Statistical analyse program, Rstudio (v.0.98.501) and the R-script language were used to examine the differences between the agents. The states in which the *P*-value was lower than 0.05 were accepted as statistically meaningful.

Results: We found that Deg. has pro-apoptotic, anti-migratory and cytotoxic potential on lung cancer cells. Deg. amplified Cis and DTX-related anti-cancer efficacy (increased apoptotic cell content and cytotoxicity, reduced migration). Also, Deguelin pre-treatment sensitized the cells DTX-treatment (reduced IC50 values). These effects were remarkable in p53-mutant cells.

Conclusion: Deguelin, solely, has anti-cancer potential on NSCLCcells. Both Deguelin pre-treatment and combinantion with standart chemotherapeutics result in enhanced anticancer efficacy.

P-05.02.2-003
Selenium pre-treatment increases the efficacy of standard chemotherapy regimens in lung cancer

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The 83% of the lung cancers are non-small cell lung cancers (NSCLC). Despite Docetaxel (DTX) and Cisplatine (CDDP) are agents used in the standard treatments of these patients and the recent improvements in the treatments, the response and remission rates observed on the patients are relatively nominal. Selenium (Se) is an essential diet component and is introduced to have a preventive impact on different levels of cancer. The aim of our study is to investigate the impacts of Selenium addition on anticancer feature and tumor prevention before or/and during NSCLC standard treatment.

The IC50 doses of DTX, CDDP and Selenium on the A549 and H1299 (P53 mutant) NSCLC cell lines were determined via the cell vitality tests in our study. The active concentrations determined and the stipulated available concentrations were applied to cell lines as DTX, CDDP, Se combinations. The impacts were compared by applying flow cytometric analyzes (apoptosis, cell cycle), glutathione and reducted glutathione, western blot analyzes on the treated cells and measuring the Oxidative Stress Index (OSI) and thioredoksin reductase activity.

Selenium pre-treatments reduced DTX-related IC50 concentrations at lower doses in both NSCLC cells. However, CDDP-related IC50 concentrations reduced dose-dependent manner. Selenium supplementation also altered cell-cycle charactheristics at several concentrations and combination regimens. The remarkably higher OSI values were observed after DTX treatment and OSI levels were found to be lower in selenium pre-treated NSCLC cells.

Selenium sensitizes NSCLC cells to DTX treatment at lower concentrations. However, this effect is obtained dose-dependent fashion for CDDP regimen.

P-05.02.2-004
HER2 overexpression drives Warburg effect in breast cancer cells

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Breast cancer is the most common female malignancy worldwide. Human epidermal growth factor receptor 2 (HER2) is overexpressed in 30% of breast cancers in association with aggressive

phenotypes. The prognosis of metastatic breast cancer remains poor in spite of advances in therapy. As such, HER2 has long been studied as a potential target for anticancer drugs. The modulation of intracellular signaling pathways leads to altered cell metabolism that triggers tumorigenesis and adapts cells to cancer cell metabolism. This characteristic hallmark of cancer metabolism is known as Warburg effect meaning energy production via enhanced glycolysis. Despite of several studies in breast cancer metabolism, little detail exists on the link between HER2 overexpression and Warburg effect. We have committed examining the nature of aerobic glycolysis in HER2 overexpression. In breast cancer cell line MCF7, HER2 overexpression (MCF-HER2) results in mitochondrial dysfunction with low mitochondrial membrane potential ($\Delta\Psi m$) and ROS accumulation. Intracellular iron levels are also higher in MCF7-HER2 cells than vector control (MCF7-vec). Additionally, MCF7-HER2 cells show enhanced levels of ATP and lactate in association with increase in glucose levels. We have found that complex I activity increases in MCF7-HER2 and decreases in knockdown of HER2 in HCC1954 cells that is HER2 positive breast tumor cell line. Based on these results, we conclude that there is a link between HER2 overexpression and metabolic indicators of Warburg effect.

P-05.02.2-005
Expression and methylation analysis revealed 11 microRNA genes deregulated by methylation and new potential target genes of miR-375 and miR-127-5p in breast cancer

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MicroRNAs (miRNAs) and methylation of miRNA genes play a great role in epigenetic deregulation in malignant tumors. The aim of our study was to assess the contribution of methylation to expression level alterations of 20 miRNA genes and to search for novel potential targets of these miRNAs.

To analyze alterations in expression we used qPCR technique with 2 references (RNU6, RNU48) and 30 paired (tumor/normal) breast cancer (BC) samples. For methylation analysis a methylation specific PCR and the same set of BC samples were used.

Significant downregulation was shown for miR-125b-5p, -129-5p, -132-3p, -193a-5p, -34b-3p, -212-3p, -127-5p, and -17-5p ($P \leq 0.05$, Fisher's exact test) in BC. We observed 10 miRNA genes to be hypermethylated and *MIR-191* – hypomethylated. Hypermethylation for 3 of these miRNA genes was shown for the first time: *MIR-132*, *-137*, and *-1258* (41–34% of BC cases). A significant correlation between methylation and expression alterations was revealed for 5 miRNAs with downregulation: miR-125b-5p, -129-5p, -132-3p, -193a-5p, and -34b-3p (Spearman's correlation coefficient (*rs*) was in the range -0.71 to -0.81, $P \leq 0.01$), and for 3 miRNAs with both scene (down- and upregulation) as well: miR-148a-3p, -203a, and -375 ($rs = -0.72$ to -0.93, $P \leq 0.01$). Comparative analysis of the data on expression alterations of 20 miRNA genes and 6 protein-coding genes, which were predicted as targets by miRWalk 2.0, revealed the negative correlation between expression levels for some potential miRNA-mRNA interaction pairs. For example, for pairs miR-375/*RHOA*, miR-375/*RASSF1(A)*, miR-127-5p/*DAPK1* ($rs = -038$ to -0.46, $P \leq 0.05$).

Thus, both miRNAs and methylation affect regulatory networks in BC. Novel potential miRNA-mRNA interaction pairs could be useful in the development of BC therapy approach.

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P-05.02.2-006
Hypermethylation of MIR-129-2, MIR-203, and MIR-107 microRNA genes is associated with metastasis of clear cell renal cell cancer

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Clear cell renal cell cancer (ccRCC) with metastases has pour prognosis: 5-year survival is about 9%. MicroRNAs (miRNAs) and methylation of miRNA genes play a great role in epigenetic deregulation in malignant tumors. The aim of our study was to find out miRNAs which methylation contributed to ccRCC progression, including metastasis, and to reveal potential target genes of these miRNAs.

To analyze methylation status, we used a methylation specific PCR as a method and a representative set of 70 paired (tumor/normal) ccRCC samples. We also used 19 post-mortal renal tissues from individuals without cancer history as additional control. For expression analysis we used qPCR method and 45 paired ccRCC samples.

We observed 9 miRNA genes (*MIR-124a-1/-2/-3, -9-1, -9-3, -34b/c, -129-2, -193a, -107*) to be hypermethylated, ($P \leq 0.05$, Fisher's exact test), 3 miRNA genes (*MIR-191, -148a, -212*) to be hypomethylated and *MIR-203a* with both scene (hyper- and hypomethylation was detected). Methylation of 7 miRNA genes (*MIR-124a-2/-3, -34b/c, -129-2, -107, -148a, -203a*) correlated with advanced stage and/or tumor size and/or dedifferentiation. Hypermethylation of *MIR-129-2, MIR-203a,* and *MIR-107* significantly correlated with metastasis presence ($P < 0.05$, Fisher's exact test). Besides, preliminary data revealed the positive correlation between hypermethylation of *MIR-129-2* and up-regulation of 3p protein-coding genes: *RARB(2), RHOA, NKIRAS1*, and *CHL1,* which were predicted as targets by miRWalk 2.0 (Spearman's correlation coefficients (r_s) was in the range 0.35–0.53, $P \leq 0.05$).

In conclusion, novel supposed interactions of *MIR-129-2* with target genes could be useful as missing chains in signaling pathways. Tests for hypermethylation of *MIR-129-2, MIR-203a,* and *MIR-107* could be suggested as markers of metastasis and pour prognosis of ccRCC.

P-05.02.2-007
ENO3 and PGM1 gene promotor methylation in hilar cholangiocarcinoma

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Hilar cholangiocarcinoma (HC) is a rare but aggressive tumor. HC accounts for about 60% of all cholangiocarcinoma cases. Because of difficulty in diagnosis and treatment HC is a clinical problem: early symptoms of HC are often non-specific and surgical resection is the only curative treatment for HC. It is well known that epigenetic alterations are linked to cancer development. The purpose of this study was to determine potential mechanisms of epigenetic regulation of genes related to energy metabolism in HC. We have performed bioinformatics analysis of The Cancer Genome Atlas (TCGA) project RNA-seq data with CrossHub software and found a number of genes involved in glycolysis and differentially expressed in cholangiocarcinoma. qPCR analysis revealed significantly decreased expression of *PGM1* and *ENO3* genes in a majority of HC samples which were known as up-regulated in other human cancers according to the literature date. On the basis of TCGA methylation dataset (450k Illumina microarrays) we supposed that CpG methylation of *PGM1* and *ENO3* promoters may play a role in their inactivation. Using bisulfite sequencing study we identified several regions within the gene promoters (*PGM1*: ~ 750 bp and ~ 330 bp upstream TSS; *ENO3*: ~ 750 bp downstream TSS) that are frequently methylated in HC samples (up to 60%, 12/20) with down-regulated *PGM1* and *ENO3* expression. Thus, we demonstrated frequent and significant *PGM1* and *ENO3* down-regulation associated with hypermethylation of the specific regions within the gene promoters in HC. The pattern of *PGM1* and *ENO3* gene promoter methylation suggests a possibility of ones to be used for the HC diagnosis and development new strategies for therapy. This work was financially supported by grant MK-8047.2016.4 from the President of the Russian Federation. The work was performed using the equipment of EIMB RAS 'Genome' center.

P-05.02.2-008
Iodine is decreased in cancerous stomach tissues in rats

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Introduction: The development of stomach cancer is a multifactorial and complex process and includes multiple epigenetic, genetic alterations and dietary/non-dietary factors. Iodine as an antioxidant may play a protective role against gastric cancer. The aim of this study was to investigate the changes in iodine level in rat with stomach cancer induced by N-methyl-N′-nitro-N-nitrosoguanidine (MNNG).

Materials and Methods: A total of 62 Sprague Dawley rats were randomly divided into six groups. 55 rats were administered with MNNG (200 μg/ml) by oral gavage on days 0, 14 and 21 to *initiate stomach cancer*. During the experiment, 28 rats died and those surviving were sacrificed in the 3rd, 5th, 7th, 10th and 12th months of the experimental period (Group I, II, III, IV, V, respectively). The control group (Group VI) contains 7 rat which are given only food and water for 12 months. The stomach tissue was examined histopathologically. And also, iodine levels in stomach tissue was determined using the Foss method.

Results: A decrease in iodine level was determined in stomach cancer tissue of rats in Group I-V compared with normal healthy stomach tissue in Group VI. When the control (Group VI) iodine level was taken as % baseline, the % iodine levels of all groups were determined as follows 71.78, 52.79, 40.76, 25.33 and 11.96 for Groups I-V, respectively. The pathological diagnosis of gastric cancer was adenocarcinoma.

Discussion and Conclusion: The iodine levels of Group I were higher than those of Group II ($P < 0.01$) and of Groups III, IV and V ($P < 0.001$) and also were lower than in the control group ($P < 0.001$). Iodine deficiency as one of the risk factors of stomach cancer strongly supports the necessity for the application of effective iodine prophylaxis in the areas with iodine deficiency. Iodine supplementation might be useful in stomach cancer therapy and therefore, further research is warranted. This study was supported by Ataturk University (project number: 2005/147).

P-05.02.2-009
Effect of water extract of Turkish propolis on mitochondrial membrane potential in human laryngeal epidermoid carcinoma cell lines

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Propolis is the generic name for the resinous substance collected by honeybees from the buds of various plant sources and it is used by bees to seal holes in their honeycombs, smooth out the internal walls, and protect the entrance of bee hive against intruders. The aim of this study is to investigate what kind of changes the Turkish propolis cause on mitochondrial membrane potential (MMP) of human laryngeal epidermoid cell lines (HEp-2), by considering its anticancer features.

Water extract of Turkish propolis (WEP, 1–3 mg/mL) and ethanolic extract of Turkish propolis (EEP, 0.075–3 mg/mL) were prepared and incubated with Hep-2 cell lines (24, 48, and 72 h). MMP was investigated with a flourometric method by using $DiOC_6$ (3,3′-Dihexyloxacarbocyanine iodide).

The most significant MMP decrease was seen on 72rd hour. Both WEP and EEP extracts at all concentrations decrease MMP according to that of control.

The recent studies have shown that propolis extracts have induced apoptotic cell death by decreasing mitochondrial membrane potential in various cancer cells. It was concluded that both WEP and EEP decreased mitochondrial membrane potentials on HEp-2 cell series according to control (0 concentration) depending concentration and time.

P-05.02.2-010
Investigation of the transcription factors involved in the regulation of the inducible gene expression at the chromatin level

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There are numerous transcription factors involved in the regulation of the inducible gene expression. Thus, transcription of proinflammatory genes, steroid hormone receptors, etc. is controlled by the group of factors triggering gene expression which includes NF-kB. Another group of factors is involved in the formation of the structure of the chromatin of the inducible genes regulatory regions, providing competence for gene expression. It is expected that this group of factors includes the proteins of NF1 (nuclear factor 1) family. There are few data suggesting that the NF1 factors maintain potentially active state of the chromatin of the hormone-dependent gene promoter regions. These findings initiated studies of the correlation between presence of the NF1 transcription factors on the chromatin of a gene regulatory region and the functional state of the gene *in vivo*. As a model we chose the rat tryptophan dioxygenase (*tdo*) gene which is expressed tissue-specifically in the liver under control of glucocorticoid hormones. Three constitutive DNase I-hypersensitive regions are identified in the regulatory region of this gene.

To conduct the study we used rat liver and kidney. The basic methods were electrophoretic mobility shift assay (EMSA), immunoblotting assay and chromatin immunoprecipitation combined with real-time PCR (ChIP-qPCR).

Using EMSA we found that the proteins of NF1 family interact with the constitutive DNase I-hypersensitive regions *in vitro*. Immunoblotting assay of the protein fraction from rat liver used in EMSA experiments showed the presence of the NF1-B1 isoform. ChIP-qPCR revealed statistically significant differences in the level of the factor NF1 enrichment of the *tdo* gene regulatory region between the rat liver and kidneys at $P < 0.05$.

These data suggest the involvement of the NF1 proteins in the formation of the chromatin structure of the rat *tdo* gene promoter region.

P-05.02.2-012
Epigallocathecin-3-gallate as a novel histone deacetylase inhibitor agent in chronic myeloid leukemia

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Reciprocal (9;22) translocation and BCR-ABL fusion protein that is responsible for developing leukemia are observed in more than 95% of Chronic Myeloid Leukemia (CML) cases. Epigallocathecin-3-gallate (EGCG) is a green-tea flavonoid and EGCG is proposed as a natural anti-cancer agent. Histone modifications which contain histone deacetylases (HDAC) and histone acetyltransferases (HAT) are parts of epigenetic regulations. HDACs play important roles in different human malignancies including leukemia via activation of abnormal signaling pathways. HDAC inhibitors have become remarkable therapeutic molecules for malignancies. The aim of this study is to determine the expression changes of leukemia-related HDACs with the treatment of EGCG in K-562 cells.

The cytotoxic effect of EGCG on K-562 cells was determined in time and dose dependent manner by WST-1 analysis. Total RNA was isolated from K-562 cells. Reverse transcription procedure was performed for cDNA synthesis and gene expressions were detected by RT-qPCR.

The expression level of HDAC4, HDAC6, HDAC11 gene that supports cell proliferation was down-regulated 2.29, 2.56, 3.81 folds in K-562 cells treated with IC50 dose of EGCG, according to control, respectively.

Our current findings suggest that is a polyphenol EGCG may be a hopeful agent in treatment of CML by HDAC inhibitory effect.

P-05.02.2-013
Serum carbonic anhydrase autoantibodies and chronic lymphocytic leukemia

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Chronic Lymphocytic Leukemia (CLL) is a disorder of morphologically mature but immunologically less mature lymphocytes and is manifested by progressive accumulation of these cells in the blood, bone marrow, and lymphatic tissues. Carbonic anhydrase (CA) is a metalloenzyme which is widely distributed in the living world, and it is essential for the regulation of acid-base balance. Anti-CA antibodies have been reported in many disorders, such as systemic lupus erythematosus, Sjögren's syndrome, rheumatoid arthritis, endometriosis, idiopathic chronic pancreatitis, type 1 diabetes and Graves' disease. The goal of this study was to investigate carbonic anhydrase I and II (CA I and II) autoantibodies in CLL.

38 patients with CLL and 39 healthy controls were included in the study and CA I and II autoantibody levels were investigated by ELISA.

The CA I autoantibody levels of CLL group were significantly higher than the healthy group ($P = 0.006$) while there was no statistical difference between serum CA II autoantibody levels of the groups ($P = 0.301$). We found a significant positive correlation between hemoglobin and hemotocrit levels in patients with CLL ($r = 0.931$, $P = 0.0001$). Cut-off value of 0.072 ABSU for anti-CA I was associated with 92% sensitivity and 41% specificity and a cut-off value of 0.047 ABSU for anti-CA II was associated with 48% sensitivity and 85% specificity for predicting CLL.

The CA I autoantibody levels in patients with CLL were found higher compared to control group and the results suggest that CA I autoantibody may be involved in the pathogenesis of CLL.

P-05.02.2-014
Epigenetic aberrations of chromosome 3 genes were revealed in breast cancer using NotI-microarrays

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Genetic and epigenetic aberrations can lead to the activation of oncogenes and inactivation of tumor-suppressor genes (TSGs) followed by the development of malignant tumors. In the present work we evaluated the frequency of alterations of CpG island methylation and DNA copy number in 47 paired (tumor/normal) breast cancer (BC) samples using comparative DNA hybridization on NotI-microarrays and original NIMAN software. The microarrays contained 180 NotI-clones associated with 188 chromosome 3 genes. Expression alterations were assessed with the use of qPCR technique, ΔΔCt method and original ATG software. In total, 35 NotI-sites with high (15–38% of cases) hypermethylation/deletion (HM/D) frequency were revealed in BC. Among genes associated with these sites, there are both known TSGs and TSG-candidates (ALDH1L1, VHL, CTDSPL, etc.) as well as genes, which involvement in breast oncogenesis was shown for the first time (LRRN1, FOXP1, PRICKLE2, etc.). NotI-microarray data were verified selectively using bisulfite sequencing for VHL, NKIRAS1, ITGA9, LRRC3B, and CTDSPL genes. Several genes with high HM/D frequency (ALDH1L1, EPHB1, ITGA9, and ROPN1) were tested for expression alterations using qPCR. Frequent (57–90% of cases) and significant (> 2-fold) down-regulation was shown for all of them in BC. The most significant expression loss was observed for ALDH1L1 gene – on the average 50-fold mRNA level decrease in 90% of samples. The involvement of the majority of genes with high HM/D frequency in breast oncogenesis was shown for the first time. These genes are novel TSG-candidates in BC. Functional hypermethylation associated with expression loss was shown for ALDH1L1, EPHB1, ITGA9, and ROPN1 genes thereby strengthening the speculation on tumor suppressor abilities of these genes. Methylation and expression analyses of genes, that were revealed by NotI-microarrays, were financially supported by grant 14-15-00654 from the Russian Science Foundation.

P-05.02.2-015
Functional hypermethylation of a number of chromosome 3 genes was revealed in colon cancer using NotI-microarrays

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Cancer is a disease of genome caused by genetic and epigenetic aberrations. NotI-microarrays, that were developed by prof. E.R. Zabarovsky, is a unique tool that allows us to simultaneously detect hypermethylation of CpG islands and DNA deletions – two major reasons of inactivation of tumor suppressor genes (TSGs). In the present work, the frequency of chromosome 3 genetic and epigenetic alterations in colon cancer (CC) was evaluated.

NotI-microarrays, that contained 180 NotI-clones associated with 188 chromosome 3 genes, were used for comparative (tumor/normal) hybridization of DNA from 24 paired CC samples. Data analysis was performed using original NIMAN software. Expression alterations were evaluated using qPCR technique and original ATG software.

In total, 24 NotI-sites with 20% and above hypermethylation/deletion (HM/D) frequency were revealed in CC. Among genes associated with these sites, there are several known TSGs and TSG-candidates (for example, *VHL*, *CTDSPL*, and *ITGA9*), but for the majority of genes, involvement in colon oncogenesis was shown for the first time (for example, *LRRN1*, *NBEAL2*, and *UBE2E2*). The highest HM/D frequency was observed for *ANKRD28*, *NKIRAS1*/*RPL15*, *ITGA9*, *CMTM6*, and *GORASP1*/*TTC21A* genes – 38–42%. Expression alterations were evaluated for 3 genes with high HM/D frequency (*PLCL2*, *PRICKLE2*, and *PPP2R3A*) and significant mRNA level decrease (> 2-fold) associated with hypermethylation was shown for all of them in the majority of samples.

A number of novel potential TSG-candidates was revealed in CC. Functional hypermethylation associated with expression decrease was shown for *PLCL2*, *PRICKLE2*, and *PPP2R3A* genes thereby enhancing the suggestion on tumor suppressor function of these genes. This work was financially supported by grant 15-34-70055 mol_a_mos from the Russian Foundation for Basic Research and the grant from RAS Presidium Program 'Molecular and Cellular Biology'.

P-05.02.2-017
Tectonic faults influence that reinforce radon emanation and cancer lung risk

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In many countries, radon is the second leading cause of lung cancer, which accounts from 3% to 14% of cases. It is obvious that the population of all the developed and industrial countries in the world spend most f their time, almost 80%. Therefore it is necessary to explore the obtained radiation dose, because of the presence of radon in a room due to the radon emanation from the soil and exhalation from a variety of building materials. The developed countries solve this problem of radon pollution as well as create a special monitoring services. The paper presents some data of 8 genes molecular-genetic analysis from patients with lung cancer who live in Almaty located in a foothill area of tectonic faults. The object of research were blood samples obtained from patients diagnosed with lung cancer who are receiving a treatment at the Almaty Oncology Center and living in the city of Almaty, where the level of radon activity exceeds the norm approved by the International Commission on Radiation Safety. As a control group relatively people living in the plains, characterized by a lower radon emanation have been considered. To determine mutations in the genes polymerase chain reaction with a subsequent analysis of restriction fragment length polymorphism has been conducted. The PCR products were subjected to hydrolysis by BstNI restriction endonucleases HaeIII, Ras I. Disturbances in the genes under consideration to variour types of cancer development. The analysis showed that examinees do not have mutations in the KRAS gene codons 12–13, which corresponds to a control group consisting of 90 people living in the city of Balkhash. On the whole, molecular genetic studies have shown that 44 examined patients do not have mutations in the KRAS gene. One mutation was been found in the EGFR gene.

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It may be connected with some peculiarities of the Kazakh population, which has not been studied earlier.

Keywords: Lung cancer, radon, mutation, RFLP.

P-05.02.2-018
The concentration of some trace elements (Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Ag, Cd, V, Hg and Pb) in the colorectal polyp tissues

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Aim: Polyps are abnormal growths of tissue that can be found in gastro intestinal system. They are most often found in the colon and rectum. Most polyps are noncancerous (benign) however, because of abnormal cell growth, they can eventually become cancerous. The aim of this study is to determine the concentrations of trace element contents in colon and rectum polyp tissues and whether there is any relationship between polyp tissue element levels and the disease.

Material and Method: The present study was conducted on total of 82 individuals including 57 patients and 25 healthy subjects. While receiving normal intestinal tissue from healthy control group; from the patient group both normal tissue and polyp samples were taken during colonoscopy procedure. The concentrations of the elements (Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Ag, Cd, Hg and Pb) were determined with Induced Coupled Plasma-Mass Spectrometer.

Results: The mean concentrations of Cr, Mn, Ni, Se and Ag in colorectal polyp tissues of patients were significantly higher than in colorectal tissues of control subjects (P is less than 0.05).

On the other hand the mean concentration of Cd and Pb in colorectal polyp tissues of patients were significantly lower than in control colorectal tissues of control subjects (P is less than 0.05).

There was no any significant difference between the groups in terms of concentrations of Al, Fe, Co, Zn, As and Hg (P is more than 0.05).

Conclusion: The differences found in some elements between polyps and a control tissues may provide an indication about the role of trace elements in the early stage (polyps) in the colon carcinogenic process and encourages further studies to confirm the involvement of such elements in neoplastic processes.

P-05.02.2-019
Initial screening the anti-cancer effect of *Vitex-agnus castus* in neuroblastoma cell lines

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The use of herbal medicines is steadily growing, with approximately 40% of the population use herbs to treat various illnesses in the western world. Vitex agnus-castus has been used since ancient times as a remedy. The aim of this study was to investigate the *in vitro* anticancer activities of *Vitexagnus-castus* oil. For this purpose, the cytotoxicity of *Vitexagnus-castus* oil in SH-SY5Y cells was investigated by crystal violet staining. EC50 was found to be 0.5%(w/w) *Vitexagnus-castus* oil for this cell line. This dose was applied to the cell for 24 h, and the cells were harvested for further studies. Vitex agnus-castus oil treatment increased Bax and p38 mRNA levels. On the other hand, Bcl-2, Bcl2 l1, Erk-2, JNK, caspase 3 and 8mRNA expression levels were reduced significantly with*Vitexagnus-castus* oil treatment while P53 and PTEN remained unchanged. These results indicate that another effector caspase such as caspase 6 or 7 may be involved apoptosis process which remains to be elucidated. Moreover, MAPK pathways, P38 and Erk, may be involved in *Vitex-agnus-castus* oil induced apoptosis in SH-SY5Y cells. These initial observations suggest that this agent might not be useful in treating cancers. Further detailed studies should be carried out to elucidate the exact mechanism of *Vitexagnus-castus* oil in neuroblastoma cell lines.

P-05.02.2-020
Investigating the role of interferon regulatory factor 4 in melanoma cell lines

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Melanoma is a skin cancer with a melanocyte origin that can occur in any part of the body that contain melanocytes. While melanoma is less common than other skin cancers, it causes the majority of deaths related to skin cancer. Several gene expression databases have shown that interferon regulatory factor 4 (IRF4) is upregulated in melanomas, and genome wide association studies linked variation at IRF4 locus with skin cancers. IRF4 was first identified to have roles in lymphocyte development and function. Studies have identified a 'non-oncogene addiction' of malignant cells to IRF4 in various hematopoietic cancer types.

The aim of this study is to investigate the role of IRF4 in melanoma cell lines. Lentiviral vectors were used to reduce IRF4 levels in melanoma cell lines. A GFP competition assay was performed to study the competitive fitness of melanoma cells with IRF4 knockdown (GFP positive cells) over melanoma cells with normal IRF4 levels (GFP negative cells). Cell cycle profiles were investigated in melanoma cells with IRF4 knockdown by propidium iodide staining. Migration potential was assessed as well by wound healing assay.

Our preliminary data showed a decreased competitive fitness for cells with decreased IRF4 levels. Cell cycle profiling showed increased G0/G1 and decreased G2/M levels in IRF4 knockdown cells compared to controls. Wound healing assay results showed no difference between controls for cells with reduced IRF4 levels.

Taken together, these results indicate that IRF4 knockdown affects the melanoma cell lines' survival and cell cycle profile, suggesting a non-oncogene addiction of melanomas to IRF4. These observations are largely similar to previous observations in hematopoietic cancers. Unravelling the role of IRF4 in melanoma will increase our knowledge about melanoma development and progression and thereby may lead to targeted therapy in melanoma treatment.

P-05.02.2-021
7-Ethoxyresorufin O-deethylase and glutathione S-transferase activities of rats treated with morin, 7,12-dimethylbenz[a] anthracene and endosulfan

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Humans are exposed to various chemicals having beneficial or toxic effects at a time in their daily lives. 7,12-Dimethylbenz[a] anthracene (DMBA) is a carcinogenic compound produced during the incomplete combustion of carbon-containing compounds. Endosulfan is an organochlorine pesticide used against insects on food. Morin is an antioxidant, antiinflammatory and

chemoprotective flavonoid. This study is aimed to determine the effect of morin in the presence of DMBA and endosulfan.

For this purpose, 56 adult Wistar male rats weighing 170–255 g were randomly selected and divided into eight groups. 25 mg/kg body weight (b.wt.) morin and 5.0 mg/kg b.wt. endosulfan were given to morin and endosulfan treated groups three times in a week. The rats in DMBA treated groups were gavaged with 30.0 mg/kg b.wt. DMBA three times during the administration period (54 days). Cytochrome P4501A (CYP1A) associated 7-ethoxyresorufin O-deethylase (EROD) and glutathione S-transferase (GST) activities were measured in rat liver cytosols and microsomes. In addition, liver tissues were evaluated by histopathological analysis.

EROD activities of control, morin, endosulfan, DMBA, morin+endosulfan, morin+DMBA, DMBA+endosulfan and morin+DMBA+endosulfan groups were 71 ± 7, 112 ± 6, 114 ± 8, 126 ± 6, 121 ± 9, 156 ± 12, 151 ± 15 and 185 ± 13 pmol/min/mg protein, respectively. All treatments increased EROD activities. GST activities of these groups were 365 ± 14, 430 ± 27, 365 ± 24, 651 ± 57, 460 ± 10, 577 ± 25, 585 ± 23 and 649 ± 33 nmol/min/mg protein, respectively. Histopathological studies showed that endosulfan and DMBA induced inflammation in the liver tissues and morin reduced their effects.

In conclusion, morin treatment increased the metabolism of DMBA and endosulfan by inducing CYP1A activity. GST activities of morin+DMBA+endosulfan group were not significantly different from those of DMBA group. Histopathological studies indicated that morin administration reduced the toxic effect of endosulfan and DMBA in the liver cells.

P-05.02.2-022
3-Deazaneplanocin A induces apoptosis through TGFβ/SMAD pathway in HepG2 cell line

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Hepatocellular carcinoma (HCC) is the sixth most common cancer and third most frequent cause of cancer-related death worldwide. Molecular mechanisms of hepatocarcinogenesis is still unclear. The impairment of epigenetic mechanisms is implicated in the development of multiple cancers, including HCC. Transforming growth factor-beta has been shown to play both tumor-suppressive and tumor promoting roles. Transforming growth factor-beta signaling pathway involves activation of Smad2 and Smad3 by the type I receptor and formation of Smad2/3/4 heteromeric complexes that enter the nucleus to regulate transcription. 3-deazaneplanocin A is an inhibitor of the histone methyltransferase EZH2. We aimed to reveal the effect of 3-deazaneplanocin A on transforming growth factor-beta /Smad pathway in HepG2 cell line.

HepG2, a human liver cancer cell line cultured in Dulbecco's minimal essential medium supplemented with 10% FBS. The cells were seeded the day before 3-deazaneplanocin A administration and then the cells were treated with 5 μM 3-deazaneplanocin A for 3 days. Expression levels of genes were analyzed by Roche LightCycler® 480. GAPDH was used as housekeeping gene. Apoptosis assay was performed by The Muse Annexin V and Dead Cell Assay kit. The unpaired *t*-test was used to compare variables and $P < 0.05$ was accepted as statistically significant.

3-deazaneplanocin treatment was significantly reduced transforming growth factor-beta, Smads 2–7 in HepG2 cells ($P < 0.05$). We also found that 3-deazaneplanocin induces apoptosis in treated cell line ($P < 0.05$).

As a result, 3-deazaneplanocin A may take place in treatment of hepatocellular cancer by its inhibitory effect on transforming growth factor-beta /Smad pathway and inducing apoptosis in liver cancer cells.

P-05.02.2-023
Brefeldin A exerts differential effects on various phenotypes of breast cancer cell lines

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Brefeldin a (BFA) is a lactone antibiotic first isolated from the fungus Eupenicillium Brefeldianium. BFA inhibits the transport of secreted proteins from endoplasmic reticulum (ER) to golgi apparatus, leading to disruption of golgi function, accumulation of unfolded and not fully incompletely processed proteins in ER. BFA also inhibits cell proliferation, phosphorylation and migration of cancer cells. Therefore in this study, we investigated the effects of BFA on breast cancer cell proliferation of various phenotypes.

In this study, MCF-7, MDA MB-231 and MDA MB-435 breast cancer cell lines were used to observe the effect of BFA on cell proliferation. The cells were cultured in 10% Fetal Bovine Serum containing Dulbecco's Modified Eagle Medium (DMEM) at 37 °C in a humidified atmosphere containing 5% CO_2. After seeding the cell suspensions in DMEM into the plate, cells were monitored every 15 min for a period of 75 h (MCF-7), 170 h (MDA MB-231), 77 h (MDA MB-435). 24 h after seeding, the cells were treated with different doses of BFA (5, 50, 500 nM). IC_{50} values were evaluated for all cell line types.

As a result of the analysis, BFA inhibited the proliferation of all cell lines as a time and dose dependent manner. The calculated IC_{50} values are as follows; 6 nM for 35 h (MCF-7), 3 nM for 110 h (MDA MB-231) and 17 nM for 50 h (MDA MB-435).

We observed that BFA inhibited the proliferation of all three phenotypes of breast cancer cells, but the effects of BFA were seen at different times and doses. According to time and dose, BFA was observed more effective to MCF-7 compared to other cell lines.

P-05.02.2-024
The blood neutrophil/lymphocyte ratio in breast cancer

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Background: It is suggested that there is the stability of neutrophil/lymphocyte ratio (NLR) compared with the absolute leukocyte subtype counts that could be altered by various

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physiological, pathological and physical factors. Moreover, NLR may represent the two opposing inflammatory and immune pathways that exist together in cancer patients. We aimed to investigate NLR in breast cancer in our population.

Methods: Using data retrieved from the medical records, 66 women diagnosed primary breast cancer met our study inclusion criteria as they had a complete blood count with leukocyte differential performed before any anti-cancer therapy. And 44 women with benign mammary neoplasm/disease, followed up in the outpatient clinics of mammary disease and confirmed with sonographical/histopathological examination, made up our controls. Exclusion criteria included laboratory evidence of white blood cells count (WBC) > 10.5×10^9/L. Differential leukocyte counts were obtained by BC 6800 (Mindray Medical International Ltd., China), we examined WBC, neutrophil, lymphocyte, platelet counts, and hematocrite, NLR, mean platelet volume values.

Results: Although there is lack of evaluation of tumor-associated neutrophils and lymphocytes, higher NLR median values and lower lymphocyte mean counts (lymphopenia) were shown in women with breast cancer ($P < 0.0001$). There was a weak negative correlation in breast cancer between NLR values and platelet counts ($r_s = -0.274$; $P = 0.026$).

Conclusion: Studying complete blood count and indices has advantages of short turn-around time, requiring no sample preparation and being cost-effective. The performance of NLR on assessing the risk of breast cancer should be investigated in pre and post-menopausal women.

P-05.02.2-025

In vitro investigaton of anti-cancer effect of *Pinus nigra* Arnold subsp. *pallasiana* (Lamb.) Holmboe essential oil in neuroblastoma cells (SH-SY5Y)

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Turkish Black Pine [*Pinus nigra* Arnold subsp. *pallasiana* (Lamb.) Holmboe] is distributed throughout southern Mediterranean Europe from Spain to the eastern Mediterranean on Anatolian peninsula of Turkey. Present study was designed to investigate the *in vitro* anti-cancer activities of Turkish Black Pine essential oil. The essential oil was extracted by steam-hydrodistillation and its chemical composition analyzed by GC-MS. The major components of the essential oil were α-pinene, β-pinene and trans-β-caryophyllene, respectively. The crystal violet staining method was used to investigate the cytotoxicity of essential oil in SH-SY5Y cells. EC50 was found to be 0.75% (w/w) essential oil for SH-SY5Y cells. Neuroblastoma cells were incubated at 37 °C for 24 h. After 24 h, cells were harvested for further studies. Bax and p38 mRNA levels were significantly elevated in essential oil-treated cells. On the other hand, Bcl-2, Bcl2 l1, Casp-3, Casp-8, Erk-2 and JNK expression were significantly downregulated. Unlike these proteins, p53 and PTEN mRNAs were not changed. In this study, apoptosis was enhanced by Turkish Black Pine essential oil treatment which was activated by the involvement of another effector caspase subfamily, like Casp-6 and Casp-7. Additionally, Erk and p38 MAPKs may be associated with upregulation of the level of bax. Based on these results, we suggest that *P. nigra* subsp. *pallasiana* essential oil might not be well-suited in cancer treatment. However, further detailed research is necessary to establish the exact role of *P. nigra* subsp. *pallasiana* essential oil in SH-SY5Y cells.

P-05.02.2-026

The protective effect of newly derivatized compound naringenin-oxime and relative to naringenin against cisplatin-induced nephrotoxicity and genotoxicity in rat

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Background: The aim of this study was to evaluate the possible protective effect potentials of newly derivatized compound naringenin-oxime (NG-Ox) relative to efficacy of free naringenin (NG) on cisplatin (Cis) induced nephrotoxicity and genotoxiticity in rat.

Methods: Totally, fifty six male Wistar albino rats were equally divided into eight groups as follows: control; Cis treatment (7 mg/kg b.w., i.p.), NG and NG-Ox (20 mg/kg b.w., i.p daily for 10 days) alone treatment; Cis + NG (20 or 40 mg/kg b.w., i.p daily for 10 days) and Cis+NG-Ox (20 or 40 mg/kg b.w., i.p daily for 10 days) combination treatment. At the end of the study total antioxidant capacity (TAC) levels, total oxidant status (TOS), lipid peroxidation (LPO), total thiol, catalase (CAT) were studied in homogenate kidney. Peripheral lymphocyte cell DNA damage was investigated with comet assay

Results: The results suggest that Cis induces oxidative stress resulting in increased TOS and LPO reduction thiol, TAC and CAT in kidney and increased Peripheral lymphocyte cell DNA damage. The treatment with naringenin and naringenin oxime alone or with Cis treatment showed a protective effect against the toxic influence of CP on peroxidation of the membrane lipids and an altering of the total thiol status in the kidney of rats. From our results we conclude that naringenin and naringenin oxime functions as a potent antioxidant and suggest that it can control CP-induced nephrotoxicity and genototoxicity and NG-Ox was found more protective than that of NG on cisplatin induced toxicity in rats.

Keywords: Naringenin, Naringenin-Oxime, Antinephrotoxic, Antigenotoxic, Comet Assay.

P-05.02.2-027

A new biosensor for rapid determining of lung cancer

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Introduction: Oxidative damage is considered to play a pivotal role in ageing, several degenerative diseases, and carcinogenesis. Lung cancer is the most common type of cancer, resulting in over 1.3 million deaths each year worldwide. Accurate and reliable determination of superoxide radicals has been widely investigated using spectrophotometric, electrochemical, amperometric, polarimetric, piezoelectric technologies. Among these methods, electrochemical detection is a most promising approach to achieve accurate, separate and rapid superoxide radicals monitoring with using biosensor system.

Materials and Methods: We used a new technic for detecting superoxide radicals in samples. Superoxide dismutase (SOD)

enzyme immobilized on the surface of gold electrode with the help of gelatin, bovin serum albumin (BSA) and glutaraldehyde (GA) crosslinker. For the biosensor preparing benzoquinone selected as a mediator in working buffer and measurements were carried out at −0.7 V.

Result: For the optimization studies, effect of the BSA, gelatin, glutaraldehyde, pH, buffer concentration on biosensor response. Characterization of the biosensor commitment to the work process and answer reproducibility were evaluated. The analytical characteristic of the biosensor were evaluated by measuring the steady state current response to superoxide radical concentrations. The electrochemical response of the enzyme electrode was linearity gradually leveled of at higher concentration. We found that crosslinking of the SOD (E.C.1.15.1.1) with glutaraldehyde could be achieved over a wide range of relative mole ratios in 50 mM phosphate buffer at pH 7.5, glutaraldehyde concentration of %2.5.

Discuss and Conclusion: In this study, a new technique for developed SOD biosensors has been developed, which features effective combination of SOD/Gelatin/BSA/GA modified electrode, trapping of SOD and glutaraldehyde cross-linking. This technique is reliable and cost effective.

P-05.02.2-028
The effect of astaxanthin on apoptosis and cell arrest in U87 brain cancer cell line

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A brain tumor is a collection, or mass, of abnormal cells in your brain. Brain tumors can be cancerous (malignant) or non-cancerous (benign). The brain is one of the least accessible organ that active pharmacological compounds cannot be delivered. The two physiological barriers control and block the entry and exit of endogenous, exogenous compounds. One of these is the blood–brain barrier and the other is the blood–cerebrospinal fluid barrier. This structures maintain protection of the brain. When there is a cancer case, it can lead to problem. Astaxanthin with potent antioxidant properties can cross blood-brain barrier.

In our study, we aimed to evaluate the effects of astaxanthin on apoptosis, cell cycle and also migration in brain cancer cell line.

In present study, XCELLigence Real-Time Cell Analyzer was used so as to determine cytotoxic effect of astaxanthin in U87 cell line. Changes of apoptosis and cell cycle in U87 cell line exposured to IC_{50} dose of astaxanthin (19.5 nM–80 μM) are detected with Annexin V-EGFP Apoptosis Detection Kit and Cycle test Plus DNA Reagent Kit with FACS, respectively. The result of apoptosis and cell cycle test was analysed in flow cytometry. The group to which active substance was not treated was used as controlled. The wound healing assay performed in order to measure migration ability of U87 cell line to which astaxanthin was treated or not.

IC_{50} dose of astaxanthin was calculated as 9.20 μM at 48 h by XCELLigence RTCA SP based on time and dose. Astaxanthin decreased the migration ability at rate of 25% in U87 cells treated by IC_{50} dose of astaxanthin. Astaxanthin had no apoptotic effect on viability in U87 cell line and astaxanthin caused an increase of G2/M phase arrest (1.15 fold) and S phase arrest (1.41 fold).

Astaxanthin has cytotoxic effects in brain cancer. It determined that astaxantin decreases cell cycle potential at G2/M even a little. The effect of anticancer of astaxanthin should be researched further.

P-05.02.2-029
Identification and analysis of interferon regulatory factor 4 target genes in melanoma via high-throughput sequencing of immunoprecipitated chromatin

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Interferon regulatory factor 4 (IRF4) is a critical transcription factor in development and survival of different cell types including immune cells and melanocytes. Furthermore, it has been demonstrated that IRF4 expression levels are elevated in several lymphoid cancers, and IRF4 is one of the key transcription factors for the survival of these cancers.

Several genome-wide association studies identified IRF4-linked genetic variants to increased melanoma incidence. In addition results from our lab and elsewhere have shown high levels of IRF4 expression in melanoma cell lines. Furthermore our preliminary results suggest melanoma cells are sensitive to IRF4 expression levels. However, there are no published studies about IRF4 target genes in melanoma cells.

In this study, we are investigating the genome-wide target genes of IRF4 in melanoma cell lines via high-throughput sequencing of immunoprecipitated chromatin (ChIP-seq). We have identified possible IRF4 binding regions in loci with known key roles in development of melanocytes from neural-crest cells. One such key factor is MITF, which is the master regulator in melanocyte development and also plays critical roles in melanoma. Integrating ChIP-seq and RNA-seq data suggests IRF4 as a transcriptional regulator of genes related to progression of melanoma.

P-05.02.2-030
The significance of some laboratory parameters as a prognostic indicator of survival in patients with primary lung cancer

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Objectives: Aim of this study was to evaluate prognostic importance of selected laboratory parameters (C-reactive protein (CRP), Gama GlutamilTransferaz, ferritin (FER), potassium, chloride, calcium, phosphorus, magnesium, total protein, Aspartat aminotransferaz, Alanin aminotransferaz (ALT), IFN-γ, IL-6, TNF-α) in non-small cell lung cancer (NSCLC).

Material and Methods: 20 patients with NSCLC who were treated with Chemoradiotherapy (CRT) prospectively evaluated. All patients were newly diagnosed tumour. Heparinized blood samples were taken from the patients before and after the completion of CRT. FER analyzed by chemiluminescence method on Beckman Coulter DxI 800; IFN-γ, IL-6, TNF-α were analyzed with ELISA kits (Boster Biological Technology) and other biochemical parameters analyzed on Abbott Architect c16000. Post-CRT and pre-CRT levels compared with survival.

Results: The LR Cox regression analysis revealed that Pre-CRT Ferritin was significantly associated with survival of patients with NSCLC (hazard ratio (HR) = 1.007, $P = 0.023$, 95%CI; 1.001–1.013). It was also demonstrated by LR Cox regression analysis, high levels of Pre-CRT CRP was associated with worse outcome of patients (HR = 1.017, 95%CI;1.001–1.032, $P = 0.035$). After CRT, mean ALT level was determined as 16.71. There was

The FEBS Journal **283** (Suppl. 1) (2016) 129–417 DOI: 10.1111/febs.13808

survival difference in NSCLC patients with high Post-CRT ALT (HR = 0.912, 95%CI; 0.841–0.990, $P = 0.027$).

Conclusions: There exists a clinically relevant relationship between Pre-CRT FER concentration and the prognosis of survival in patients with NSCLC. Elevated FER is the result of inflammation rather than body iron overload. Ferritin showed negative correlation with survival so it could be a useful biomarker to indicate bad prognosis of the patients with NSCLC. Additionally, CRP which is easy to detect and feasible for the use in the routine clinical practice should be considered in the prognosis of NSCLC patients.

Keywords: ferritin, nonsmall cell lung cancer, survival, C-reactive protein.

P-05.02.2-031
Differences of global methylation profiles in L929 and HeLa cells treated with a serial benzoxazole and benzamide derivatives

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Epigenetic therapy tries to reverse the aberrations followed to the disruption of the balance of the epigenetic signaling ways through the use of natural and synthetic compounds, active on specific targets, such as DNA methyltransferases (DNMTs). We previously synthesized some benzoxazole and benzamide derivatives which might have anticancer activities on account of their heterocyclic structure. Our studies showed that not only these compounds caused selective cytotoxicity towards cancer cells (HeLa) with little or no toxicity on normal cells (L929) but also were not genotoxic. In this study, we aimed to test whether these compounds changed global demethylation profile of normal and cancer cells.

We used methylation specific comet assay (MSC assay) to determine global methylation levels of cells. Cells were treated with the tested compounds at IC_{50} concentrations for 48 h. Slides were prepared as did in alkaline comet assay, then they were incubated with methylation specific restriction enzymes (MspI, HpaII) before electrophoresis. Differences in global methylation levels between nontreated control cells and cells treated with compounds were compared by using tail moment data. 5-aza-C, a demethylating agent, was used as reference drug.

MSC assay results revealed that none of the tested 9 compounds caused hypermethylation on both cell lines. However, global methylation levels decreased statistically ($P < 0.05$) through both cells treated with c-2 and c-8. Only c-3 decreased methylation level on L929 but not on HeLa.

Consequently, c-2 and c-8 caused demethylation on HeLa cells similarly with 5-aza-C at low concentrations. For the reason that DNA methylation is regulated mainly DNMT enzymes in the cell, c-2 and c-8 might cause global demethylation in the cell by inhibiting DNMT activity. Further studies will be done to support this prediction.

P-05.02.2-032
How immune cells can affect miRNAome and nudge the epigenetic disorganization leading to the tumour promotion

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Overall, macrophages and some subtypes of lymphoid cells are found in tumour stroma. These cells secrete a variety of growth factors, proinflammatory cytokines and chemokines, esp. TNF-a, IL-1b and IL-6, causing the formation of inflammatory microenvironment around tumour cells. TNF-a and IL-1b signaling increases activity of NF-kB pathway. At the same time, IL-6, triggers JAK-STAT signaling pathway, which effector is STAT3. NF-kB and STAT3 activity facilitates hyperexpression of miRNAs miR-155, miR-181 and miR-21 as well as down-regulates expression of miRNAs miR-15/16, miR-199 and let-7. This investigation aims to identify in what way these shifts in miRNAome can lead to epigenome reorganization supporting the cell transformation.

MiRNA targets within gene transcripts were predicted *in silico* using TargetScan software.

Transcripts of *HDAC2/4/8/9* and *SIRT1/5* genes encoding histone deacetylases carry targets for at least one of up-regulated miRNAs miR-155, miR-181 or miR-21. Also, these miRNAs can silence *EZH1*, *MLL*, *MLL3*, *NSD1*, *SETD6/7/8*, *SMYD1*, *SUV39H2* genes encoding histone methyltransferases. MiRNA miR-21 suppresses gene encoding *de novo* DNA methyltransferase DNMT3B. At the same time, down-regulation of miRNA miR-15/16 can allow hyperexpression of gene encoding acetyltransferase Elp3.

These shifts impair DNA and histone methylation, cause the increase of overall level of chromatin acetylation and expression and, therefore, create epigenetic background for reactivation of silent transposons, oncogenes as well as other genes important for cell transformation.

Immune system can paradoxically facilitate the tumour growth instead of healing. Cancer-related inflammation leads to the miRNAome and epigenome shifts contributing to the tumour promotion and progression.

P-05.02.2-033
Discovery of BET bromodomain inhibitors with novel scaffolds and its application to treat cancers

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Lysine acetylation is one of the key mechanisms to regulate chromatin structure and transcriptional activation. Acetyl-lysine modifications are recognized by bromodomains, which are small interaction modules found on diverse proteins including histones. Among these acetyl-lysine reader proteins is the family of the BET (bromodomain and extra-terminal) proteins which contain tandem bromodomains (BD1 and BD2). The recent discovery of potent and specific inhibitors for the BET family proteins has stimulated intensive research activity in diverse therapeutic areas, especially in oncology, where BET proteins regulate the expression of key oncogenes and anti-apoptootic proteins. Several BET inhibitors are currently in clinical trials and reported to exhibit promising clinical activities. However, pleiotropic nature of BET proteins regulating tissue-specific transcription has raised safety concerns and suggested that attempts should be made for domain-specific targeting. Here, we report the recent progress in the development of BET inhibitors in Korea Research Institute of Chemical Technology (KRICT). We have identified the BET inhibitors with a novel scaffold different from the previously reported diazepine and azepine scaffolds and specific for first bromodomains (BD1s). A medicinal chemistry effort is currently made to optimize the pharmacokinetic properties of these lead compounds for further drug development. The experimental data from the biochemical and cell-based assays for these BD1-selective BET inhibitors will be presented.

P-05.02.2-034
Overexpression of miR-183-96-182 family is able to lead to the simultaneous inactivation of potential tumor suppressors, CTDSPL/1/2 phosphatases, in non-small cell lung cancer

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Family of small C-terminal domain serine phosphatases (SCP), which includes CTDSPL, CTDSP1, and CTDSP2, plays a regulatory role in a number of vital processes. In particular, it is shown that CTDSPL is capable to activate the retinoblastoma protein (Rb) which is well-known tumor suppressor and one of the key cell cycle regulators. Although the question on whether CTDSP1 and CTDSP2 dephosphorylate Rb is open, high similarity of sequences and three-dimensional structures of phosphatases may indicate the similar function of these enzymes.

In the current study expression of SCP genes was evaluated by quantitative PCR in 28 non-small cell lung cancer (NSCLC) samples. Using original CrossHub software, that combines an analysis of high-throughput sequencing data of The Cancer Genome Atlas project (TCGA) and databases of mRNA-miRNA interactions (TargetScan, miRTarBase, *etc*), the involvement of miR-183-96-182 microRNA cluster in co-regulation of *CTDSPL/1/2* genes in NSCLC was predicted.

The significant (5-fold on the average) and simultaneous decrease of mRNA levels of *CTDSPL/1/2* genes was revealed in the majority of NSCLC samples (82%, 23/28). Such unidirectional expression change and strong positive correlation between phosphatase expression levels (r_s = 0.53–0.62, $P \leq 0.01$) allowed us to suggest a common mechanism of their inactivation. We evaluated the expression of predicted co-regulators of SCP gene expression, miR-183-96-182 family, in examined NSCLC samples. As a result, the simultaneously increased levels of all three miRNAs in most NSCLC samples (82%, 23/28) and negative correlation with phosphatase gene expression was shown.

The results suggest the ability of investigated phosphatases to exhibit tumor-suppressive activity and the involvement of miR-183-96-182 microRNAs in the regulation of Rb protein activity *via* inactivation of CTDSPL/1/2 in NSCLC.

P-05.02.2-035
The clinical use of tumor markers

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Cancer is one of the leading causes of death in all around the world. Cancer is defined as a disease involving abnormal cell growth with the potential to invade or spread to other parts of the body. Tumor markers are substances that are produced either directly by the tumor or as an effect of the tumor on healthy tissue. Tumor markers can be used for screening, determining prognosis and monitoring effectiveness of therapy and disease recurrence. The aim of this study is to investigate the frequency of tumor markers orders and the appropriateness of these requests.

Laboratory information systems data for 2015 were reviewed. For 2015, a total of 35874 patients and 55539 tumor marker requests were included. Carbohydrate antigen 19-9, cancer antigen 125, cancer antigen 15-3, prostate specific antigen, alphafetoprotein and carcinoembryonic antigen were measured by chemiluminescence method.

According to the data from the year of 2015, both positive tumor markertest resultsratio and the positive patient ratio were 14%. In the patients group with increased marker levels, 48% of the patients had no history of cancer. In the patients group with tumor marker levels in referenceranges, 40% patients with diagnosed cancer history in remission. The ratios of positive tumor markers were 13% forCA 19-9, 19% for CA 125, 7.9% for PSA, 25%for CA 15-3, 8.4%for AFP, and 9.56% for CEA.

In conclusion; unnecessary test requests increase laboratory work load and health expenses. Laboratory and clinical staff collaboration is crucial to increase the appropriate use of tumor markers.

P-05.02.2-036
Optimization strategy for DNA methylation measurements

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DNA methylation is an epigenetic modification that is involved in both normal biological and disease states. Hypermethylation of promoter regions of tumor suppressor genes have a role in tumor development. Therefore, the measurement of promoter methylation of genes can be used for diagnosis and prognosis purposes of cancer. To detect DNA methylation alterations in a sample (biopsy, blood, saliva, etc.), sensitive detection systems and optimization of the methods are needed.

As a part of a collaboration project between National Metrology Institute of Korea (KRISS) and National Metrology Institute of Turkiye (TUBITAK UME). DNA methylation status of APC and GSTP1 genes were studied. DNA methylation measurements were performed using StepOne Real-Time PCR system and results were analyzed using HRM (High Resolution Melting) software.

The parameters effecting the quantification of DNA methylation were found as primers, annealing temperature, PCR cycle number, fluorescence dye and the commercial DNA methylation standards used for quantification of DNA methylation.

Since, the accurate measurement of DNA methylation is very critical in early diagnosis of cancer and choosing the right therapy, optimization of the method is required.

P-05.02.2-037
The effects of resveratrol gain importance according to p53 mutation in HCT116 colon cancer cell lines

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Cancer is a disease that includes heterogenic and complex molecular changes. Anti-carcinogenic effects of Resveratrol, a natural polyphenol, have been proved in a variety of cancer cells. Considering the effects of Resveratrol, the influence of the signal transduction pathways in the presence or absence of p53 of colon cancer cells is gaining importance. Our aim was to investigate the effects of Resveratrol in the presence or absence of p53 on cell viability, apoptotic cell death ratio and fold changes of proliferative or anti-proliferative gene expressions, which may have important effects on colon cancer, in HCT116 colon carcinoma

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cells. IC50 doses of Resveratrol were determined by WST-1 assay. The apoptotic cell death ratios in treatments of Resveratrol were determined by Annexin-V-FITC/PI assay for flow cytomety. The changes of CCND1, FRA1, PPARD, EGFR, BIRC5, PCNA, MCL1, STAT3, FOS, JUN, P27, ATF4, TRAIL, PUMA, GADD45A, RB1, FASLG, TNF, SOCS3, STAT1 gene expressions were evaluated by Real Time PCR. All data were statistically analyzed by Student's *t* test. Our research has revealed that Resveratrol (60 μM) causes decrease in cell viability and increase in apoptotic cell death in HCT116 p53(+/+) and HCT116 p53(−/−) cells significantly ($P < 0.05$). The fold changes of the gene expressions have shown that Resveratrol has significant ($P < 0.05$) and different effects on the expressions of the genes related with the existence of p53 in HCT116 cell lines. Therefore we proposed that Resveratrol might show proliferative or apoptotic effects related with p53 mutation of colon cancer cells and we predicted that unconscious consumption of Resveratrol in colon cancer patient might cause adverse effects.

P-05.02.2-038
Effect of *Lactobacillus lactis* on AKT signaling pathway in HT29 cancer cell line

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Introduction: Colon cancer occurs due to genetic and epigenetic changes. Probiotics are microorganisms in the intestine led to increase in the amount of useful bacteria, which can affect on cancer in the colon. Aim of this study was the evaluation of *Lactobacillus lactis* effect on AKT gene expression in HT29 cell line.
Method: *Lactobacillus lactis* bacteria and HT29 cancer cell line were cultured and the MTT assay was used for cytotoxicity evaluation of bacteria onto HT29 cancer cell line culture. The total cell RNA was extracted for cDNA synthesis after coculture of bacteria and cancer cell line, then AKT gene expression was evaluated using Real time PCR.
Results: MTT assay test showed that bacteria had cytotoxic effect on HT29 cell line after 24 h. Real time PCR results showed that AKT gene expression was decreased after bacteria and HT29 coculture.
Conclusion: The reduction of HT29 cancer cell line proliferation may be related to effect of bacteria onto AKT gene expression.
Keywords: Colorectal Cancer; *Lactobacillus lactis*; AKT gene; HT29 cancer cell line.

P-05.02.2-039
Evaluate the effect *Lactobacillus lactis* on PTEN signaling pathway in HT29 cancer cell line

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Introduction: Studies have been shown that genetic and epigenetic changes on colon cancer have a very important role. Probiotic bacteria present in the intestine directly related to reduce colorectal cancer, that in the context, researchers opinion is involve several of cell signaling in this process.

In order to, in this study the effect of *Lactobacillus lactis* in PTEN gene expression was evaluated.
Method: The *Lactobacillus lactis* and HT29 cancer cell line, each separately cultures in specific media, then *the* bacteria was added to HT29 cell line culture. To evaluate the toxicity of these bacteria on HT29 cancer cell lines MTT assay was perform. Total RNA was extracted from HT29 cell line and cDNA synthesized, followed by performation the Real time PCR.
Results: The results of MTT assay indicated that *Lactobacillus lactis has* toxic effect on HT29 cell line and reduced proliferation. Analysis of the results of the Real time PCR was shown that there was positive effect on PTEN gene expression .
Conclusion: overexpression of PTEN gene in the presence of *Lactobacillus lactis* reduced the proliferation of HT29 cancer cell line, the possibility that this could be done through inhibition of AKT gene.
Keywords: Colorectal Cancer; *Lactobacillus lactis;* PTEN gene; HT29 cancer cell line.

P-05.02.2-040
Hypermethylation analysis of 25 tumor suppressor genes in colorectal cancer tissues

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Introduction: Colorectal cancer (CRC) is the third most common cancer worldwide. Alterations in methylation profiles of tumor suppressor genes (TSGs) have been recognized as a key mechanism in colorectal cancers. In the current study, we investigated the hypermethylation status of 25 TSGs in colorectal cancer tissues.
Materials and Methods: Formalin-fixed paraffin-embedded (FFPE) tissue samples obtained from patients with CRC. Methylation specific-multiplex ligation dependent probe amplification (MS-MLPA) technique was used to assess the methylation status of 25 TSGs. The findings were evaluated in terms of age, mortality, survival, positive lymph node status, lymphovascular invasion, and perineural invasion.
Results: Hypermethylation-detected patients and hypermethylation-undetected patients were called as group 1 and group 2, respectively. Hypermethylation was detected in ATM, CDKN2A, and GATA5 genes. Mortality rate was (12.5%) in group 1 and group 2 ($P > 0.05$). Mean 5-years survival rate in group 1 was 45 ± 3 months and mean 5-years survival in group 2 was 44 ± 3 months ($P > 0.05$). Positive median lymph node count was 10 ± 2 for group 1 and 5 ± 1 for group 2 and the difference was statistically significant ($P < 0.05$). Frequencies of perineural invasion and lymphovascular invasion rate in two groups were 25% ($P > 0.05$).
Discussion and Conclusion: Our findings suggest that TSG hypermethylation found in CRC patients may increase the lymph node metastasis. Further investigations with larger sample size are required to support our results.

P-05.02.2-041
Cytotoxic effects of boron compounds in P53 (+/+) and P53 (−/−) HCT-116 colon carcinoma cells lines

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Boron (B) is known to be important for cell replication and development, but the underlying mechanism remains obscure. Recently B has also become important in some specific anticancer processes. Some recent reports advise using of some Boron compounds for the treatment of specific forms of cancer. For instance, Boron-based drugs (Bortezomib) are now being developed for use as therapeutic agents with anticancer activities and several other boron-based compounds are in various phases of clinical trials. It has been shown that Bortezomib disrupts the regulation of cell cycle and induces apoptosis in both hematologic and solid tumor malignancies except for colon carcinoma. Colorectal cancer (CRC) is the third most common cancer in men and the second in women, accounting for 10% of all tumour types worldwide. Cytotoxic effects of boron compounds on CRC cells and changing of its effects related with p53 mutation, which is mutated 50% of cancer cases, have not take part in literature yet. For this purpose; the aim of the study was designed to investigate the effects of Borax pentahydrate and Disodium pentaborate decahydrate compounds on cell viability, apoptotic cell death ratio and PARP protein expressions in $p53^{(+/+)}$ and $p53^{(-/-)}$ HCT116 colon carcinoma cells lines. The effects of the Boron compounds on cell viability were assessed by XTT assay and apoptotic effects and PARP protein expression of the compounds were evaluated by flow cytometry and western blot analysis respectively. Our results showed that Borax pentahydrate (6 mM) and Disodium pentaborate decahydrate (12 mM) significantly causes nearly 50% reduction of cell viability at 48 h ($P < 0.05$). Apoptotic cell death ratios and PARP expressions revealed that both of the compounds might have a potential for a candidate of anticancer agent.

Keywords: Colon Cancer, Boron, Borax Pentahydrate, Disodium Pentaborate Decahydrate.

P-05.02.2-042
The crosstalk between p38 and Akt signaling pathways orchestrates EMT in NSCLC cells via regulation of SATB2 expression

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Epithelial-mesenchymal transition (EMT) is a significant event for metastasis, and could be mediated by several pathways such as PI3K/Akt, MAP kinases and many epigenetic regulators. SATB2 is an epigenetic regulator involved in EMT and osteoblastic differentiation. Since preliminary results indicate that there is a crosstalk between p38 and Akt pathways in NSCLC cells, we aimed to determine whether this crosstalk has a regulatory effects on EMT and SATB2 expression in NSCLC cells.

We used A549 and H1650 cells as a model to evaluate the effects of the crosstalk between p38 and Akt on EMT of NSCLC cells. Therefore, cell culture, inhibition of p38 activation via SB203580, transient expression assay for (CA-Akt), Western blot analysis, siRNA transfection for SATB2, wound healing and invasion assay were performed in this study.

Firstly, the expression statues of E-cadherin, SATB2, p-p38, p38, p-Akt and Akt was examined in A549 and H1650 cells by Western blot analysis. We observed that E-cadherin and SATB2 are downregulated in A549 cells (highly active p38, lowly active Akt) compared to H1650 cells (lowly active p38, highly active Akt), suggesting that E-cadherin and SATB2 are associated with the crosstalk between p38 and Akt pathways. Our results demonstrated that p38 inhibition in A549 cells leads to decreased PTEN expression and subsequently increased Akt activation. Then, we found that p38 inhibition upregulated SATB2 expression, and reversed EMT in A549 cells. Furthermore, alone SATB2 knockdown is sufficient to induce EMT, and prevented the effects of p38 inhibition on EMT.

All these results strongly indicate that the crosstalk between p38 and Akt pathways might determine SATB2 expression and epithelial characters of NSCLC cells, and SATB2 is a critical epigenetic regulator for EMT in NSCLC cells. Therefore, it is also need to explore how p38 and Akt signalling pathways could regulate SATB2 expression.

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P-05.02.2-043
The role of endothelial nitric oxide synthase gene polymorphisms in patients with lung cancer

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Introduction: Lung cancer is a disease characterized by uncontrolled cell growth in the lung tissues. The most common causes of lung cancer are tobacco smoke, radon gas, asbestos, air pollution, and genetic factors. Nitric oxide (NO) has potential mutagenic and carcinogenic activity and may play important roles in lung cancer. Endothelial NO, synthesized from L-arginine by endothelial NO synthase (eNOS), inhibits apoptosis and promotes angiogenesis and tumor cell proliferation. The aim of the present study was to examine the possible relationship between eNOS gene intron 4 VNTR and exon 7-G894T (Glu298Asp) (rs1799983) polymorphisms and lung cancer risk.

Material and Methods: DNA was extracted from peripheral blood leukocytes of 107 lung cancer patients and 100 controls cases. Restriction length polymorphism (RFLP) method for detection of eNOS gene G894T gene polymorphisms (206 bp (G allel), 119 bp (T allel), 87 bp (T allel)), Polymerase chain reaction (PCR) protocol was performed for eNOS gene intron 4 VNTR (27 bp repeat).

Results: Our study showed that the b/b genotype and b allele frequency of eNOS gene intron 4 VNTR polymorphism were significantly higher in the lung cancer patients than in controls ($P < 0.05$). However, there was no significant difference between eNOS gene G894T polymorphism and lung cancer risk ($P > 0.05$).

Discussion: We concluded, eNOS intron 4, the risk of developing lung cancer 27 bp again polymorphism and found a significant relationship between and this polimorfizmin lung cancer genetic contribution has been concluded. But eNOS Glu298Asp polymorphism does not increase the risk of developing lung cancer.

Conclusion: These result suggest that the presence of the intron 4 VNTR* b allele and bb genotype may be a genetic risk factor

for development of lung cancer. Further larger-scale studies are needed to confirm these findings.

P-05.02.2-044
L-proline homeostasis, substrate channeling and mitochondrial flavine turnover in microenvironment: phenotypic landscape of Saccharomyces cerevisiae for pharmacological screening *in silico*/*in vitro*

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The stressful ecosystems exert strong adaptive pressure and proteins that facilitate these adaptation processes are candidate drug targets. Nucleotides are the core of biochemical pathway required for cancer cell growth and replication and genetic changes will lead in oscillation in their pools. Although it is questionable whether the Warburg effect actually causes cancer, impairing D-glucose uptake and metabolism induces oxidative metabolism. L-proline (Lproline) homeostasis is critical in a constellation of human diseases, in parametabolic linkage between cancer, epigenetics (Phang et al. 2013) and bioenergetics (Pallotta 2013) where degradation and biosynthesis are robustly affected by oncogenes or suppressor genes that can modulate intermediates involved in epigenetic regulation. Lproline-fueled mitochondrial metabolism involves the oxidative conversion to L-glutamate by a flavin dependent Lproline Dehydrogenase/Oxidase and a NAD^+-dependent L-Δ^1-pyrroline-5-carboxylate dehydrogenase. In *Saccharomyces cerevisiae* an important test tube, Put1p and Put2p respectively help cells to respond to changes in the nutritional microenvironment by initiating Lproline breakdown after mitochondrial uptake (Pallotta 2014). In this preclinical study, low molecular weight compounds were tested for inhibiting Lproline mitochondrial transport and Put1p/Put2p catalytic activities. Thus, in seeking for natural bioactive compounds targeting Lproline pathway and its substrate channeling (Becker's group 2016), we report data using *in silico* screening and *in vitro* researches in *Saccharomyces cerevisiae* with genetic background ATCC18790 but different phenotypic landscape induced by nutritional stress/pH changes. Cells vitality, $\Delta\Psi$ measurements, $NAD(P)^+$/NAD(P)H pool and flavine turnover were determined in spectrofluorimeter microplater reader and via HPLC (Pallotta et al. 1998, 1999, 2004; Pallotta 2011; Di Martino Pallotta 2011) thus in supporting of future cancer therapies with decreasing side effects.

P-05.02.2-045
Evaluation of lymphocyte to monocyte ratio (LMR) in patients with colorectal cancer

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Introduction: Inflammation may play an important role in cancer progression and a high neutrophil to lymphocyte ratio (NLR) has been reported to be a poor prognostic indicator in several malignancies. The aim of this retrospective study was to evaluate the prognostic value of NLR, lymphocyte to monocyte ratio (LMR) and platelet to lymphocyte ratio (PLR) in patients with colorectal cancer (CRC).

Materials and Methods: 107 patients who were diagnosed with colorectal cancer between January 2010 and January 2016; were evaluated retrospectively. The cutoff value was determined using receiver operating characteristics curve analysis. Survival analysis was performed using the Kaplan–Meier method and log-rank test. The Cox proportional hazard model was used to identify the influence of factors related to survival. (TNM stage, tumor differentiation, age, tumour size and LMR)

Results: Receiver operating characteristic curves showed that LMR was superior to PLR and NLR as a predictive factor in patients with colorectal cancer. The cutoff value for LMR was 1.82. Cancer-specific survival was not significantly different between the high- and low-LMR groups ($P = 0.786$). Age was identified as independent prognostic factor in colorectal cancer (hazard ratio: 3.50; 95% confidence interval: 3.14–351.82; $P = 0.004$).

Discussion and Conclusion: Our preliminary study showed that the LMR was not an independent prognostic factor in CRC patients, but additional large sample sized prospective studies will be needed to confirm these findings.

P-05.02.2-046
Investigation of the possible role of luteolin in breast cancer

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The aim of this study is to investigate the effects of luteolin treatment on enzymatic activity of arginase, and ornithine and polyamine levels (putrescine, spermidine spermine) in serum and cancer tissues of Ehrlich ascites breast cancer model. Balb/c female mice were divided randomly into following groups: healthy control, healthy treatment, cancer control, treatment 1 and treatment 2. 0.2 mL Ehrlich ascites tumor cells was inoculated subcutaneously to medial part of left hind leg. Healthy treatment and treatment 1 groups, and the treatment group 2 were given 5 mg/kg and 10 mg/kg dose of luteolin, intraperitoneally, for a 12 days period, respectively.

Luteolin has a hydroxylated flavonoid structure and shows potent antioxidant, anti-inflammatory, and anticarcinogenic properties. Luteolin not only leads to cell death in various tumors by suppressing cell survival pathways and stimulating apoptotic pathways, but also sensitize them to cytotoxic therapy. Supporting various previous studies, tumor implantation to healthy mice resulted in statistically significant elevation of serum arginase and polyamine levels ($P < 0.05$) indicating the tumor cells as the main source of this production. Furthermore, luteolin treatment abolished this increase in serum arginase and polyamine levels ($P < 0.05$).

Tissue measurements of arginase and polyamine levels indicated that luteolin treatment resulted with an increase in these parameters of tumor tissue while the serum levels of them showed a significant decrease. Our results revealed that increased tissue arginase and polyamine levels might be related with estrogenic agonistic effect of luteolin on utilized tumor model in this experiment; and decreased serum levels of these parameters while there is a significant increase of them in tissue levels might be a result of a suppression of polyamine efflux from the tumor tissue by inhibitory effect of luteolin on plasma membrane polyamine transporters.

P-05.02.2-047
TXNIP overexpression promotes aggressive phenotype in HCC cell lines

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Hepatocellular carcinoma (HCC) is the third most common cause of cancer-related deaths. Around 30–40% of HCC patients are diagnosed at an early stage of the disease. Hepatic resection, liver transplantation are common strategies in HCC treatment. Even if, most of the patients present advanced-stage tumors and have a restricted survival rate. For the reason, resistance against existing tumor stress conditions have been demonstrated in HCC. Hypoxia, hyperglycemia are general stress sources in HCC and result in aggressive cell phenotype, resistance to apoptosis and therapeutic drugs. Thioredoxin interacting protein (TXNIP) regulates cellular responses under *stress conditions*. Over-expression of TXNIP results activation of oxidative stress and apoptosis. In cancer models TXNIP is considered as a tumor suppressor gene. However, its role in the development, progression of HCC and mechanisms behind it warrant further investigation.

In this study *expression* levels of TXNIP were examined in 12 HCC cell lines by RT-PCR and Western blotting. *TXNIP expression was significantly high in* poorly-differentiated *SNU-182, SNU-387 and SNU-423 than the well-differentiated HCC cell lines such as HuH-7, HepG2 and PLC/PRF/5*. Besides, expression of TXNIP was examined in 16 non-HCC and 79 HCC tissue samples by Immunohistochemical staining. TXNIP positivity was observed in 40% of well and 80% of poor differantiated HCC tissues. However, no TXNIP positivity was observed in non-HCC tissues. To investigate whether TXNIP might be involved in biological responses such as cell proliferation, motility and invasion, we used overexpression and silencing strategies. Overexpression of TXNIP minimally inhibited adhesion and proliferation, whereas boyden-chamber motility and invasion assay showed that invasiveness of cells were increased.

Our findings suggest that TXNIP expression is increased in HCC and TXNIP over-expression is important for invasive phenotype during hepatocarcinogenesis.

Monday 5 September
12:30–14:30

Novel signaling pathways controlling the cardiac function

P-06.01.2-001
The role of the JAK/STAT pathway in the cardioprotective mechanism of adaptation to chronic hypoxia

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Cardiovascular diseases are the leading cause of morbidity and mortality in the Western world. It was shown that ischemic tolerance of the heart can be enhanced not only by ischemic or pharmacological conditioning (pre- and postconditioning), but also by adaptation to chronic hypoxia. Different studies have indicated that these cardioprotective phenomena may at least partly share the same signaling pathways. The JAK/STAT signaling pathway has been demonstrated to participate in the development of cardioprotection by conditiong apparently through the inhibition of GSK-3β. The aim of our present study was to determine whether this pathway also takes part in cardioprotection induced by adaptation to chronic hypoxia.

We investigated the effect of inhibitor of JAK2 kinase (AG-490) on myocardial infarct size and the JAK2/STAT3 signalling pathway and other effector molecules that may participate in cardioprotection conferred by adaptation to hypoxia. Adult male rats were adapted to intermittent normobaric hypoxia (10% O_2, 3 weeks, 8 h/day) and part of them recieved AG-490 (3 mg/kg) 15 min before ischemia. Control rats were kept under normoxia. Infarct size was assessed in isolated perfused hearts. Relative expression of the key components of the JAK2/STAT3 signalling system and other proteins was detected using Western blotting.

Preliminary data indicate that administration of the JAK2 inhibitor AG-490 caused a significant increase in infarct size in hypoxic rats. Western blot analysis revealed changes in phosphorylation of JAK2, STAT3 and some other proteins involved in cardioprotection (Akt, ERK1/2, GSK3β).

These results suggest that the JAK/STAT signaling pathway could participate in the development of a cardioprotective phenotype in rats exposed to chronic hypoxia. However, further research will be needed to clarify in more detail the role of this signalling pathway in the cardioprotective mechanism.

P-06.01.2-002
Detrimental effect of hypertension on myocardium was reversed by liver X receptor agonist GW3965

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Hypertension is a cardiovascular disease that causes functional and structural changes in the heart. Nuclear liver X receptors (LXRs) are involved in the control of cholesterol and lipid metabolism. However, effect of LXR activation on the hypertensive heart is not well characterized. In this study, the effects of LXR agonist GW3965 on hypertension-induced damage of myocardium were investigated.

Hypertension was induced by deoxycorticosterone acetate (DOCA) injection (20 mg/kg, twice a week) following the unilateral nephrectomy in male 8-week-old Wistar albino rats for 6 weeks. Blood pressure was measured by using tail-cuff method. GW3965 (10 mg/kg/day, i.p.) was administered last 1 week. Expression of various markers (GRP78, PERK, p-PERK, IκB-α, NF-κB p65, TNF-a, Bax, Bcl-2, MMP-2) in the ventricular tissue were examined by Western blotting. Inflammation and fibrosis were evaluated in histopathological examination.

GW3965 treatment reduced systolic blood pressure of hypertensive animals. Expressions of endoplasmic reticulum stress markers GRP78 and p-PERK were increased by hypertension and GW3965 treatment reversed them. Hypertension-induced increase in nuclear NF-κB p65 expression and decrease in IκB-α expression were reversed by GW3965 treatment. While Bcl-2

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expression was lower, Bax level was higher than control in the hypertensive animals. In hypertensive group, fibrosis marker MMP-2 expression was augmented and GW3965 treatment reversed this elevation. Hypertension-induced increase in interstitial and perivascular collagen deposition and inflammatory cell infiltration in left ventricle were prevented by GW3965 treatment.

These results suggest that LXR activation by GW3965 restored the hypertension-induced structural changes of heart in the DOCA-salt hypertension.

Monday 5 September 12:30–14:30

Mechanism of neurodegenerative diseases

P-09.02.2-001
Blood-brain barrier dysfunction induced by chronic methylphenidate treatment: control vs. hyperactive rats

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Methylphenidate (MPH) is a psychostimulant prescribed for the treatment of attention deficit hyperactivity disorder (ADHD), one of the most common neurobehavioral disorders of childhood and adolescence. In fact, despite the widespread use of MPH the full comprehension of its cellular/molecular mechanisms is still elusive, including its effect on blood-brain barrier (BBB). This barrier is a key structure in the Central Nervous System since it protects the brain and its dysfunction has been described as a critical event in several brain diseases.

Thus, the aim of the present study was to clarify the effects of MPH on the BBB function in both physiological and ADHD conditions. For that, we used a rat model of ADHD, the Spontaneously Hypertensive (SHR) rats, and Wistar Kyoto (WKY) animals as inbred comparator strain. Also, to mimic a clinical dosing schedule for ADHD treatment, rats were administered for Monday–Friday with vehicle or MPH (1.5 mg/kg/day or 5 mg/kg/day, per os) from P28-P55.

Chronic MPH treatment (5 mg/kg/day) promoted cortical BBB permeability in both WKY and SHR animals; however, more prominent in WKY rats. This effect can be explained by the downregulation of claudin-5 and collagen-IV, tight junction and basal lamina protein, respectively. Noteworthy, WKY animals also showed an increase in the expression of caveolin-1 and in both vascular cell and intercelular adhesion molecules. These BBB alterations led to subsequent infiltration of peripheral immune cells, including $CD169^{+}$ macrophages. Furthermore, hippocampal BBB disruption was only observed in WKY rats with 5 mg/kg of MPH. Here, MPH decreased collagen IV expression and upregulated caveolin-1, with no alterations in claudin-5.

Overall, our results show that chronic exposure to MPH can led to brain vascular alterations particularly under physiological conditions. This highlights the importance of an appropriate MPH dose regimen for ADHD, and also that MPH misuse can have a negative effect.

P-09.02.2-002
A novel regulatory role of RGS4 in neural precursor proliferation and STAT5B-mediated gene transcription

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Regulators of G proteins signaling (RGS) serve several cellular functions varying from tolerance, dependence, neuroprotection, transcription and tumorgenesis. Despite their initial role as GTPase activating proteins, evidence suggests that RGS proteins are localized in the nucleus, interact with transcription factors thus regulating transcriptional responses. It was shown that RGS4 directly interacts with and interferes in opioid receptor (OR) signaling. RGS4 is mostly expressed in brain and is implicated with brain structural alterations; however, the molecular mechanisms of how RGS4 could be involved in cellular differential functions remains unclear. Based on these observations we examined whether RGS4 can regulate transcriptional responses mediated by the STAT5B transcription factor.

Isolated neural stem cells from $RGS4^{-/-}$ mice were immunostained for the mitosis marker PH3 and the mRNA levels of anti-apoptotic genes were determined. Proliferation assays were performed with BrdU staining in Neuro2A cells stably expressing RGS4. The functional assays of STAT5B transcriptional activation were performed in HEK293 expressing either the erythropoietin receptor (EPO-R) or the delta opioid receptor (δ-OR).

The present data demonstrate that RGS4 blocks STAT5B phosphorylation and transcriptional activation by interfering in STAT5B heterodimerization upon EPO-R or δ-OR activation triggered by cytokines or opioids administration. Lack of functional RGS4 results in increased mRNA levels of STAT5B target genes such as the members of the Bcl anti-apoptotic family Bcl-2, Bcl-6 and Bcl-xl. This upregulation of STAT5B inducible gene transcription results in an increased proliferation rate of neural stem cells.

This study demonstrates for the first time a non-canonical function of RGS4 in STAT5B mediated transcriptional responses and a novel selective role of RGS4 in transcription.

P-09.02.2-003
Role of the pre-molten globule structure in amyloid fibril formation

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The major factor that caused extensive research on the protein fibrillation is their crucial roles in important diseases known as the amyloidosis diseases. Neurodegenerative diseases, including Alzheimer's, Parkinson's, diabetes and Huntington are the most important types of this disease. Understanding the mechanisms of fibril formation and ways of treatment can be useful in reducing this type of disorder. In this project, the fibrillation of carbonic anhydrase protein was investigated as a model. Carbonic anhydrase creates two stable intermediated known as pre-molten and molten globule, in different pH solution. This protein at pH between pH 3-4 molten globule structures was formed while the pre-molten form took place under pH 3. In our tests at pH 3.5 when the protein in molten globule structures only the amorphous aggregates were formed. Instead, at pH 2.4 in pre-molten globule structure amyloid fibrils formed in the protein. There

some reports, indicated the protein from pre-molten globule structure go toward amyloid assembly. Even intrinsically unstructured proteins such as alpha-Synuclein first took a structure similar to pre-molten globule and then made amyloid fibrils. It seems pre-molten globule structure have the major role in promoting to amyloid fibrils. Perhaps drugs that prevent the formation of pre-molten globule structure have an important role in inhibiting amyloid fibrils.

P-09.02.2-004
Myo-inositol prevents the cell loss and biochemical changes induced by kainic acid status epilepticus

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Identification of compounds preventing the biochemical changes that underlie the epileptogenesis process and understanding the mechanism of their action is of great importance. We have previously shown that myo-Inositol (MI) daily treatment for 28 days prevents certain biochemical changes that are triggered by kainic acid (KA)-induced status epilepticus (SE), [1,2]. However in these studies we have not detected any effects of MI on the first day after SE. In the presented study we broadened our research and focused on KA induced other early molecular and morphological changes and influence of MI treatment on these changes. The increase in the amount of voltage-dependent anionic channel-1 (VDAC-1), mitochondrial-plasma membrane cofilin and caspase-3 activity was observed in the hippocampus of KA treated rats. Administration of MI 4 h later after KA treatment abolishes these changes, whereas under the same time schedule diazepam treatment has no significant influence. The number of neuronal cells in CA1 and CA3 subfields of hippocampus is decreased after KA induced SE and MI post-treatment significantly lessens this reduction. No significant changes are observed in the neocortex. Obtained results indicate that MI post-treatment after KA induced SE could successfully target the biochemical processes involved in apoptosis, reduces cell loss and can be successfully used in the future for translational research.

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P-09.02.2-005
Azure B reduces extracellular amyloid-β levels in PS70 cells

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Extracellular deposits of amyloid-β peptide (Aβ) in brain parenchyma via proteolytic processing of amyloid precursor protein (APP) are one of the typical characteristics of Alzheimer's disease (AD). These aggregates mainly occur as a result of an increase in Aβ production or a decrease in its degradation. It was found that the neurotoxicity of Aβ aggregates is accelerated by acetylcholinesterase (AChE). Besides, Aβ-AChE complex has a prominent neurodegenerative effect in brain. Thus, cholinesterase inhibition and preventing Aβ production are current treatment strategies for AD. Recent studies have shown that methylene blue (MetB), a cholinesterase inhibitor with phenothiazine structure, inhibits the formation of amyloid plaques and neurofibrillary tangles. Azure B, the major metabolite of MetB, has been shown to inhibit AChE and BChE with IC50 values of 0.486 μM and 1.99 μM, respectively.

In the present study, we tested whether azure B, may effectively lower the levels of $A\beta_{40/42}$. We treated Chinese hamster ovary cells, which stably express human wild type APP and presenilin-1 (PS70) with 0–15 mM azure B or vehicle for 24 h. To determine the effect of azure B treatment on $A\beta_{40/42}$levels, we used separate sandwich-based ELISAs and normalized to total protein levels, determined by BCA protein assay. Azure B treated PS70 cells were also assessed by propidium iodide in flow cytometry for cellular toxicity.

We observed a significant decrease in both extracellular $A\beta_{40}$ and $A\beta_{42}$ levels with a dose range treatment of azure B in PS70 cells. $A\beta_{40}$ levels were reduced by 89.2% in 10 μM and 94.1% in 15 μM azure B-treated cells when compared to control. Additionally, $A\beta_{42}$ levels were decreased by 83% in 10 μM and 93.5% in 15 μM azure B-treated cells when compared to control. Overall, these preliminary results suggest that azure B may have beneficial effects for the treatment of AD.

P-09.02.2-006
The effect of green silver nanoparticles (AgNPs) on the amyloid formation in alpha-lactalbumin and chaperone action of alpha-casein

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Formation and deposition of protein fibrillar aggregates in the tissues is associated with several neurodegenerative diseases such as Alzheimer's and Parkinson's disorders. Molecular chaperones are a family of proteins that are believed to have ability for inhibiting protein aggregation. In the present study the effect of different concentrations of green synthesis silver nanoparticles (AgNPs) from *Pulicaria undulate* L. on the amyloid formation of α-lactalbumin (α-LA) and chaperone action of α-casein have been investigated. The effects of the AgNPs were determined using light scattering absorption, ThT binding assay, intrinsic fluorescence assay, ANS binding assay, CD spectroscopy and SDS-PAGE. Light scattering and ThT assay results showed that AgNPs have the ability in preventing aggregation of α-LA in a concentration-dependent manner. Consistent with these results, SDS-PAGE results represented that by increasing the concentration of AgNPs the adsorption and interaction between AgNPs and protein have increased. Light scattering and ThT assay results, also, revealed that the amyloid fibrilation decreased in the presence of both AgNPs and α_s-casein compared to presence of α_s-casein alone. Fluorescence results, however, show that AgNPs have no effect on the chaperone ability of α-casein and in fact they perform their protection of protein aggregation action independently. Consistent with the above experiments, CD spectroscopy also revealed that AgNPs have decreased structural changes in reduced α-LA in absence and presence of α-casein, both the tertiary and the secondary structure of the proteins. Our finding represented that AgNPs have preventing effects on protein aggregation and have no effect on the chaperone ability of α_s-casein. In the main, results of this study show that biosynthesized AgNPs mediated by >*Pulicaria undulate* L. maybe could be affective as a therapeutic agent for inhibiting aggregation in treatment of amyloidosis disorders.

P-09.02.2-007
PINK1 is involved in intercellular, bystander communication following genotoxic stress

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PINK1 is a mitochondrial kinase with multiple cellular functions. While loss-of-function mutations of *PINK1* gene lead to early onset Parkinson's disease, its over-expresion is associated with cancer development. Parkinson is a multifactorial neurogenerative disease, with a complex aethiology including various cellular stressors. It is now known that genotoxic stress also triggers the release of soluble factors able to induce changes in neighboring cells enhancing the initial lesions, process known as bystander phenomena. Althrough the mechanisms are still unclear, recent studies point towards a role for mitochondria in this process.

Our work investigates PINK1 role in intracellular and intercellular stress response, comparatively in various models: fibroblasts (MEFs) and neuroblastoma (SH-SY5Y) used as a tumoral model or differentiated to a neuronal phenotype. PINK1 role in this process was analyzed using genetically engineered PINK1 deficient cells exposed to a genotoxic agent, bleomycin.

The modified cell lines showed a reduced level of basal ATP production. PINK1 proved to be involved in cellular vulnerability to stress. Despite differences in cellular sensibility between our models, genotoxic treatment of PINK1 deficient cells induced consistently higher lesions compared to corresponding wild type variant. PINK1 deficient cells showed altered intercellular signaling of stress, impairing bystander phenomena induction, by suppression of signal formation in treated cells, but also by altering the capacity to respond to the signals in neighboring cells.

Our hypothesis is that PINK1 contributes to the management of cellular stress being involved in bystander transmission of detrimental effects through intercellular communication. This is determined mainly by its role in maintaining mitochondrial homeostasy and ATP levels, PINK1 deficient cells lacking the amount of energy required for rapid DNA repair and stress signaling transmission.

P-09.02.2-008
Intranasal administration of synthetic fragments from receptor for advanced glycation end products prevents memory loss in olfactory bulbectomized mice

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The receptor for advanced glycation end products (RAGE) is a member of the immunoglobulin protein superfamily. Activation of RAGE causes brain inflammation, oxidative stress and secretion of beta-amyloid that has been recognized as an essential phase in the development of Alzheimer's disease.

It is known that the receptor soluble isoform (sRAGE) which lacks the transmembrane and cytosolic domains binds to ligands and prevents negative effects of the receptor activation in *in vivo* and *in vitro* experiments. We proposed that potential ligand-binding peptide fragments from sRAGE would demonstrate the same biological activity.

We have selected and synthesized 10 peptide fragments from unstructured surface-exposed regions of RAGE. Synthetic peptides were intranasally administrated into olfactory bulbectomized (OBE) mice with neurodegeneration of Alzheimer's type. We have found that only administration of RAGE fragment (60–76) effectively prevents the OBE murine memory from impairment, leads to decrease of beta-amyloid level and blocks the development of neuronal pathology in the brain of experimental mice. Six overlapping fragments of RAGE (60–76) peptide were synthesized in order to find a site, responding for the therapeutic effect. Tests in OBE mice carried out with these fragments showed that only the N-terminal part of the molecule is responsible for preventing OBE mice memory from impairment. All fragments which do not include N-terminal 60–61 dipeptide have been fully inactive in these experiments. We have proposed that active peptides can interact with beta-amyloid or S100B protein preventing these ligands from binding with RAGE. This interaction can inhibit the development of neurodegeneration. Our proposal is currently under investigation. Supported by RFBR Grant 15-04-01360.

P-09.02.2-009
Effects of social isolation, enriched environment and exercise on learning in rats

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The aim of this study was to examine effects of social isolation, enriched environment and exercise on learning in rats. The study included 36 female 25 day old Wistar rats. The rats were randomly divided into four different groups; control, exercise, social isolation and the enriched environment groups. The social isolation group and the enriched environment group were housed under their specific conditions and the exercise group and the control group were housed in standard conditions during 6 weeks. The rats in the exercise group swam for 6 weeks. After 6 weeks, the rats were evaluated in the Morris water maze. Brain and blood samples were taken and the hippocampus tissue was dissected. BDNF and NGF levels were measured in these samples. In conlusion, while enriched environment was a positive effect on spatial learning, social isolation was a negative effect on spatial learning and increase thigmotactic behaviors. According to the analysis results NGF and BDNF levels in the hippocampus and plasma did not change with environmental conditions and exercise. Time of exposure to social isolation, procedures of the enriched environment, time of exposure to the environment, type and duration of exercise and gender may affect the results.

P-09.02.2-011
Design, synthesis and anticholinergic effects of some chiral benzimidazole derivatives

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Alzheimer's disease (AD) was characterized by dementia that typically begins with subtle recognition failure and poor memory. It slowly becomes more severe and, eventually, incapacitating. The cholinergic system seemed particularly susceptible to synapse loss, especially in cortical regions associated with memory and executive function (1). Recent studies showed that the main cause of the loss of cognitive functions in AD patients was a continuous decline of the cholinergic neurotransmission in cortical and other regions of the human brain (2). Acetylcholinesterase (AChE) and

butyrylcholinesterase (BChE) are hydrolytic enzymes that act on acetylcholine (ACh) to terminate its actions in the synaptic cleft by cleaving the neurotransmitter to choline and acetate. Both enzymes are present in the brain and detected in neurofibrillary tangles and neuritic plaques. It was suggested that AChE predominates in the healthy brain, with BChE considered to play aminor role in regulating brain ACh levels. However, BChE activity progressively increases in patients with AD, while AChE activity remains unchanged or declines. Both enzymes therefore represent legitimate therapeutic targets for ameliorating the cholinergic deficit considered to be responsible for the declines in cognitive, behavioral, and global functioning characteristics of AD (3). We initiated a study to screen their acetylcholinesterase (AChE, EC 3.1.1.7) inhibitory activities, which are the key enzymes taking place in pathogenesis of AD. Newly synthesized chiral benzimidazole derivatives with thioure structure showed IC_{50} values in the range of 11.55–36.00 nM for AChE.

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P-09.02.2-012
F3-A13 h, novel fingolimod derivative, activates cAMP-dependent signalling pathway in SK-N-SH cell line

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FTY720, a sphingosine 1-phosphate (S1P) receptor modulator, is the first oral disease-modifying therapy to be approved for the treatment of relapsing-remitting multiple sclerosis.

In this study, we have synthesized and characterized novel derivative of FTY720, namely F3-A13 h, and have determined its underlying cAMP regulation in SK-N-SH cell lines. For this purpose, we first determined the regulation of the cAMP response element (CRE) activity and cAMP concentration by F3-A13 h along with FTY720 using pGL4.29 luciferase reporter assay and cAMP immunoassay, respectively. Then, we have determined their effect on cAMP/PKA-related gene expression profiles using custom arrays along with FTY720 treatment at non-toxic doses.

It was found that F3-A13 h significantly activate CRE and increase cAMP concentration in the SK-N-SH cell line, indicating that it activates cAMP pathway through cell surface receptors as FTY720 does. Furthermore, F3-A13 h modulates the expression of the pathway related genes that are important in cAMP signaling pathway.

In summary, our data demonstrate that the novel FTY720 derivative act as a modulator of cAMP ultimately by influencing the gene expression via the cAMP and downstream transcription factor CRE pathway. In conclusion, F3-A13 h might contribute future therapies for multiple sclerosis.

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P-09.02.2-013
Alpha 7 nicotinic acetylcholine receptor deficiency in the brain results in the development of Alzheimer-like symptoms in mice

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Alzheimer disease (AD) results in memory impairment and accompanied by neuroinflammation, cholinergic deficit and amyloid-beta (Ab_{1-42}) accumulation in brain. We found that bacterial lipopolysaccharide (LPS) injections or mice immunizations with extracellular a7 nicotinic acetylcholine receptor ($a7_{1-208}$ nAChR) domain resulted in astrogliosis, decrease of a7 nAChR density, accumulation of Ab_{1-42} in brain and episodic memory impairment. The aim was to reveal main event triggering AD-like symptoms development.

C57Bl/6 mice were injected with LPS, immunized with recombinant $a7_{1-208}$ or $a7_{1-208}$ endoglycosidase treated to remove carbohydrates. Two immunizations with 3 week interval were performed. Control mice obtained complete Freund's adjuvant injections. Mice were tested for memory performance, blood sera were examined for presence and fine specificity of $a7_{1-208}$-specific antibodies and brain preparations were studied for a7 nAChR, Ab_{1-42} and IL-6 levels.

The original $a7_{1-208}$ ('glyc') was more immunogenic than 'deglyc', and their epitopes were recognized with different efficiency. In contrast to LPS and 'glyc' $a7_{1-208}$ immunization with 'deglyc' $a7_{1-208}$ did not stimulate IL-6 elevation in brain and had no pro-inflammatory effect. Immunizations with 'glyc' or 'deglyc' $a7_{1-208}$ resulted in similar a7 nAChRs decrease and Ab_{1-42} accumulation in brain and significant episodic memory decline comparable to those after LPS injections.

a7 nAChR interacts directly with amyloid-beta precursor protein and facilitates its proper processing and metabolism. Our data indicate that decrease of a7 nAChR density caused by $a7_{1-208}$-specific antibody is critical for Ab_{1-42} accumulation and episodic memory impairment while pro-inflammatory capacity of $a7_{1-208}$-specific antibody plays secondary role in AD-like symptoms development.

P-09.02.2-014
In vitro antioxidant and anti-acetylcholinesterase activity of *Achillea millefolium*

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Alzheimer diseases (AD) is a neurodegenerative condition without a current effective treatment. Increase in reactive oxygen species and lipid peroxidation or decrease in total antioxidant capacity causes oxidative stress-induced tissue damage. It has been suggested that decrease in oxidative stress and inhibition of acetylcholinesterase (AChE) activity play a major role in the prevention and slowing of cognitive symptoms of AD. Recently, studies have been directed for the discovery of medicinal plants and natural substances that are known to have natural antioxidants. *Achillea millefolium (A. millefolium)* is a traditional herbal medicine that contains natural compounds with antioxidant activity and has been used as a carminative, diuretic, menstrual

regulator and wound healer, however the mechanism of its actions are unclear. The aim of our study was to investigate the effects of *A. millefolium* extracts on free radical production, acetylcholinesterase (AChE) activity and lipid peroxidation in vitro.

Methanol (ME) and ethanol (EE) extracts of *A. millefolium* were prepared to determine (a) *in vitro* antioxidant capacity (by using 2,2-diphenyl-1-picrylhydrazyl assay, radical scavenging activity, phosphomolibdenum-reducing antioxidant power, ferric-reducing antioxidant power, and total phenolic-total flavonoid contents), (b) effects on AChE kinetics (by using a colorimetric assay) and (c) effects on sodium nitroprusside-induced lipid peroxidation in mice brain homogenates.

ME showed a higher antioxidant activity compared with EE in the biochemical assays tested. Similarly, ME demonstrated significant inhibition of AChE activity that was potent than EE. Both extracts dose-dependently decreased malondialdehyde content in mice brain homogenates suggesting a strong inhibition of lipid peroxidation.

These results showed that *A. millefolium* has a high antioxidant capacity and antiAChE activity, indicating a potential use as an adjuvant therapy in AD.

P-09.02.2-015
Potential mechanisms of CXCR5 promoter regulation associated with SNP rs630923 which is responsible for high risk of multiple sclerosis

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B-cells are known to play a key role in multiple sclerosis (MS) progression and autoimmune response. CXCR5 is the main B-cell chemokine receptor that under normal conditions directs their migration to specific areas of secondary lymphoid organs. In MS, areas of demyelinating lesions have been reported to attract B-cells due to overexpression of CXCL13, the CXCR5 ligand. We aimed to determine whether SNP rs630923 located in the promoter of *CXCR5* gene and associated with high risk of multiple sclerosis could have a direct effect on of CXCR5 promoter activity.

MEF2C binding to DNA was assessed using pull-down assay. B-cell stimulation was performed using LPS, PMA and ionomycin. Activities of variants of CXCR5 promoters containing different rs630923 alleles were estimated using luciferase reporter assay.

We determined that minor rs630923 allele creates functional MEF2C-binding site within one of the regions required for the basal activity of the *CXCR5* promoter. *CXCR5* promoter containing minor rs630923 variant that is statistically associated with low risk of MS showed significantly decreased activity in stimulated human B-lymphoblastoid cell lines.

MEF2C has been reported to play an essential role in B-cell survival and B-cell responses. We determined MEF2C as the main regulator of rs630923-dependent modulation of CXCR5 promoter activity in B-lymphoblastoid cell lines. This link may be directly related to pathogenic B-cell activities in multiple sclerosis.

P-09.02.2-016
Paving the way towards personalized treatment of Parkinson's disease by integrated biomarker research

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Introduction: Parkinson's disease (PD) is the second most common neurodegenerative progressive brain disease with increasing prevalence in aging population. The etiopathogenesis involves many cellular procesess, but is not fully elucidated yet. Treatment of PD is based on levodopa and dopamine agonists, but MAO-B inhibitors, COMT inhibitors, amantadine or anticholinergics may be used as initial monotherapy or as adjuvant therapy. Treatment related adverse drug reactions (ADRs) are frequent, but cannot be predicted and/or prevented. Non-motor ADRs, such as nausea, somnolence, hallucinations and hypotension are frequent in dopamine agonist therapy, while dyskinesias along with motor fluctuations are the most common late ADRs with levodopa. The aim of our study is to combine clinical data with genetic and epigenetic biomarkers in the algorithm for personalized approach to PD management.

Materials and Methods: We are planning a clinical study to assess the combined impact of selected clinical, genetic and epigenetic factors on the progression of PD, ADRs and treatment response. Our study will have a retrospective and prospective arm. We will collect peripheral blood samples of PD patients and clinical data. Single nucleotide polymorphisms (SNPs) in the genes involved in dopamine, neurotransmitter and drug metabolism and transport, receptors and signalling pathways will be genotyped. SNPs within inflammatory, neurodevelopmental, antioxidative defense, synaptic transmission and immune response pathways will also be analysed. In the prospective arm we will isolate the exosomes and check their miRNA content at the time of diagnosis and after the treatment initiation. The combined effects of clinical, genetic and epigenetic factors will be analyzed using lasso penalized regression analysis.

Conclusions: We hope to identify genetic and/or epigenetic biomarkers that may predict progression of PD, ADRs and treatment response and may support personalized tratment of PD.

P-09.02.2-017
Glutamate NMDA, dopamine D1 and histamine H3 receptors form heterotrimeric complexes in brain

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Most evidence indicates that G protein-coupled receptors form heteromers between them and with other receptors. By allosteric mechanisms, them acquire a multiplicity of unique pharmacological and functional properties. Recently, we discovered that dopamine D1 receptors (D1R) and histamine H3 receptors (H3R) form heteromers through which H3R ligands can inhibit D1R function. D1Rs also physically interact and modulate ionotropic glutamate NMDA receptors (NMDAR). In the present work, we investigated if NMDAR, D1R and H3R form a heterotrimeric complex in brain.

The heteromer expression was studied in slices from both rat and mouse brain cortex by co-immunoprecipitation (Co-IP) and proximity ligation assays (PLA). The ability of D1R and H3R to

interact with NMDAR in transfected HEK cells was analyzed by bioluminescence resonance energy transfer (BRET) with bimolecular fluorescence complementation (BiFC) experiments. Heteromer properties were studied by analyzing ERK1/2 phosphorylation and cell death in cortical slices.

Endogenous D1R-H3R heteromers were detected in rat and mouse cortical slices, where H3R ligands decreased D1R signaling (ERK1/2 pathway) and were also able to block the cell death induced by overstimulation of either D1R or NMDAR. By BRET experiments in transfected HEK cells, we demonstrated that both D1R and H3R form heteromers with NMDAR subunit 1A in the presence of subunit 2B. D1R-H3R-NMDAR heteromers were detected by BRET with BiFC. Endogenous D1R-H3R-NMDAR heteromers were observed in rat and mouse cortex by PLA.

Many systems, including the glutamatergic and dopaminergic, are involved in neurodegeneration. Our innovative finding is that D1R, H3R and NMDAR form heteromers that may be a point of intervention for cognitive disorders in neurodegeneration.

D1R-H3R-NMDAR heteromers are expressed in brain cortex and a complex interaction exists between protomers in the heteromer, where H3R ligands act as a 'molecular brake' for D1R and NMDAR signaling.

P-09.02.2-018
Obesity and effects on hypothalamic vascularity

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Studies conducted on obesity and HFD (high fat diet) revealed hypothalamus have crucial roles on development of metabolic diseases. After chronic over nutrition or high fat diet, as a neurodegenerative condition, premise inflammation, neural stress and development of functional impairments are observed. These studies generally focused on changes in neurons, but it's effects on brain vessels are still unknown. In this study, as a neuronal damage infrastructure, changes in hypothalamic vascularity investigated.

Experiment initiated with 5 weeks old total 40 male Wistar rats. In order to acquire obese phenotype, the rats were fed either cafeteria diet as HFD, or normal/chow (standard diet, SD) for 9 months. Intravenous glucose tolerance tests performed before sacrification. Animals were exposed for 10 s to CO2 and then decapitation was performed with guillotine. Isolated brains were directly immersed into liquid nitrogen and stored at −80 °C. The hypothalamic sections were acquired with the cryostat instrument at different. Immunofluorescence was performed on serial sections through the hypothalamus using the antibody SMI-71 and CD31. Changes in tight junction (TJ) proteins (occluding and zone occludin-1) are evaluated via western blot (WB) analysis.

The HFD-treated consumed significantly more food than did control animals, when examining average food consumption per day and rats that received the HFD diet weighted significantly more at the end of 9 month diet treatment. There were no differences acquired for glucose tolerance tests. However, after HFD treatment, WB analysis have shown that TJ proteins decreased even if hypothalamic micro vessel number increased and SMI-71 staining have shown that increased.

Our primary results have shown that HFD diet can affect hypothalamic vascularity and such changes might initiate neurodegenerations and functional impairments as observed in neuroretinal degeneration in relation to vasculopathy in diabetic patients.

P-09.02.2-019
Mitochondrial distribution and trafficking in primary fibroblast derived from patients diagnosed with sporadic form of Alzheimer's and Parkinson's diseases

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Defects of mitochondrial trafficking are common problem in many neurodegenerative diseases. Its dysregulation can contribute to changes in bioenergetics profile of the cell and can lead to cell death. In our study we investigate distribution of mitochondria and their transport in primary fibroblasts derived from patients with sporadic form of Alzheimer's (AD) and Parkinson's (PD) diseases. Our data revealed that in the most cases the velocity of mitochondrial movement is lower in AD and PD cells in comparison to the control. The most intense differences between AD, PD patients and control group are observed in the case of movement of large mitochondria. Owing to the fact that mitochondrial trafficking depends on mitochondrial state, we investigated the 'age' of mitochondria. We observed a diminished mitochondrial turnover in AD and PD fibroblast. Evaluation of the mitochondrial distribution within the cell in all 3 groups (AD, PD and control) showed that in the perinuclear area are accumulated 'old' and 'worn out' mitochondria, probably dedicated to remove from the cell. Because mitochondrial biogenesis, shape and size depends on fusion/fission proteins we assessed their level within the cell. To summarise, our results revealed alterations in mitochondrial trafficking in fibroblasts derived from patients with Alzheimer's and Parkinson's diseases in comparison to the healthy control cells.

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P-09.02.2-020
The determination of the carbonic anhydrases activators

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Carbonic anhydrases (CAs, EC 4.2.1.1) is a zinc metalloenzyme that catalyzes the reversible reactions of CO_2 and water. Carbonic anhydrases (CAs, EC 4.2.1.1) form a family of metalloenzymes that play an important function in various physiological and pathological processes. Therefore, many researchers work in this field in order to design and synthesize new drugs. Carbonic anhydrase activators are important as much as inhibitors.

CAAs have polar groups to make hydrogen bond in the main body and the activation property of enzyme increaase in this way. CAAs are have polar groups to make hydrogen bond in the main body and the activation property increaase in this way. Furthermore, recent studies suggest that CA activation may provide a novel therapy for Alzheimer's disease.

In this study CA activators are determined. Human carbonic anhydrase isozymes CA I and CA II are isolated from human blood erythrocyte. hCA-I and hCA-II isoenzymes were purified using Sepharose-4B-L tyrosine-sulfanilamide affinity colum. Finally, hCA-I and hCA-II isoenzymes were eluted with appropriate elution buffers. Enzyme purity was checked by SDS-PAGE. The enzyme activity system contained 0.05 M Tris-SO_4 pH 7.4, r-nitro phenol in 1 ml total volume. Effects of some macrocyclic thiacrown ethers derivates were investigated. Enzyme

The FEBS Journal **283** (Suppl. 1) (2016) 129–417 DOI: 10.1111/febs.13808

activities were measured at constant substrate and different activator concentrations to find AC_{50} value.

These compounds are thought to be useful for treating Alzheimer's disease.

P-09.02.2-021
Social isolation and predator scent tests alter brain BDNF levels, differentially according to gender, in rats

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Introduction: Gender differences in stress models are not studied in detail. We compared different stress conditions on brain BDNF levels, in social isolation (SIT) and predator scent tests (PST) in rats. BDNF levels in cortex, hippocampus and amygdala were compared, effects of chronic fluoxetine (FLU) treatment were evaluated.
Methods: 128 rats were used. For SIT, animals were kept individually for 1 month and for PST, rats were exposed to dirty cat litter for 10 min at the first day of 1 month stress. FLU was given (5 mg/kg, ip) through stress. Controls, stress and treated groups were evaluated in elevated plus maze (EPM), anxiety scores were calculated. Brain BDNF levels were determined in cortex, hippocampus and amygdala by Western blot. $P < 0.05$ were considered significant.
Results: SIT and PST induced anxiety in both male and female rats, females having greater anxiety scores than males ($P < 0.05$). FLU restored anxiety scores in both sexes ($P < 0.01$) in two settings. Male and female rats exhibited reduced cortical BDNF levels in SIT ($P < 0.001$). PST reduced cortical BDNF in females, but increased in males. Hippocampal BDNF expression was lowered in SIT ($P < 0.01$) and PST ($P < 0.001$) in both sexes. Female rats had 40% lower BDNF expression than males in amygdala in SIT. FLU did not restore cortical BDNF in females in both tests, but reduced incresed BDNF levels in males ($P < 0.001$). FLU did not restore reduced brain BDNF in males in hippocampus and amygdala, but restored in hippocampus, in females.
Discussion: Our findings indicate that sex differences must be considered in studies related to mood disorders of animal models, and suggest that BDNF expression in different brain regions are altered differentially in a gender-dependent manner in rats. Antianxiety effect of FLU is not mediated through increasing BDNF activity in cortex in both genders. Increased BDNF in hippocampus and amygdala may reflect its antidepressant effect in female rats, but not in males.

P-09.02.2-022
Role of hyaluronan and proteoglycan link protein 1 during neuritogenesis

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Perineuronal nets (PNNs) are special forms of neural extracellular matrix found around neuron bodies and neurites. Hyaluronan and proteoglycan link protein1 (HAPLN1) is one of the major elements of PNNs. HAPLN1 interacts with tenascins and aggrecan which are other essential PNNs components. In most of neurodegenerative disorders caused by neuritogenesis defects, disrupted PNNs structure and decreased expression of HAPLN1 were observed. However, the role of HAPLN1 in neural differentiation is unknown.

The aim of this study is to determine mRNA and protein levels of HAPLN1 during differentiation using PC12 cell line as a neural differentation model, derived from rat pheochromocytoma. After PC12 cells were stimulated to differentiate into neurons by nerve growth factor on days 3, 5 and 7; cells were collected, qRT-PCR and western blot were performed. Also, in order to find out whether there is a physical interaction between HAPLN1 and proteins related to neuritogenesis defects, spinal muscular atrophy (SMA) was used as a neurodegenerative disease model. Therefore, a detailed HAPLN1 and survival motor protein 1 (SMN1) network analysis were performed *in-silico*.

As a result, we analyzed 3 fold increase in HAPLN1 mRNA level compared to undifferentiated state. On the other hand, a decrease in protein level was detected. This decrease in cellular HAPLN1 level suggests that, HAPLN1 is required for formation of PNNs structure, thus secreted to extracellular environment at early stage of differentiation. In addition, according to *in-silico* analysis, an indirect path between HAPLN1 and SMN1 through fibulin2 (FBLN2) was detected. FBLN2 was also found to be an interaction partner between different matrix molecules such as aggrecan and HAPLN1 which form a macromolecular meshwork.

The results of this study will pave the way for investigating the role of HAPLN1 and FBLN2 in neurodegenerative disease models. Also it will help us to understand the mechanism of neuritogenesis defects.

P-09.02.2-023
Determination of properties of bone marrow and tissue-derived mesenchymal stromal/stem cell population in neurofibromatosis type 1 patients

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Neurofibromas, complex tumors deriving from Schwann cells and containing fibroblasts, vascular structures and mast cells, are part of the clinical picture in NF1. The risk of malignancy is increased in NF1, wound healing is delayed and keloid formation is frequent. Because multiple tissues are involved in malignant and non-malignant manifestations of NF1, we considered the mesenchymal stem/stromal cells (MSC) carrying the NF1 mutation might play a role in the microenvironment. MSCs affect the biological behaviour of other cells: they alter their proliferation, apoptosis and migration through various secreted growth factors, cytokines, chemokines, or by direct contact. We examined the MSC of NF1 patients.
Methods: The adipogenic and osteogenic differentiation potential of MSCs from NF1 and healthy subjects was examined

in vitro and by RT-PCR. MSC's migration potential was measured in the scracth assay. MSCs' interaction with Schwann cells and their effect on tumorigenesis was examined in co-culture by apoptosis markers on Flow Cytometry.

Results: NF1-MSCs' adipogenic and osteogenic differentiation potential was lower than healthy controls as assessed by staining Aizerin Red S and Oil Red O and RT-PCR for osteopontin and collagen1. MSCs cultured from dermal neurofibroma showed faster closing of the scratch compared to the same patient's normal and café au lait skin. On the other hand, MSCs obtained from plexiform neurofibroma healed late, while MSCs derived from the same patient's café au lait skin showed the fastest healing. Schwann cell-MSC co-cultures showed no specific effect of MSC on Schwann cells' Annexin V and Propidium iodide expression, neither any effect of Schwann cells on the MSC surface markers was observed.

Conclusion: These results support particular behavior of MSC form NF1 patients in terms of differentiation and cell motility in vitro that might have implicaitons on clinical behavior of their tissues.

P-09.02.2-024
Assay of the key enzymes of glutamine-methionine bicycle activity of the tissues in the animals with induced hepatic encephalopathy

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Hepatic encephalopathy with ammonium ions accumulation is accompanied by some disorder in the brain due to toxic material concentration being usually detoxified in the liver. One of the reasons for hyperammoniemia could be some imbalance in brain glutamine metabolisation induced by the key enzymes glutamine transferases (GTs), which catalyze the reaction of glutamine transamination resulting in neurotoxic product of α-ketoglutaramate (αKGM). αKGM is hydrolyzed to α-ketoglutarate and ammonia by ω-amidases.

In the study, the dynamics of the enzymes activity in the tissues and biological liquids of experimental animals with hepatic dysfunction induced by thioacetamide (TAA) was under investigation. White laboratory rats of Wistar line (female, weight of 140 g) chronically intoxicated with hepatotrophic toxine of TAA. Every 2 weeks, some biological samples were collected to assess GT-K and ω-amidase activities. ω-Amidase activity was the highest in the kidney tissue in the control and decreased by 70% in the experimental group. In the experimental hepatic ω-amidase activity decreased by 240% compared to those in the control. The average ω-amidase activity in the blood serum (0.015 nmols/mg/min) and in the brain (0.005 nmols/mg/min) differed faintly. Maximal GT-K activities were revealed in the kidneys; in the controls, it was about 250% higher than those in the experimental animals. The difference between average enzyme activities in the liver of the control and experimental animals reached 350%. The average GT-K activities in the blood serum and brain of the control and experimental animals were rather similar.

The decrease in ω-amidase and GT-K activities obtained in the study during hepatic pathology development could testify to imbalance of glutamine metabolism, possibly aimed at declining the level of αKGM neurotoxicant under the hepatic dysfunction.

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P-09.02.2-025
Functional characterization of clinically relevant novel mutations in ATP7B gene using the *Saccharomyces cerevisiae* model

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Wilson disease is an autosomal recessive disorder of copper metabolism characterized as neurodegeneration and liver abnormalities. It is caused by defects in the ATP7B gene.

ATP7B is responsible for the sequestration of Cu into secretory vesicles, and this function is exhibited by the orthologous Ccc2p in the yeast. We aimed to characterize clinically-relevant novel mutations of p.T788I, p.V1036I and p.R1038G-fsX8 in yeast lacking the CCC2 gene.

The patients with these mutations have copper storage abnormalities in different parts of their bodies; p.T788I mutation mainly affects the liver and the nervous system, p.V1036I mutation affects the nervous system, and p.R1038G-fsX83 mutation causes damages to the liver. To better understand the effects of these mutations on normal functions of ATP7B, we cloned human ATP7B gene onto a yeast expression vector and created the same mutations by site directed mutagenesis. Then, wild type and mutated forms of ATP7B genes were transformed into yeast cells lacking the homologous CCC2 gene for functional comparison.

First, we analyzed the expression of ATP7B and its variants in yeast cells by a real time PCR approach and Western Blot to make sure that transformed cells express the plasmids.

Expression of human wild type ATP7B gene in ccc2Δ mutant yeast restored the growth deficiency and copper transport activity, however, expression of the mutant forms did not restore the copper transport functions and only partially supported the cell growth.

Our data support that p.T788I, p.V1036I and p.R1038G-fsX83 mutations cause functional deficiency in ATP7B functions and suggest that these residues are important for normal ATP7B function.

Monday 5 September 12:30–14:30

Education, training, and career planning in molecular life sciences

P-EDU-001
An all solid-state urea biosensor based on ammonium-selective PVC membrane electrode

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In recent years, attempts were made to develop miniaturized potentiometric biosensors which is particularly important to reduce the amount of enzyme and reagents needed. The miniaturization of a biosensor is possible by using an all solid-state polymer membrane ion selective electrode which is cheap, easy to prepare and allow micro-sized construction. The use of all solid-state polymer membrane ion selective electrode as the basic sensing element also has the advantage of providing biosensors that are easy to fabricate, exhibit rapid response and have long life-

The FEBS Journal **283** (Suppl. 1) (2016) 129–417 DOI: 10.1111/febs.13808

times. They are also mechanically stable and allow flow-through configuration.

Genetical and chemical modifications for the alteration of enzyme molecule characteristics are gained considerable importance. Enzymes can be modified chemically by using water-soluble polymers or some chemicals. Conjugates of natural and synthetic macromolecules with enzymes provides wide usage in medicine and in many fields of biotechnology.

In this study, enzyme-polymer conjugates with different molar ratios were synthesized using urease enzyme. In this study micro sized potentiometric urea sensitive biosensor has been developed in which urease-polymer conjugates were immobilized on polymeric membrane ammonium ion selective electrodes whether PVC or derivatized-PVC via glutaraldehyde cross linking reaction. Biosensor is not include inner reference electrode and inner reference solution. Potentiometric performance of biosensor will be examined with a computer-controlled measurement system designated.

The most important features of the obtained micro sized urea biosensor by using enzyme-polymer conjugations were being highly sensitive, having long life-time, easily built at a low cost, and having short response time when compared with conventional potentiometric urea biosensor. Also, these biosensors were easily built at micro-construction.

This study was supported by grant from the TUBİTAK research fund (Project number: 114Z138).

P-EDU-002
Creative drama technique as a new tool to increase enthusiasm and to achieve learning objective for medical students

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Recently drama in education techniques have been implemented successfully in education program of primary and secondary high school and positive effects of these techniques on learning ability and attitude of students have been shown. The aim of this study was to organize an education program based on drama in education techniques in a special module of Ege University Medical Faculty and to test any effect of this technique on achievement of learning objectives and student's perspectives on drama.

The special module program was on the oxidative stress and antioxidants. The program covered the drama in education sessions (improvisation, role play, game) linked with learning objectives (understanding of free radical generation and free radical reactions in body, evaluation of the effect of free radical reactions in diseases as well as increase the ability usage of scientific information), laboratory work (antioxidant activity determination) and searching a special scientific topic on literature. 12 students (in 3rd year of Faculty) who had taken theoretical lecture on this subject a year ago, participated in this special module.

The opinions of the students on the program were obtained through a questionnaire form and the increase in knowledge was evaluated via pre/posttest. The mean of pretest point was 2.7/10, that increased to 7.4/10 in the posttest evaluation. 50% of students pointed that they enjoyed participating in drama activities in the pre-questionnaire, this rate was 100% in the final questionnaire. They all remarked that implementation of drama in education was beneficial for their communication skill, helping them to learn more about science and increased their enthusiasm to learn and discuss the scientific information.

Although the preparation process might take more time and need to hard work for teachers, we concluded that the drama methods as a new tool to increase of participant's interest might be proposed for students in higher education.

P-EDU-003
Laboratory-based performance assessment in medical education: an opportunity for connection between scientists and medical students

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Number of medical students who interested in basic medical sciences is declining and medical sciences literacy is falling, it is crucial to develop ways for students and scientists to connect. Students need to know that science is an intensely human endeavor, and scientists need mechanisms to bring that truth to the community at large.

Based on continued interest and experience on the part of faculty, and on student feedback, the development of a more effective and stimulating interactive learning tool was undertaken. An in-depth knowledge of laboratory medicine principles is vital to all practicing physicians. Great variation exists in the ways that medical students learn the principles of laboratory medicine. There are a number of programs for electronic media that emphasize laboratory-related skills. Some of these are appropriate for medical students in the clinical years. Programs that teach skills in common laboratory procedures, such as interpretation of peripheral blood smears and microscopic examination of urine sediment, have been shown to improve student performance.

To ensure that important principles are addressed, medical schools should establish goals and objectives specifically related to laboratory medicine and experiment with optimal teaching and assessment methods. We also hope that this study will inspire dialogue among primary care and specialist physicians as to the proper degree of education in this area. Ideally, it will encourage scientific studies that address evidence-based possibilities for improving critical laboratory medicine educational outcomes, that is, the training of physicians who optimally use laboratory diagnostics and therapeutics. Engaging medical students in scholarly scientific activities and producing clinically competent and research-oriented medical workforces are essential demands, particularly in developing countries.

P-EDU-004
An experimental special study module for medical undergraduate students: learning Western blot analysis and detection of β-actin protein expression in tissue and cell culture samples

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Engaging medical undergraduate students in designing and executing original research should be accompanied by technique training. Special Study Modules (SSMs) are educational activities which provide students to develop independent study and self-education skills and to learn basics of scientific methodology. The objectives of this SSM were to train students in independent

learning, introduce basic principles of laboratory research and to present the results.β-actin is one of six actin isoforms which is mainly expressed in all eukaryotic cells. Western blotting is a widely used laboratory technique to determine specific proteins and to evaluate protein expression in tissues and cells. In our study, different concentrations of rat spleen homogenates (25, 50, 75 μg/well) and 50 μg protein/well of human lung and ovary cancer cell lysates were used. The proteins were seperated by 10% SDS-PAGE, transferred to PVDF membrane, incubated with specific β-actin antibody and then with HRP-conjugated antibody. Protein bands were detected with ECL and densitometric analysis of proteins was quantified by ImageJ software. Differences in protein band intensities were compared using one way ANOVA.A value of $P < 0.05$ was considered statistically significant. We detected β-actin expression in rat spleen homogenates, human lung and ovary cancer cell lysates, as a 43 kD protein. The protein band intensities were in correlation with protein concentrations. The highest concentration resulted in the highest signal intensity in rat spleen homogenates.β-actin level was higher in ovary cancer cells than in lung cancer cells, although both proteins were loaded equally. The feedback showed that students were very satisfied with this laboratory SSM. They developed their independent study skills, planned a research, worked in a laboratory, learned and performed a technique, Western blotting. The hands-on experience is very important for medical undergraduate students for their future scientific career.

P-EDU-005
Three-dimensional structure of truncated human Kv10.2 ion channel studied by cryoelectron microscopy and molecular modeling

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Voltage-gated potassium channel Kv10.2 belongs to the ether-a-go-go family. It has been proved that its mutants are involved in development of neurological diseases and some types of tumor. Directed drug design needs knowledge of details of the three-dimensional channel structure. The members of the Kv10-12 subfamilies are characterized by extremely long N- and C-terminal intracellular tails, which possess a number of structural domains. The N-terminal PAS domain in Kv10 plays an important role in activation, and is thought to alter the rate of deactivation, possibly by binding at or near the S4-S5 linker at the inner mouth of the pore. Here we present an improved 3D structure of the truncated human Kv10.2 channel, obtained by single particle EM. This channel lacks its cytoplasmic PAS domain but it still forms tetrameric particles. Earlier we showed that the full length Kv10.2 channel is build according to the 'hanging gondola' type, and its cytoplasmic and transmembrane parts are connected by linkers. The cytoplasmic part includes the interconnecting PAS and cNBD domains. Deletion of the PAS domain leads to the conformational change in the cytoplasmic part of the channel. For interpretation of the 3D structures we used homology modeling and molecular dynamics simulation. There are several templates available to the moment including eag domain-CNBHD complex of the mouse EAG1 channel, full-length Shaker potassium channel Kv1.2, C-linker/CNBHD of zELK channels and others. But there are still no templates for many fragments that led to necessity of partial *de novo* modeling. Analysis of molecular trajectory allowed estimating dynamical characteristics of channel, supposing interdomain interactions. Results of the conducted investigation have a great interest at both the academic and the industrial levels.

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P-EDU-006
A cultivating experience of Ege University Faculty of Medicine, with research training program; AEP

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The objective of this task program is to enable students to gain scientific attitude and skills for them to be able to deal with the problems they'll confront in future research careers. It's been emphasized in various studies that medical students are keen on conducting scientific research, and it's been stated that they need to be supported in this respect, as they lack the adequate fund of knowledge.

This study aims to share information throughout a 5-year performance of the Research Training Program (RTP) conducted at Ege University, Faculty of Medicine.

RTP is an educational program applied in addition/parallel to the bachelor's degrees for establishing scientific thought, attitude, proceeding and knowledge of the willing medical students, and enabling them to adopt study skills. The dynamic program produced by the RTP committee in 2011 has been developing each and every year via feedback received from the students. An operating principles, a manual for advisor and an introductory booklet have been laid out.

Applications are being accepted at the end of first academic year, making announcement to the freshmen in advance. Students are being admitted to the program, taking the assessment criterias into consideration. Second and third year medical students attend the didactic part of the program during the terms devoted to special study modules. Thereafter, they go through the project management phase, and accomplish their projects under supervision of a faculty member until their graduation.

12 first graduates of the program, accomplishing their projects, received their certificates at the graduation ceremony of 2015 graduation. Currently, there are 61 students in total from all classes who perpetuate their studies within the program.

An inventive training pattern of Ege University Faculty of Medicine, RTP experience is being maintained as a dynamic process and successfully keeps the students advised of conducting scientific researches, cultivating scientific awareness.

P-EDU-007
Comparison of Ayset tubes with references tubes for routine clinical chemistry laboratory testing

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Objectives: Objectives selection and verification of blood collection tubes is an important preanalytical issue in clinical laboratories. In this study comparison with the reference glass tube of Ayset plastic tubes containing separator gel and assessment for routine clinical chemistry laboratory testing in samples were aimed.

Materials and Methods: Thirty-four volunteers were included in the study. Samples were taken into two different tubes by two experienced technologists according to the CLSI protocol [Tube1: Z (Becton Dickinson and Company, Franklin Lakes, NJ, USA); Tube2: AYSET (lot10069, TURKEY)]. Glucose, Urea, Creatinine, AST, ALT, Total-Cholesterol, Triglycerides and High density lipoprotein-Cholesterol were analyzed subsequently (Olympus AU2700) and randomised samples stored at 2–8 °C for 24 and 48 h. 0th hour sample was analyzed immediately after collection and accepted as the reference for the comparison of the other samples. A paired *t*-test and Wilcoxon signed rank sum test were used to test the significance of differences between the reference tube and test tubes.

Results: The difference between the results of reference tube and test tubes for glukose, urea, creatinine, AST, ALT, Total cholesterol, TG and HDL-cholesterol at 0, 24 and 48 h were statistically no significant ($P = 0.09$, $P = 0.07$, $P = 0.20$, $P = 0.16$, $P = 0.13$, $P = 0.09$, $P = 0.05$, $P = 0.54$, $P = 0.58$, $P = 0.46$, $P = 0.19$, $P = 0.13$, $P = 0.66$, $P = 0.72$, $P = 0.34$, $P = 0.11$, $P = 0.16$, $P = 0.06$, $P = 0.08$, $P = 0.39$, $P = 0.23$, $P = 0.63$, $P = 0.58$, $P = 0.54$, respectively).

Conclusions: No Significant difference was observed between Ayset tubes' results and the reference tube's results.

P-EDU-008
Inflammation based insulin resistance and obesity can be treated by anti-inflammatory diet, dietary supplemental support and regulation of life style-analysis of PubMed Publications, 2005–2016

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Insulin resistance underlies the development of obesity which is a global health problem. Obesity is a main concern of scientists because it's associated with Type 2 diabetes, hypertension and some cancers. Recently, inflammation centered mechanisms are deeply investigated as well as the effects of anti-inflammatory diets which are highly rich in vitamins, minerals, fibers and healthy oils. These diets are proposed to inhibit or supress the secretion of the inflammatory mediators and also improve the intestinal microflora. The aim in this study is to highlight the increasing trend of publications in regard to insulin resistance and inflammation based obesity along with the effects of anti-inflammatory diets used for its treatment in the last decade.

We performed a PubMed search with key words of 'obesity AND insulin resistance AND inflammation' (01/01/2005–15/05/2016) (Search #1). Besides, we performed another search with key words of '(anti-inflammatory diet OR dietary supplement) AND (obesity OR insulin resistance)' (Search #2) to highlight the value of anti-inflammatory diets and dietary supplements in combating inflammation based obesity and insulin resistance.

Search #1 revealed 6763 articles; of these 341 were clinical trials, 18 were observational studies. Human studies were 4 552 while animal studies were 2 580. Overall, there were 2 229 review articles and 5 meta-analysis in the field. Search #2 revealed 4 255 articles of which 796 were clinical trials, 958 were review articles, 46 were meta-analysis. Human studies were 2 610 and other animal studies were 1 931.

The relationship of metabolic diseases with a low grade inflammatory state has opened a new area of research to understand the consequent causes of inflammation in the human body. The increasing scientific data in the field indicates that anti-inflammatory diets may serve as powerful tools to solve the inflammation and the consequent insulin resistance and obesity.

P-EDU-009
Medical and biological illustrations for life sciences education: Is 'a picture worth a thousand words' in visualizing medicine and science?

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Medical and biological illustrations (MBI) convey complex ideas with just an image and they are powerful intersections of science and art. The clarification of complex pathways via illustrations can be effective means in education as they help the student to visualize the biomolecular world and understand the mechanisms. Our aim is to illustrate how a MBI is developed over the example of mechanism of insulin action, via the phosphoinositide (PI) 3 kinase-protein kinase B (Akt) pathway.

Organising one's thoughts and clarifying relationships and then using the optimal complexity level to illustrate the pathway most clearly are the basics of MBI. Thus, we made a thorough investigation of insulin mechanism on glucose uptake in skeletal muscle and adipose tissue; a biochemical process that includes insulin receptor (IR), IR substrate, PI3 kinase, PI-dependent kinase 1 and Akt. Then, we found the 3D structures of molecules via protein databanks and accordingly created drawings and diagrams of each component in both molecular and macrolevels by Adobe Photoshop® software. Graphics tablets and a compatible PC were also used in the production phase.

The use of computer hardware/software enables unlimited detail in images and provides the flexibility that classical drawing techniques can not. Thus, the final diagram clarifies the underlying molecular mechanisms of a biochemical pathway along with the physiologic actions.

Recent improvements of computer technology have resulted in the creation, and reproduction of high-quality lower cost medical art. MBI's can be used in education to explain concepts/pathways to students to enhance learning. Similarly, MBI's are great tools to show mechanism/procedures to enhance patients' understanding of their medical condition.

Considering their unquestionable contribution to education, research and patient care, the creation of MBI's should be promoted as a graduate level course and the discipline should be represented at academic level.

P-EDU-010
Scientometric analysis of the last decade's postgraduate theses in the subject area of biochemistry in Turkey: evidence from Turkish Higher Education Council's Theses Database

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Biochemistry is a compelling field with broad applications in many disciplines like medicine, dentistry, pharmacy and bioengineering. Biochemical research increasingly combines ideas from genetics, molecular biology, etc. and collaborates with many disciplines. Our objective is to conduct a scientometric analysis of the last decade's postgraduate theses in the field of biochemistry (PTB) in regard to number, collaborations, subject area distribution, etc. to discover the characteristics and trends in Turkey.

We searched the Turkish Higher Education Council's Theses Database (2006–2015) which includes master of science (MSc.), doctorate (Ph.D.) and specialization (S) theses in all disciplines. An electronic search with the keyword of 'biochemistry' (in the thesis subject area) was conducted, thus it brought all theses either in the biochemistry discipline or theses in other disciplines but have a biochemistry component. We performed data cleaning and further quantitative analyses in Excel. We also executed word count analysis on the titles of theses to derive the main subject areas in PTB.

Of the total of 6374 PTB (2215 S, 2781 MSc, 1339 PhD theses) 37.6% was in Natural Sciences while 62.4% was in Health Sciences. The theses output-growth measured by the Compund Annual Growth Rate was 82% over the 10-years. The top 3 clinical disciplines in collaboration with biochemistry were pediatrics, surgery and cardiology, and the top 3 science disciplines were biotechnology, bioengineering and biology. Oxidants-stress and antioxidants (1008), endocrine-metabolism (655) and enzymology (608) were the top research areas in PTB, followed by genetics (305) and cancer (252).

Scientometrics is a powerful tool to understand the direction of science and research. Our PTB analysis indicated that prominent areas like stem cell, biosensors, geriatrics are somewhat lagging in Turkish biochemistry research while postgraduate education and research in total is growing fast with sound collaborations.

P-EDU-011
The 1st Turkish *in vitro* diagnostic symposium evaluation

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Objective: *In vitro* diagnostic (IVD) medical laboratory tests and the equipment are closely related the public health, patient safety and the safety of all who utilize these tests. It depends on auditing of the process from the production to the consumption of these materials, that they do not pose a risk to individuals and society. Upon these basic requirements; 'Turkish in vitro Diagnostic Symposium: Medical Laboratory Tests' was held in February 2016, organized by the cooperation of Turkish Biochemical Society Branch of Izmir, and Dokuz Eylul University Health Sciences Institute. It was intended to shed light on some questions such as, what is the place and importance of IVD in Turkey? What are the responsibilities of educational institutions?, What is the role of Ministry of Health? to put across the conditions of preparing a substructure that may provide achieving success in producing IVD medical devices and in this sector, in our country.

Material-Method: 39 invited speakers attended the symposium, along with the participation of both as lecturers and attendees; Ministry of Health, Turkish Standards Institution; representatives of manufacturer enterprises; representatives of enterprises manufacturing in Turkey; scientists conducting considerable researches on health technology; students and representatives from some of the non-governmental organizations. In addition to the presentations, gathering up the written opinions of the participants, a report was prepared.

Results: The symposium that lasted for 3 days was realized with 120 participants in total,55 of which from universities;38 representatives of their companies;17 from chamber-institute-public establishments and 10 of which from public hospitals. 94 of the participants were from Izmir,26 of them were coming from out of Izmir.

Conclusion: At the end of the symposium,40% of the participants gave feedback. Among the feedback selected; 86.2% of the participants evaluated the symposium overall, as successful. 75.6% of them found the symposium successful with regard to its scientific content. Their feedback were 55.2% positive in terms of the symposium's contribution to the situation assessment on IVD in Turkey, and 44.8% of them stated they would consider participating in the second of the IVD symposium if it is to be organized.

P-EDU-012
Perceptions of molecular life science Master's students on their scientific and academic competencies and prospective plans for professional development

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The Master's education (ME) in molecular life sciences (MLS) is aimed at strengthening the knowledge and skills base of the young scientist, preparing him for the competitive academia/industry positions. The rapidly developing pace of science and research forces the master's student (MS) to play a central role in monitoring and guiding his scientific education and professional development (PD). Thus, the aim of our study was to examine the perceptions of MS of MLS, regarding their scientific and academic competencies. With this data, we planned to analyse if this awareness channels MS to take action and/or matches with their prospective plans.

We developed a 15 item online survey with 3 sections (demographic data, current data-contributions of ME, competencies and prospective data-action for PD, future plans) and distributed it via e-mail to various postgraduate institutes.

At the end of the 10-day period, 138 MS students (in the thesis phase) answered the questionnaire (Female: 66.7%, Male: 33.3%). The most highly rated activities that contributed to their

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scientific knowledge and skills gained in the ME were laboratory work (73%), visits with their mentors (70%) and theoretical lessons (65%). MS expressed low levels of sufficiency both in theoretical scientific knowledge and laboratory skills (only 43% and 33% sufficiency, respectively). Communication skills (80%) and team work (78%) were rated as the highest professional competencies followed by literature search and research planning (both 73%). It was striking that MS perceived themselves as quite insufficient in scientific writing (50%), data analysis (60%) and project writing (61%) while proficiency in English (55%) was the first area they wanted to take action. Despite their perceptions of insufficiencies in many areas, a majority (69%) wanted to continue to PhD education.

These and similar surveys may lead to an increase in self-awareness in MS and the data may contribute to the revision of ME.

P-EDU-013
The report of the 1st Turkey *in vitro* diagnostic symposium results

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'1. Turkish in vitro Diagnostic Symposium: Medical Laboratory Tests' was held on 18–20 February 2016, organized by the cooperation of Turkish Biochemical Society, Izmir Branch, and Dokuz Eylul University Health Sciences Institute.

The following aspects and suggestions took place in the results report prepared after the symposium:

About the National IVD-TC R&D, Production, forming Quality Assurance and Innovation Strategy and Policy
The cooperation of the university and the industry is not sufficient
Most of the industrialists cannot take enough advantage of the support provided by the institutions like
TUBITAK, the Ministry of Science, Technology and Industry and the Ministry of Development
The statistics on the IVD-TC in Turkey should be carried out as soon as possible
National standards should be determined in parallel with the international standards
The VAT rate of the exported raw materials that would be used in IVD production should be decreased.
About the Education and Training, the Job Titles and Positions
The related graduate programs, which would focus on all steps of the whole life cycle related to the IVD-TCs one by one, are not widespread
There is no 'postdoc' application in Turkey. 'Postdoc' staff is needed for insufficient component human resources
The lecturers should not be restricted to one discipline only
Graduate programs on Laboratory medicine are needed to be established and spread, in order to train component labor specified on the IVD-TC
About Research and Development
There are almost no researches related to product development. This should be associated with the education and training institutions
About the Research Centers
CURRENTLY, there is a real infrastructure on Health Technology in Turkey, but there are difficulties in its instituonalism
The insufficient cooperation of the university and the industry does not allow the inventions to turn into products
The cooperation supports of R&D, being restricted to TechnoPark and R&D centers, are open fields for improvement

P-EDU-014
PhD training in medical education: career profiles and satisfaction levels of graduates from Dokuz Eylul University Graduate School of Health Sciences

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Dokuz Eylul University, Izmir, Turkey

This survey was carried out within the scope of Special Study Modules that is entitled 'PhD Training in Medical Sciences' by a group of medical students in DEU. The purpose of the survey to investigate the members of health sciences that have successfully completed their PhD training in terms of the levels of satisfaction and the status of their career. From this scope we generated two hypothesis: We expected that graduate PhD graduates are mostly involved in academy and find their satisfaction levels at moderate level as to PhD education.

The study was designed as cross-sectional. We reached 166 PhD graduates who had graduated from DEU Graduate School of Health Sciences between 1991 and 2002 from 12 different departments via e-mail. The survey was included 27 questions, which were prepared in the light of the existing literature.

Among the 166 PhD graduates, 55 (30%) participated in the study. Through this survey, perception of PhD students on supervisors' scientific and educational abilities, opinions on PhD training, productivity of PhD training, number of articles published, their position and related satisfaction levels after graduation were investigated. According to the results, more than half of the graduates (%52.7) are well satisficed from the education they had taken. Beside this, interestingly we found that %94.5 of graduates prefers staying in the academic positions and %64.8 of them sustains their communication with their supervisors.

In conclusion, most of PhD graduates were contented with PhD training and their career profiles. As a result of this survey, we produced a novel and precise contribution to the literature. In a further study, this survey may extend to other parts of Turkey and compile the results in order to get more accurate and inclusive data.

P-EDU-015
PhD training in biomedicine and health sciences

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This study is a literature analysis based on a special study module, which has been done by a third year medical student, Mert Artuk.

PhD is an international degree that is reached by conducting an original research after finishing bachelor or master's degree. Doctoral degree (PhD) can open the door of academic career; on the other hand, a person with a doctoral degree is equipped to carry out important work in research, industry, or public sector. Today, gradually increasing number of PhD students have brought some problems in PhD training. The purpose of this study is to investigate and review activities that have been done by the following international organizations:

ORPHEUS: (Organization for PhD Education in Biomedicine and Health Sciences)
EUA-CDE: European University Association-Council on Doctoral Education.
FEBS Education Committee: Federation of European Biochemical Societies

These three organizations have done workshops on PhD training to pay attention to the following points:

*A PhD student must take some courses and trainings outside her/his institute, should not be limited to the institute.
*The PhD training programme should include transferable skills courses.
*Clinicians, if involved in PhD training, should be given free time from their clinic duties.
*With regards to potential problems with the supervisor, the institute should provide the student an advisory system.
*Students should be encouraged to participate in the management of doctoral programmes in the institute.
*The students should be given be opportunity to select their own supervisor (thus their thesis area).

The PhD training has gained quality thanks to these organizations. It is advised that graduate school of health sciences should follow the recommendations and report from these organizations.
Keywords: PhD training; ORPHEUS; EUA-CDE; FEBS Education Committee.

Tuesday 6 September 12:30–14:30

MicroRNAs and noncoding RNAs

P-01.03.3-001 Computational analysis and experimental confirmation of ITSN gene family posttranscriptional regulation by microRNAs and RBPs

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ITSN1 and ITSN2 are genes encoded adaptor proteins with multiple isoforms participating in clathrin-mediated endocytosis (CME), MAPK signaling and reorganization of actin cytoskeleton. Changes in ITSNs expression can lead to different neurodegenerative disorders and cancers. To date little is known about regulation of ITSN genes on posttranscriptional level. The aim of our work was to predict and experimentally confirm target sites for microRNAs that could potentially regulate ITSNs expression.

Using 8 web servers we analyzed 3′UTRs of short and long isoforms of human ITSN mRNAs and found conservative target sites for miR-34, miR-19, miR-129, miR-103/107, miR-194, miR-181 and miR-30 in 3′UTR of ITSN1-S, predicted by 5 servers, miR-203 predicted by 5 servers for ITSN1-L, and miR-153, miR-148/152, miR-27, miR-144 and miR-128 predicted by 5–6 servers for ITSN2-L. To elucidate potential impact on CME, MAPK signaling and actin cytoskeleton regulation by these miRNAs we performed enrichment analysis by Diana-MirPath server and found that miR-34, miR-19, miR-103/107, miR-181, miR-30 and miR-148 were highly enriched for all analyzed pathways.

Using RegRNA 2.0 and Scan for Motifs services we predicted 12 types of different regulatory elements in 3′UTR of ITSN1 and ITSN2: K-Box and Brd-Box, Musashi binding element for RBPs Musashi1 and Musashi2, GU-rich element (GRE) and AU-rich elements (ARE) that regulate mRNA decay.

To confirm ITSN1-S regulation by microRNAs we cloned 3′UTR of ITSN1-S into luciferase reporter vector and transfect HEK293 cells by this construction and miR-181a, miR-30a and miR-19a. For miR-181 transfected cells, we found 25–40% decrease of expression of ITSN1-S 3′UTR-bearing construction. For other miRs we did not obtain strong reproducible effect in luciferase assay. These data may confirm miR-181 target site in 3′UTR of ITSN1-S mRNA but needed additional research.

P-01.03.3-002 Expression levels of some microRNAs in ischemic stroke and relations to prognosis

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Objective: Stroke is an acute neurological disorder that is mostly caused by ischemia in central neural system. 30% of stroked patients lose their life in 1 year, and remaining 1/3 of the living patients continue to their lives as dependent to others. NIHSS and mRS are two scales which are used in prognosis studies because they can show stroke intensity and after stroke functional recovery. microRNA's which have effects on transcription and posttranscription gene regulations are small RNA molecules. Their roles have been investigated on pathophysiology and treatment of diseases. In this study, it was aimed to detect changes in blood serum levels of mir-146a, -155, -210, 181b, -31, 126, -92a and let-7f of ischemic stroke patients and to investigate role in predicting prognosis
Methods: 21 patients diagnosed by acute ischemic stroke admitted to neurology service of Göztepe Hospital and 16 healthy individual were included in the study. After stroke patients' blood samples were taken periodically in 1st day, 1st week, and 3rd month, and at the same time NIHSS and mRS scores were determined. Set 8 miRNA blood serum levels were measured by RT PCR
Results: When compared to the control group, we found that after stroke 1st day peripheric blood levels of miR-146a,-31,-92a and let-7f were significantly low; when 1st week and 1st day serum records were compared there was a significant increase in miR-126 level; and when 1st week and 3rd month records were compared we noted that there was a significant increase in miR-146a,-126 and let-7f levels. From prognosis point of view; after ischemic stroke measurements showed that miR-181b in the 1st day, miR-146a and miR-210 in the 1st week showed positive significant correlation with 3rd month mRS scores ($P = 0.002$, $P = 0.05$, $P = 0.002$, respectively)
Conclusion: According to the outcomes of this study, after stroke 1st day miR-181b, 1st week miR-146a and 1st week miR-

210 levels can be stipulated to use in predicting patients' 3rd month prognosis

P-01.03.3-004
Inhibitory RNA aptamer against lambda cI repressor showed the transcriptional activator activity

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Because of the variety of functionalities on gene regulation and the easiness of molecular engineering, functional RNAs are promising parts for the construction of genetic circuits. Artificial affinity RNA, or RNA aptamer, is one of such genetic parts.

In the previous study, an inhibitory RNA aptamer against a repressor protein, TetR, was developed as a transcriptional activator [1]. The expression of this aptamer abolishes the repressor activity of TetR, resulting in the elevated gene expression under the control of TetR. Because of simplicity of the mechanism, similar transcriptional activators can be generated by using RNA aptamers against other repressor proteins. Here, we examined the generation of an activator based on an RNA aptamer against one of the most frequently applied repressor proteins, lambda phage cI.

In vitro selection (SELEX) was performed targeting a recombinant cI protein employing an RNA pool containing 40-nucleotides of a random region. After 6 rounds of SELEX, the pool RNA showed the affinity, as well as the inhibitory activity, against cI in vitro. Then, RNA aptamers with the transcriptional activator activity were screened from the enriched pool in vivo employing a reporter plasmid on which the expression of a reporter gene, LacZ, is repressed by cI. When the variants of the RNA pool were transformed to *E. coli* cells harboring the reporter plasmid, about half of the transformants showed the elevated reporter expression. Interestingly, all of these desired RNA clones shared the same sequence. Quantitative analysis indicated that 35-fold induction of the reporter expression was achieved upon the aptamer expression.

Our results suggested that diversity of artificial transcriptional activators can be extended by employing RNA aptamers against repressor proteins to broaden parts for the construction of genetic circuits.

[1] Hunsicker, et al. (2009) An RNA aptamer that induces transcription. Chem. Biol., 16: 173-180.

P-01.03.3-006
microRNA expression signatures between non-atherosclerotic plaque and atherosclerotic plaque in CAD with humans, and parallels whole blood

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Aim: Coronary artery disease (CAD) is caused by atherosclerosis of the coronary arteries. Atherosclerosis is an inflammatory disease of the arteries characterized by the formation of atherosclerotic plaques. MicroRNAs (miRNAs) are small endogenous RNAs and represent a new important class of gene regulators. The present study was designed (i) to investigate the miRNA expression profile in human atherosclerotic plaques from peripheral arteries aorta as compared to non-atherosclerotic left internal mamary artery (LIMA); (ii) to examine the expression levels of miRNA in whole with correlation miRNAs of aorta tissue.
Material and Methods: Thirty-one patients with CAD were enrolled in study. LIMA and aorta tissue samples were obtained during coronary artery bypass surgery. Whole blood samples were collected before CABG surgery. Each patient with CAD was obtained from whole blood, aorta and LIMAs tissues. These tissue samples were immediately soaked in RNALater solution and homogenized using a MagnaLyser. The RNA was extracted using the Trizol reagent and the miRNEasy® Mini-Kit. The expression profiles of 738 miRNAs were evaluated using high-throughput qRT-PCR.
Results: We found that miR-497-3p was expressed only in aorta. miR-431-5p and 433 were expressed both aorta and whole blood. 6 miRNAs were significantly up-regulated in aorta when compared to LIMA tissue (FC > 2). 59 miRNAs were significantly down-regulated in aorta compared to LIMA.
Conclusion: In conclusion, our study suggests that miR-497-3p, miR-431-5p and 433 might be a potential for cardiovascular disease development. Also miR-431-5p and 433 might serve as novel non-invasive biomarkers for CAD

P-01.03.3-007
miR193b regulates cell proliferation and colony formation in pancreatic ductal adenocarcinoma

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Due to the strong metastatic potential, pancreatic cancer has the highest mortality rate of all major cancers. Therefore, the investigators are in urgent need of developing the new alternative therapeutic approaches for the prevention of pancreatic cancer. miRNAs, small noncoding RNAs, regulates as an inducer or inhibitor in expression of key mediators related molecular mechanisms in cancer promotion.

To investigate the effect of miR193b on pancreatic ductal adenocarcinoma cells, we performed the cell viability and clonogenic assays by MTS and crystal violet dye, respectively, in Panc-1 and MiaPaCa-2 cells transfected with miR193b mimic.

Our data revealed that miR193b led to decrease the cell viability depending on enhanced miR193b doses, which are 25, 50, 100 and 150 nM, as the ratio of % 23, 60, 82 and 87, respectively, in MiaPaCa-2 cells and as the ratio of % 40, 58, 77 and 89, respectively, in Panc-1 cells compared with control condition of each cell. This inhibition mediated by miR193b was also obtained in colony formation both of pancreatic cancer cells. When the induced effect of miR193b on the death of pancreatic cancer cells was compared with gemcitabine, which is currently used as a clinical drug for pancreatic cancer patients, we determined that miR193b was more effective than gemcitabine.

Based on our findings, it is clearly shown that miR193b serves as a tumor suppressor in pancreatic ductal adenocarcinoma cells. We strongly believed that miR193b gene therapy might be more effective and targeted approach than classical gemcitabine therapy for pancreatic cancer patients.

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P-01.03.3-008
A miRNA-mediated network reveals trastuzumab responsive key players in breast cancer

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Breast cancer is the most common cause of cancer death in women. Trastuzumab is a therapeutic agent frequently used against HER2+ breast cancers, which has role in approximately 20% of invasive breast cancers. With the discovery of their activity in cancerogenesis, microRNAs (miRNAs) have become potential candidates to mediate therapeutic actions by targeting genes that are effective in drug response. Recent studies have showed that miRNAs are induced by targeted therapies.

In this study, we aim to find out miRNA-mediated mechanism, which is driven by common trastuzumab responsive microRNAs in HER2+ breast cancer. For this purpose, the common trastuzumab responsive miRNAs were determined in treated BT474 and SKBR3 cells by microarray profiling. Two datasets were intersected to find out common miRNAs for both cell lines. The overlapping predicted targets of common miRNAs were provided by two different miRNA-target prediction databases and then a miRNA-gene network was built in Cytoscape by using NetworkAnalyzer Plugin. The most shared target genes were chosen to be analyzed in the EBI-EMBL Gene Expression Atlas for their expression patterns in breast cancer.

25 common miRNAs were found to have overlapped targets in two target prediction algorithms that were used to build the miRNA-Gene regulatory network. 14 overlapped targets were determined as the most shared genes in the miRNA-Gene network. Expression pattern of each shared gene in the Gene Expression Atlas showed that 12 out of 14 the most shared target genes were strongly dysregulated in several breast cancer types.

Our results suggest that miRNAs might show a common response to the targeted therapies and network analysis can be profitable to have a better explanation of the regulatory mechanisms between drug responsive miRNAs and their target genes. Revealing the miRNA-potential target interactions might provide novel key players that mediate the mechanisms of action in drug treatment.

P-01.03.3-009
Resveratrol up-regulates tumor supresor miR-214-3p expresion via regulation of histon deacetylase 1 and p62/SQSMT1 gene expressions in chronic myeloid leukemia

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Chronic myeloid leukemia (CML) is a clonal disease of primitive pluripotent stem cells that identified with a specific *t*(9;22) reciprocal translocation that encoding Bcr-Abl oncoprotein. Resveratrol (RES) is a natural phytoalexin found in grapes and induces apoptosis, erythroid differentiation and autophagy in leukemic cells. MicroRNAs are small, single strand, non-coding RNA molecules that regulate post-transcriptional gene expression via disrupting the stabilization of target transcripts or inhibiting protein translation.

In our study we aimed to determine cytotoxic effect of RES in K562 human CML cell line and to evaluate the expressions of miRNAs that are associated with genetics of leukemia after treatment with RES; to investigate target genes of miRNAs which show significant expression alterations and molecular mechanisms of RES treatment.

K562 cells were treated with 100 μM (IC50 dose) RES during 72 h and cytotoxicity was evaluated by using WST-1 assay. The RT-qPCR is used for miRNA and gene expression analysis.

Results showed that; RES up-regulated tumor suppressor miR-214-3p level 2.87 fold and significantly downregulated HDAC1 gene expression ($P = 0.003$) and upregulated p62/SQSMT1 gene expression ($P = 0.001$), according to the control cells.p62/SQSTM1 interacts with LC3 and plays role as a critical player in the autophagic degradation of the BCR-ABL fusion protein. Our findings showed that Resveratrol acts as a HDAC inhibitor targeting HDAC1 and p62/SQSMT1 gene expression level. Treatment with HDAC inhibitors results apoptosis, cell-cycle arrest, cell differentiation, anti-angiogenesis and autophagy. Downregulation of HDAC1 provides post-translational modification for expression of tumor supressor genes and leads to cell cycle arrest and increases apoptosis.

These results could be linked to HDAC1 dependent induction of gene associated with autophagy like p62/SQSMT1 and resveratrol could be a therapeutic candidate as a HDAC inhibitor for CML treatment.

P-01.03.3-010
Effects of various miRNAs and their combinations on cell viability to A549 cell line was detected

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miRNAs are small RNAs which regulate gene expression. Lung cancer is the most common cancer causing 1.6 million deaths around the world every year. miRNAs play crucial role in cancers. The aim of this study is to detect the effects of various miRNAs and their combinations on A549 cell line viability.

The miRNA used in this study are hsa-mir-145, hsa-let-7a, hsa-mir-29b and hsa-mir-155. Thereafter, we bought pre-miRNAs and their miRNAs commercially. We apply them to the A549 cell line in different combination and different concentrations. These miRNAs applied solely onto cells or in combination as; four of them, let7 + 145, let7 + 29b, let7 + 155, 145 + 29b, 145 + 155, 145 + let7 + 29b, 155 + let7 + 29b. The cell viability was detected by WST-1 kit in a 96 well plate ELISA reader. Cells were seeded as 10 000 per well, miRNAs incubated with cells for 24 h in an appropriate atmosphere.

According to our results some combinations and miRNAs didn't alter viability, however 145 + 29b and 145 + 155 combination increased the cell viability dramatically. On the other hand let7 + 29b and 155 only applications decreased the cell viability. The other applications' viability results are not different from the control cells significantly.

In this study, we used A549 cell line also called non-small lung cancer (NSCLC) cell line and possibly effective miRNAs on lung cancer. It is important to exhibit the miRNA combinations should be effective on cancer cells' viability. The prospect combinations were determined which is crucial to develop new strategies for cancer treatment.

P-01.03.3-011
Competing endogenous RNA of SIP1 in hepatocellular carcinoma

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Competing endogenous RNAs (ceRNAs) act as molecular sponges for the same pool of microRNAs through their miRNA response elements (MRE), titrate miRNA levels and thereby regulate gene expression post-transcriptionally. SMAD interacting protein 1 (SIP1), a member of the ZEB family is a regulator of epithelial-to-mesenchymal transition (EMT) program, which is active during embryogenesis and tumor invasion and metastasis. Hence, we investigated the regulation of SIP1 by ceRNAs in hepatocellular carcinoma (HCC) cells. Among hundreds of SIP1 ceRNAs listed at Competive Endogenous mRNA Database (ceRDB), 14 transcripts (*PTEN, ZEB1, PTCH1, CREB5, ACVR2B, ENAH, ROBO2, ERBB4, E2F3, FOXO1, RICTOR, KLF3, ETS1, CDK6*) sharing at least 9 common MRE sites with SIP1 were selected and their expression in 9 HCC cell lines were determined by qRT-PCR. *ETS1* was found to be highly correlated with *SIP1* in HCC. Furthermore, repressing *SIP1* expression by shRNA in HCC cells resulted in decreased expression of *ETS1, PTEN* and *ZEB1*. Our results suggest a possible bidirectional and post-transcriptional regulation of SIP1 and its ceRNAs in HCC.

P-01.03.3-012
A meta-analysis for the identification of common microRNA signatures in colorectal cancer

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Colorectal carcinogenesis (CRC) has quite frequent incidence and mortality rates worldwide, despite being studied for decades now. New biomarkers are needed to be identified in addition to the existing ones, due to heterogeneous nature of this disease. The regulatory molecular machinery of a cell, including microRNAs (miRNAs), contributes to this heterogeneity upon aberrant expression.

Herein, for a mechanistic understanding of differential gene expression in CRC tissue we analyzed miRNA expression profiles of 78 CRC tumors against 62 normal colorectal mucosa samples, using raw data from E-MTAB-752 and E-GEOD-35834 (Affymetrix microarrays), and GSE35982 and E-MTAB-813 (Agilent microarrays) datasets obtained from Gene Expression Omnibus and ArrayExpress. Raw samples were normalized (different platforms separately) using quartile normalization in BRB-ArrayTools. Differential expression of miRNAs was identified using cut-off values of $P \leq 0.05$, fold change ≥ 1.5 and stringent false discovery rates. miRTarBase and miRWalk2.0 databases were explored to identify validated targets.

We found thirty (including miR-21 and miR-183) and thirteen (miR-1, miR-139, miR-375, etc.) miRNAs commonly upregulated and downregulated respectively, in both Affymetrix and Agilent microarray results. Predicted targets of these miRNAs frequently belong to pathways related to cancer like β-catenin, Phosphoinositol-3Kinase, and Transforming growth factor-β, to name few. Moreover, the target genes were significantly enriched in clusters related to cell cycle, cell differentiation and regulation of apoptosis.

These promising results will further be compared with differentially expressed gene profiles from a cohort of Turkish CRC patients. Hence the integrated study will refine the panel of diagnostic and prognostic CRC markers.

P-01.03.3-013
Hsa-miR-X modulates motility and invasion in triple breast cancer cell line

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Breast cancer is a heterogeneous disease and expression levels of certain receptors have demonstrated subtypes which characterize clinically distinct breast tumors. A triple-negative phenotype lacks expression of ER, HER2 and PR and is known as basal-like carcinoma. MicroRNAs are a class of small non-coding RNAs that participate in the gene expression in many biological processes. E-cadherin is an important mediator of adhesion in epithelial tissues, and loss of E-cadherin can play a critical role in tumor invasive behavior. Another key player of cell integrity PIP (3,4,5) triphosphate is generated at the leading edge of the cell and leads to cell polarization. PIP3 is generated by hydrolysis of PIP (4,5) bisphosphate, which is synthesized by PIP5K1. Any dysregulation in these molecules may support the invasive behavior of the cells. The aim of this study is to find out the role of miRNA precursor (hsa-miR-X) in invasion and motility in triple negative breast cancer cells.

In this study a triple-negative breast cancer cell line BT-20 was transfected with hsa-miR-X or scrambled control siRNA. To check its role in motility and invasion, wound healing and invasion assays were performed respectively. Cell invasion was monitored over a period of 24 h by xCELLigence real-time cell analyzer using a double-plate and measuring impedance-based signals. Additionally EMT markers were analyzed by qRT-PCR to explain the molecular mechanisms beneath motility and invasion.

We observed that cell motility and cell invasion diminished after transfection of BT-20 cells with mimic for hsa-miR-X. Furthermore, qRT-PCR experiments indicated that transfection of hsa-miR-X decreased the expression level of PIP5K1C while increasing the E-cadherin expression level.

Wound healing and invasion assays together with qRT-PCR results support the role of hsa-miR-X in cell motility and invasion. This process might be explained via E-cadherin mediated MET or GSK-3-beta related inhibition of invasion.

P-01.03.3-014
Expression level of five microRNAs as diagnostic markers in glioblastoma

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Glioblastoma or glioblastoma multiforme (GBM) is the most frequent and most aggressive type of glioma. Generally, GBM is

situated in the main brain lobes, but can also be found in other brain regions. While microRNA (miRNA) as non-coding RNAs, play a crucial function in the post-transcriptional regulation of gene expression by mRNA degradation or translational repression. In the present study, we aimed to investigate the contribution of gene expression of the five miRNAs and to unravel their role in brain tumor cell lines, the miRNAs to the risk of GBM tumor.

The five GBM cell lines (CRL-2365, CRL-2366, CRL-2948, CRL-1690 and HTB-15) were evaluated with non-malignant (normal) brain cell line (HCN-2). Determinations of expression level of five miRNAs (miR-21, miR-101, miR-138, miR-196, and miR-222) were evaluated by monitoring quantitative RT-PCR (qRT-PCR) technique.

The expression levels of four miRNAs (miR-101, miR-138, miR-196, and miR-222) were significantly decreased while the expression level of miR-21 was increased in GBM cell lines according to HCN-2 cell line.

Consequently, these five miRNAs can potentially be used as biomarkers for GBM tumor; further studies are mandatory to a better understand and confirm our preliminary findings.

P-01.03.3-015
Prediction of ANRIL long non-coding RNA sponge activity in neuroblastoma and Alzheimer disease

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Background: Noncoding RNA are known to be crucial molecules with diverse regulatory roles in neural oncology and neurodegenerative disease. The recent study suggested that lncRNA ANRIL play role in the development of neuroblastoma and Alzheimer disease via binding disease-specific microRNAs.
Material and Methods: We used LncRNADisease, HMDD v2.0, Mir2disease to predict lncRNA- and miR-associated disease in our study. In addition, we utilize TardetScan to search lncRNA-miRNA interaction.
Results: Disruptions of lncRNA ANRIL expression (also named as CDKN2B-AS, locus CDKN2a/b (INK4/ARF), chromosome 9p21) have been associated with the development of neuroblastoma and Alzheimer disease. Here, we predicted interactions between noncoding transcripts encoded by locus CDKN2a/b and their molecular partners – microRNA. ANRIL can act as decoy while containing sequences that mimic miRNA target sites to titer these miRs away from their primary targets thereby act as molecular sponge. Using TargetScan 7.0, we predicted target sites for hsa-mir-15-p/16-p/195-p/424-p/497-p/6838-p and hsa-mir-125-5p/4319 in ANRIL 3′UTR. Then, we used HMDD v2.0 and Mir2Disease databases to define if any of these miRs participate in Alzheimer disease and neuroblastoma. According to both databases, miR-125 is implicated to Alzheimer disease and miR-15 to neuroblastoma. As soon as ANRIL participate in the development of both abovementioned disorders and can have microRNA sponge activity, it could potentially positively regulate miR-125 and miR-15 targets by competing with them for microRNA binding sites thus restoring the expression of target genes. In our further research we plan to experimentally validate predicted microRNA sites in ANRIL 3′UTR.
Conclusions: We predicted sites for miR-125 and miR-15 in 3′UTR of ANRIL lncRNA that could uncover its possible sponge activity in the development of neuroblastoma and Alzheimer disease.

P-01.03.3-016
hsa-miR-376b-3p expression levels decreased during the development of matrine resistance in acute lymphoblastic leukemia cells

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Aim: Matrine excracted from saphora flavescens root and demonsrated that indicates pro-apoptotic and anti-proliferative effect in many types of cancer. Acute lymphoblastic leukemia (ALL) is an acute form of leukemia, or cancer of the white blood cells which characterized by the overproduction and accumulation of lymphoblasts. miRNAs play important roles in deregulated cell death mechanisms. We aimed to investigate the effects of critical miRNAs during the development of matrine resistance on ALL cell line CCRF-CEM.
Material-Method: CCRF-CEM cells were treated with different (0.5–3 mg/ml) concentrations for 72 h and cell viability measurements were carried out with xCELLigence device to determine the cytotoxic effects of Matrine. miRNAs were extracted from treated and untreated CCRF-CEM cells using the miRNA Isolation Kit. cDNA was synthesised using Allin-One First strand cDNA Synthesis Kit. Expressions of 44 selected miRNAs were analysed with miProfileTM Custom miRNA qPCR Array using the Applied Biosystem 7500 Fast Real-Time PCR System.
Results: According to the cytotoxicity assay, it was determined that 'treatment with increasing concentrations of Matrine, decreased the viability of the CCRF-CEM cell line. Expression levels of 44 different miRNAs were studied for indicated passages in two replicates. Our results showed that hsa-miR-376b-3p (-37,099 fold, $P = 0.008$), hsamiR-106-3p (-16,6795 fold, $P = 0.028$), hsa-miR-20a-3p (-15,926 fold $P = 0.0148$), hsa-miR-519a-3p (-11,7398 fold, $P = 0.00534$), hsa-miR-204-5p (-10,9536 fold, $P = 0.0012$), hsamiR-30b-5p (-9,0631 fold, $P = 0.0221$), hsa-miR-15b-5p (-8,8971 fold, $P = 0.0339$), hsamiR-106b-5p (-8,8561 fold, $P = 0.021$), hsa-miR-885-3p (-8,6139 fold, $P = 0.00006$), hsamiR-30a-5p (-8,594 fold, $P = 0.009$) expression were decreased during the development of matrine resistance.
Conclusion: These data suggested that especially hsa-miR-376b-3p plays a critical role in the matrine response.

P-01.03.3-017
Opposite roles of lncRNA ERICD (E2F1-regulated inhibitor of cell death) and ARID3A (AT-rich interaction domain 3A) in osteosarcoma, glioblastoma and lung cancer

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ERICD (E2F1-Regulated Inhibitor of Cell Death) is a newly found lncRNA. It is located at 8q24.3. It has two exons and its transcript size is 1745 bp. ERICD is regulated by E2F (transcription factor 2) and modulates the cellular response to DNA damage. ARID3A is a family member of the AT rich interaction domain (ARID) DNA-binding proteins that participate in diverse biological processes like development, cell cycle control, chromatin remodeling and epigenetic regulation. Both, ERICD and ARID3A have just opposite roles in apoptosis in case of DNA damage indicating a probability of reciprocal interaction between

each other. Till now, there is no work related to the interaction between lncRNA and ARID3A in cancers. Herein we try to find a probable interactive role between these in cancers.

In this study, 12 different cancer cell lines, 1 osteoblast cell line and 19 different types of normal human tissues RNAs were selected for expression analysis of ERICD and ARID3A. After RNA isolation, cDNA was converted from their RNAs. Expression profile analysis of ERICD and ARID3A in different cancer cell lines and normal tissues was done using ImageJ Program for semiquantitative and $2^{(-\Delta\Delta Ct)}$ method for quantitative RT-PCR.

Among used cancer cell lines, ERICD was highly expressed and ARID3A had lower expression in U-2OS (osteosarcoma), A-172 (glioblastoma) and A549 (lung cancer). At the same time, ERICD expression was lower and ARID3A had high expression in hFOB 1.19 (osteoblast cell line) and normal tissues like brain and lung .

Both ERICD and ARID3A are cell cycle regulated and are commonly regulated by E2F. They have just opposite roles in apoptosis during DNA damage. These two genes have a probability of reciprocal interaction between each other in cancer.

Our results indicate that both ERICD and ARID3A might have opposite roles in lung cancer, glioblastoma and osteosarcoma. ERICD and ARID3A might act as cancer promoting and tumor suppressor genes respectively in these cancers.

P-01.03.3-018
The importance of miRNA expressions in Infertilty

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Implantation process is controlled with endometrium, factors secreted by the embryos and in accordance with these factors embryo and/or endometrium via receptors on. More than 700 human MicroRNA (miRNAs) that are small noncoding RNAs were shown to play an important role in intracelluler cycle regulation in both normal and pathological conditions. In this study we aim to identify miRNAs and controlling molecules expressions in different time period of endometrium in fertile and infertile cases.

The endometrial samples were taken from fertile and infertile patients in proliferation and early secretion periods. The samples are fixed and stained either with hematoxylen-eosin for morphological analysis or with immunohistochemistry for distributions of anti-dicer, anti-drosha, anti-eIf2α and anti-eIF2C. miR-17-5p, miR-23a, miR-23b, miR-542-3p, miR-21, miR-199a*, miR-705, miR-20a, miR-26a, miR-125b, miR-200a/b/c were analyzed with qRT-PCR.

While Dicer immunoreactivity was detected weakly only proliferation phase of fertile group, this immunoreactivity were detected strongly in both proliferation and early secretory phases of infertile group. Drosha immunoreactivitiy was also weakly detected in the proliferation phase of fertile group, it was moderately detected in both proliferation and early secretory phases of infertile group. eIF2α immunoreactivitiy was similar in each groups but there were a few differences between fertile and infertile group. eIF2C immunoreactivitiy was negative in all groups. miR-21, miR-199a* and miR-23a were highly expressed in proliferation phase of fertile group, miR-23a and miR-125b were highly expressed in early secretion phase of infertile group.

In conclusion, Dicer and Drosha immunoreactivities and different expression of miRNA's were detected in all groups. Implantation problems may be reason for different miRNA expression which controlling with Dicer and Drosha in the infertile endometrium in both proliferation and early secretory phases.

P-01.03.3-019
Identification of conserved microRNA molecules in einkorn wheat (*Triticum monococcum* spp. *monococcum*) by deep sequencing analysis

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Wheat is an important agricultural crop with an over 615.8 million metric tons harvesting capacity annually. Drought and salinity are environmental stress factors that affect yield and quality of wheat, dramatically. There are different defense mechanisms against these stress conditions in plants. Altering gene expression profiles by microRNAs at post-transcriptional level is one of the most conserved mechanisms among plants. microRNAs are an extensive class of noncoding RNAs, approximately 22 nucleotide length which regulates the expression of genes by binding to the 3′-untranslated regions of specific mRNAs. microRNAs implicated under salt and drought stress have widely been reported in numerous plant species and wheat genomes in the last years; however, studies on einkorn wheat (*Triticum monococcum* spp. *monococcum*) are not yet available. The goal of this study is identification of conserved microRNAs from einkorn wheat using next generation sequencing technology and bioinformatic analysis. In this study, small RNA molecules were extracted from pooled plant samples grown under normal, drought and salinity conditions. Sequencing analysis revealed 75 164 unique small RNA sequences obtained from 15 139 448 raw reads. After bioinformatic analysis based on comparative genomics approaches, we identified 168 putative mature microRNA sequences belonging to 142 distinct microRNA families. Since chromosomal sequence data is not available for *Triticum monococcum* spp. *monococcum*, we used available sequences from *Triticum urartu,* a close relative, as template to extract precursor microRNA sequences. 111 of precursor sequences showing 100% homology to *Triticum urartu* genome were analyzed for secondary structure prediction using Mfold software. Data provided in this study is critical to investigate transcriptional regulation of genes involved in stress metabolism in einkorn wheat.

P-01.03.3-020
The role of VIM-AS1, a natural antisense transcript, in cancer

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Introduction: A mass of indication suggest that the majority of human genome is transcribed into functional non-coding RNA transcripts called lncRNAs. Of these transcripts, natural antisense transcripts (NATs) are of special interest because they are oriented in the opposite strand complementary to either a protein-

coding or non-coding transcript. In this regard, VIM-AS1 is NATs located antisense to vimentin gene. In the present study, we aimed to determine tissue and cell line distribution of VIM-AS1.

Materials and Method: For the tissue expressions analysis, Human Total RNA Master Panel II (containing 20 different human tissue samples) was used. Total number 14 cancer cell lines and 5 normal cell lines included in the study. For the expression analysis RT-PCR and qPCR methods were used.

Results: As a result, expression levels of VIM-AS1 were found to be tissue specific. Particularly, while VIM-AS1 was highly expressed in lung and thyroid gland tissues, its expression was not observed brain, stomach and adrenal gland tissues. Also, VIM-AS1 was also found to be differentially expressed in cancer cell lines. VIM-AS1 expression was found to be lost in CAL29, PC3, and HCT116 and highly diminished A549 cancer cells. Also, it is found to be highly expressed in BCPAP, PANC1 and BEAS2B cells.

Discussion: Results of the current study suggest that VIM-AS1 may have significant role in the regulation of VIM gene in thyroid gland tissues, as it is highly expressed in both thyroid gland tissues and BCPAP thyroid cancer cells. Moreover, VIM-AS1 and VIM axis can be involved in the formation of lung tumors because VIM-AS1 was highly expressed in normal lung tissues and BEAS2B cells and expressed very low levels in A549 lung cancer cells.

Conclusion: In conclusion, VIM-AS1 gene can be important regulator of VIM gene and can be involved in the formation of lung tumors. Further functional analysis is need to reveal its comprehensive role in the thyroid gland and lung tissues.

P-01.03.3-021
miR-548a-3p, miR-548as-3p and miR-8078 are responsible for NSCLC invasion

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Lung Cancer is the leading cause of cancer related deaths in the world and approximately 90% patients with lung cancer ultimately die from metastatic disease. Metastasis is the most dangerous step of cancer. In our recently published work showed that Akt/NF-kB pathway is continuously active and induces cellular invasion and PTEN suppresses cellular invasion via inhibition of Akt/NF-kB pathway. In this study we aimed to show NF-kB mediated induction of miRNA expression can responsible for inducing NSCLC invasion.

We used Chromatin Immunoprecipitation (ChIP) Assay Kit for detection of TNF-α induced NF-kB mediated miRNAs. Therefore, H1299 and PC14 cells treated by TNF-α (30 ng/ml) for ChIP assay. Chromatin regions, reading with ChIP-Seq, were analyzed using bioinformatics tools. We also performed additional bioinformatics search to find NF-kB related miRNAs which potentially take a role in NSCLC invasion. We investigated the effects of miRNA which determined at the bioinformatics analysis results on invasion using invasion chamber method.

We found 16 miRNAs which potentially induced by NF-kB and related with NSCLC invasion. Our invasion results indicate that miR-548a-3p, miR-548as-3p, miR-8078, miR-1915, miR-6814-3p, miR-548q mimics can induce cellular invasion on H1299, miR-548v, miR-548 h-5p, miR-138-5p, miR-548a-3p, miR-548as-3p, miR-8078 mimics can induce cellular invasion on PC14. We also verified our results by qRT-PCR, because we want to sure that miRNAs which can induce invasion, can also transcriptionally regulated by NF-kB or not.

We found that miR-548q, miR-548a-3p, miR-584as-3p, miR-1915, miR-8078 in H1299, miR-138-5p, miR-548a-3p, miR-548as-3p, miR-8078 in PC14 can induce cellular invasion by NF-kB. As a conclusion, our investigation indicate that NF-kB can induce NSCLC invasion via miR-548a-3p, miR-548as-3p and miR-8078.

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P-01.03.3-022
TNF-alpha induced PI3K/Akt/NF-kB pathway regulate EMT via inhibition of PTEN by induction of miR-548as

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To understand of the molecular mechanisms of cellular invasion is very important for fight against cancer mechanisms and first step of invasion is EMT. Cells change phenotypical and genotypical in EMT progress. In this study, we focussed on the explore molecular mechanism of TNF-alpha induced cellular invasion of NSCLC. We use western blot, qRT PCR and miRNA transfect methods for this purpose. We find that TNF-alfa treatment reduce the expression of PTEN and induce E cadherin expression in A549 cells. When we inhibit NF-kB activity by p65 targeted siRNA TNF-alpha can not reduce PTEN expression means that TNF-alpha inhibits PTEN expression through on NF-kB. Because TNF/NF-kB pathway change the transcriptional level of miR-548as and PTEN 3′ untranslated region has recognition site for miR-548as. Therefore; we transfected A549 cells by miR-548as. miR-548as transfection leads to inhibition of PTEN expression. Our results indicate that TNF-alpha mediated activation of NF-kB can inhibit PTEN expression on induction of EMT through on miR-548as.

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Tuesday 6 September 12:30–14:30

Autophagy: Regulation mechanisms

P-02.03.3-001
Autophagy as a possible component of corpus luteum regulation that is critical for the maintenance and termination of rodent pregnancy

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Introduction: The corpus luteum (CL) is an ephemeral tissue whose regulated secretion of progesterone is essential for maintenance and/or timely termination of pregnancy in rodents. Our previous finding that CL of pregnant rats does not possess Fas/FasL system suggests that this tissue may undergo autophagic, but not apoptotic, cell death during regression. We here investigated the presence of autophagic system in CL and its any implications in rodent pregnancy and parturition.

Materials and Methods: LC3 (–I and –II) expression in CL was estimated by Western blot analysis in comparison with progesterone secretion and luteal mass throughout pregnancy. LC3 was also tested by immunocytochemistry. Functional implication

of autophagy in this tissue was examined by local treatment with Bafilomycin A1 (autophagy and V-ATPase inhibitor, 6.23 pg/0.1 ml/ovary) on day 19 of pregnancy. Reproductive, biochemical, and morphological outcomes were evaluated.

Results: While the expression of cytosol-associated LC3-I was not significantly altered throughout pregnancy, that of autophagosome-associated LC3-II increased significantly from day 15, showed a peak on day 21, and decreased on day 23 (day of normal parturition). LC3-II / I ratio had positive correlations with steroidogenic activity and cell size. Immunoreactive LC3 was found to be present in the cytosol of steroidogenic cells and showed marked aggregation in cells on day 21. In vivo treatment with Bafilomycin A1 resulted in unaltered delivery, but in significant reductions in steroidogenic cell size and neutrophil infiltration compared to vehicle-treated control groups.

Discussion and Conclusion: The ratio of LC3-II / I in CL was markedly up-regulated in the late phase of pregnancy. Manifestation of this autophagy parameter was temporally matched with further structural and functional activation of CL. Autophagy may contribute to activation, but not regression, of rodent CL and thus their female reproduction.

P-02.03.3-002
Apoptotic and necrotic effects of low dose bisphenol A in SHSY5Y neuroblastoma cells

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Bisphenol A (BPA) is a commonly used chemical in industry to make plastics. 'Low-dose' term has been expressed for the first time in studies with BPA in 2001. The value of low dose BPA was received as $< 1\mu M$.

In this study, matrix metalloproteinases (MMPs) together with their tissue inhibitors (TIMPs), involved in tissue remodeling after I-131 therapy, have been examined in 51 patients (8M/46F) with PTC and 38 (3M/38F) with PTC+HT. Peripheral blood samples were collected just before and, subsequently, at 4 days after I-131 administration (3.7 GBq). PTC+HT patients had positive titers of anti-thyroglobulin autoantibodies (TgAb). The serum levels of TgAb, MMP-2, MMP-9, TIMP-1 and TIMP-2 were measured by ELISA.

There were no significant changes in serum concentrations of MMP-2, TIMP-2 and MMP-2/TIMP-2 ratio after I-131 in the two groups. In PTC patients, I-131 administration resulted in an increase with 26% in TIMP-1 level ($P = 0.005$) and a reduction with 44% in MMP-9/TIMP-1 ratio ($P = 0.003$). In PTC+HT patients it has been observed an increase with 18% in TgAb level ($P = 0.001$), 5% in MMP-9/TIMP-1 ratio ($P = 0.003$) and unchanged TIMP-1 serum concentration. TgAb titers were positively correlated with MMP-9/TIMP-1 ratio ($r = 0.51$, $P < 0.001$).

Our data suggest that radioiodine therapy for PTC patients, but not for PTC+HT, modulates the balance of MMP-9/TIMP-1 for anti-invasion and anti-migration by augmenting TIMP-1.

Tuesday 6 September 12:30–14:30

Extracellular matrix and metalloproteinases

P-02.07.5-005
Role of extracellular matrix proteases for determination of post mortem interval

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In criminal cases, the determination of the time of death of the bodies step in the analysis of events is making a big contribution. Today, forensic and molecular methods utilized time of death rather than provide clear information offers potential death time interval. Principally, 'time of death' is a term that should be avoided. In forensic science, the postmortem 'interval' is the term to be considered. Nowadays, there is no precise molecular method that can be used alone in the determination of PMI.

Aim of this study is to analyze the usability of ECM components, ADAMTS1,5 and 9 and MMP1,2 and 9 to determine PMI. For this purpose, with iliopsoas muscle tissues provided by Ethical Board of the Ankara Institute of Forensic Medicine, 21 cases have been included in this study, divided into 3 groups according to their PMIs: '0–12 h', '12–18 h' and '18–27 h'. From these tissues, Western Blotting technique is used to analyse the expression of ADAMTS1,5 and 9 and MMP1,2 and 9.

It is determined that when PMI increases; ADAMTS-1 and 9 amounts decrease. On the other hand ADAMTS-5 amounts are examined to increased related to the interval., especially on the '12–18 h' dataset. Additionally, considering metalloprotease levels, MMP-2 and 9 amounts decrease as PMI increases. Unlike MMP-2 and 9; MMP-1 levels increase proportional to the interval, especially on the '18–27 h' dataset.

These results are the first data on PMI determination from iliopsoas muscle tissue. In this study, it is suggested that ADAMTS-5 and MMP-1, proteases responsible for ECM degradation, can be used to determine PMI as markers.

Here we present the first observations of postmortem variation of MMP and ADAMTS activities in skeletal muscle. In recently, popular MMPs and ADAMTS s pathways of the relationship between time-dependent changes to investigate the post mortem time interval determination to shed light on the future.

P-02.07.5-006
The role of functional polymorphisms of matrix metalloproteinases 2 and 9 in spontaneous abortion samples

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Abortion is one of the most common gynecological problems. After diagnosis of pregnancy, 15–20% of pregnancies result in abortion. In early stages of pregnancy, the zygote starts to attach the endometrium wall, and then invades the endometrium to start maternal nutrition. It is known that pathologies related to

capillaries, which are responsible for maintenance of implantation and placental nutrition, have major effects on mechanisms underlying abortion. Matrix metalloproteinases (MMPs) function in various cellular pathways. They play a role in patholohical conditions, metastasis; as well as normal physiological functions like tissue and bone regeneration, physiologic function of uterus, ovulation, embryogenesis and embryo implantation. MMP2 and MMP9 (gelatinases) have key roles at organisation of extracellular matrix and affect endometrial implantation. Previous studies have shown that MMP2 -1306C>T and -735C>T polymorphisms cause loss of gene function, and MMP9 -1562C>T polymorphism causes a decrease in gene expression, and also gene expression levels are lower in abortion specimens, compared to control specimens. The goal of this study was to investigate the potential effects of functional MMP2 -735C>T and -1306C>T polymorphisms, and MMP9 -1562C>T polymorphism on etiology of abortion.

Restriction fragment length polymorphism (RFLP) method was used to analyze the polymorphisms those evaluated in the study. Study group consisted of samples collected from 80 spontaneous abortion specimens, and control group consisted of peripheral blood blood samples collected from 100 healthy subjects.

The risk of abortion was 2.2-fold higher in patients with heterozygous -1306C>T polymorphism ($P = 0.043$). Combined genotype analysis showed that the risk of abortion was 3.7-fold higher for patients with normal -735C>T polymorphism, and heterozygous -1306C>T polymorphism ($P = 0.021$).

Functional polymorphisms of MMP2 and MMP9 may have a role in etiology of abortion.

P-02.07.5-011
Changes in the specific extracellular matrix proteins and expression of ADAMTS proteinases and effects of hypoxia in the adriamycin-induced renal fibrosis model

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Renal fibrosis is a common cause of renal dysfunction with chronic kidney diseases. This process is characterized by excessive production of extracellular matrix (ECM) or inhibition of ECM degradation. ADAMTS proteinases, which are widely presented in mammals, have very critical roles in ECM remodeling. We aimed to study the role of ADAMTS proteinases in the pathogenesis of renal fibrosis and the effets of hypoxia by studying ADAMTS expressions in rat kidney after adriamycin (ADR) administration. ADR was administered intravenously in consequtive two doses (2.5 and 4 mg/kg) to the rats. In addition to control and ADR groups, another rats were assigned into three groups as sham, 15 min and 45 min ischemia-reperfusion. After 2 months following the first dose, the expression of ADAMTSs were determined in the renal tissues using Western blot analysis. Additionally, renal nephropathy and fibrosis were assessed pathological and immuno-histochemical staining methods. In the ADR group rats developed renal dysfuntion and tissue damage in favor of renal fibrosis pathologically. It is observed that occurred remarkable changes in the expression of ADAMTSs. It is showed that hypoxia and hypoxia time enhanced tubulointerstitial fibrosis in the rat kidney tissues. Expression differences were absorved also in the hypoxia groups, and especially the expression of ADAMTS-1 and -15 genes were showed an increase in various rates. The restricted and different expression pattern of ADAMTS protein profiles in the ADR-induced renal fibrosis suggest that ADAMTS family members are related with development and progression of fibrosis. Moreover, our findings raise the possibility that the hypoxia promotes renal fibrogenesis. The monitoring of ADAMTS proteinases might contribute to the early diagnosis of renal fibrosis and chronic kidney disease.

P-02.07.5-013
ADAM9, ADAM10 and ADAM17 differentially regulated in male infertility

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ADAMs (a disintegrin and metalloproteas) are transmembrane proteins that contain a pro-domain, a metalloprotease, a disintegrin, a cysteine-rich, an epidermal-growth factor like and a transmembrane domain, as well as a C-terminal cytoplasmic tail. They are involved in cell adhesion and they have protease activities.

Previous studies showed that some ADAM proteins act in a highly diverse set of biological processes, including fertilization, neurogenesis, myogenesis, embryonic TGF-α release and the inflammatory response. Although there are more researches about ADAM proteins, still the function of all ADAM proteins remain unclear.

We aimed to investigate the potential link of infertilty with ADAM9, -10, and -17. In this study twenty four patients were included. The patients were classified as normozoospermia (NS; $n = 8$), oligozoospermia (OS; $n = 8$), azoospermia (AS; $n = 8$) groups. ADAM9, -10 and -17 protein levels in infertility indviduals were analysed by Western blot.

ADAM9 protein level was 1.7 fold lower in the OS and AS groups than in the NS group. ADAM10 protein level was 1.36 fold higher in the AS group than in the NS group. ADAM17 protein level was 2 fold lower in the AS group accourding to NS group. We observed no change between protein level of ADAM10 and ADAM17 of OS and NS groups .

In conclusion, ADAM proteins may have a potential role in male infertility. Our study is a preliminary and first study on this issue.

Keywords: ADAM, infertility.

P-02.07.5-014
The role of tissue metalloproteinase inhibitors thymus chemokine and thrombospondin-1 on prognosis of Crimean-Congo hemorrhagic fever

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Crimean-Congo hemorrhagic fever (CCHF) is a disease which is caused by an arbovirus carried by ticks and characterized by the sudden onset of high fever, severe headache, dizziness, back and abdominal pain. CCHF pathogenesis is still not resolved today to fully open. Therefore, in this study, to determine the level of TCK-1, TIMP-1 and TSP serum samples obtained from CCHF patients and the control group is intended to be examined for the pathogenesis and prognosis of the disease.

The study sample was created 45 patients with diagnosis of CCHF. 45 healthy volunteers were chosen control group matched for gender and similar to in terms of age. TSP, TPC-1 and TIMP-1 levels of patients and a control group were analyzed using the Human ELISA kits.

Serum TIMP-1 TCK-1 and TSP levels in CCHF patients were measured significantly higher than the in the control group.

CCHF pathogenesis of today still have not provided fully open. Therefore, it reveals the importance of this work. In our study, presence of high TIMP-1, TCK-1 and TSP levels indicate important direction for pathogenesis and prognosis of CCHF disease.

P-02.07.5-015
Activation of calpain 1 and protein kinase Cα promotes a constitutive expression and release of matrix metalloproteinase 9 in peripheral blood mononuclear cells from cystic fibrosis patients

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Matrix metalloproteinase 9 (MMP9) is physiologically involved in remodeling the extracellular matrix components but its abnormal release has been observed in several human pathologies, including cystic fibrosis.

We have studied if the alteration in intracellular Ca^{2+} homeostasis, observed in peripheral blood mononuclear cells (PBMCs), isolated from cystic fibrosis (CF) patients homozygous for deletion of phenylalanine 508 in gene of cystic fibrosis transmembrane conductance regulator (F508del-CFTR), could be involved in the abnormal presence of MMP9 in the extracellular fluids of CF patients.

PBMCs were isolated from 23 healthy donors and 26 CF patients homozygous for F508del-CFTR. MMP9 levels were evaluated following 2 h of cell incubation.

Our investigations show that all CF PBMCs analyzed constitutively express and release high levels of MMP9; conversely, in PBMCs from healthy donors, expression and secretion of MMP9 are undetectable but both events can be evoked, after 12 h of cell culture, by a possible paracrine stimulation. We have demonstrated that in CF and 12 h-cultured control PBMCs MMP9 secretion is prevented by chelation of intracellular Ca^{2+} and mediated by the concomitant activation of calpain and protein kinase Cα (PKCα) and also that MMP9 expression is mediated by the sequential activation of PKC and extracellular signal-regulated protein kinases 1 and 2 (ERK1/2). Moreover, the rescue of active F508del-CFTR reduces the extent of MMP9 secretion, correlating the channel defect to the $[Ca^{2+}]_i$ dysregulation of CF PBMCs.

Our results indicate that the high level of intracellular Ca^{2+} concentration in CF PBMCs, promoting the aberrant activation of both calpain and PKCα, induces a constitutive release of MMP9.

These data characterize new alterations in mononuclear leukocytes of CF patients that may be of primary importance in the progression of the disease and indicate that PBMCs may contribute to the accumulation of MMP9 in fluids of CF patients.

P-02.07.5-016
AEBP1/ACLP is upregulated in differentiation, injury repair and fibrotic degeneration of skeletal muscle

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AEBP1/ACLP is an ambiguous gene with several attributed functions and cellular events, adipogenic differentiation, cell adhesion, pattern development and fibrosis are the well-understood. AEBP1 is the short isoform that acts as a transcriptional repressor by targeting the AP2 promoter and ACLP, which is the long isoform that harbors a leader sequence that directs the peptide to the extracellular compartment. The latter is known to be associated with development of the connective tissue, injury repair and fibrosis in certain pathological conditions. AEBP1/ACLP displays a moderate expression in skeletal muscle where the role is not known. We have investigated the spatial and temporal expression of AEBP1/ACLP in defined models of skeletal muscle differentiation, injury repair and fibrosis. AEBP1/ACLP expression is present in steady state dividing myoblasts. This basal expression is upregulated 4 folds upon the induction of differentiation in C2C12 cells. Considering that differentiation and post-natal injury repair share several common aspects, we also investigated the expression of AEBP1/ACLP in acute injury-repair model. In the course of cardiotoxin induced injury, AEBP1/ACLP expression peaks up to 5 folds in the 6th day of regeneration. This time point concomitantly corresponds to tissue remodelling. Since AEBP1/ACLP is also known to be associated with fibrotic events in chronic pathological conditions, we also have investigated its expression in tenotomy induced skeletal muscle degeneration which mimics endomysial and perimysial fibrosis. AEBP1/ACLP expression is upregulated up to 10 folds in early time-point samples. These results depict a novel role for AEBP1/ACLP in extracellular remodeling of the skeletal muscle during injury repair as well as fibrotic degeneration. The source of this expression might come from fibroadipogenic precursers which reside in endomisial area of muscle. Our current efforts are focused on presenting of this endomysial expression.

P-02.07.5-018
The regulation of *Bacillus pumilus* 3-19 metalloendopeptidase expression

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The *mprBp* gene from *B. pumilus 3-19* encoding a novel secreted metalloproteinase was identified. Based on the primary structure the enzyme has been classified as metzincin metalloproteinase that combines the features of two families: astacin and adamalysin. Representatives of the adamalysin family previously have not been described for bacilli. The aim of the work was to elucidate the mechanisms of the gene expression regulation of a new *Bacillus pumilus* 3-19 extracellular metalloendopeptidase. Promoter region analysis revealed the presence of potential binding sites for transcription factors Spo0A (sporulation) and DegU (biodegradation). Study of *mprBp* expression in strain defective in regulatory proteins DegS and DegU shows that the productivity of metalloproteinase biosynthesis decline in average 60% compared with the strain with a complete DegS-DegU system. We also studied *mprBp* expression in strains with a mutation in

the gene *degU*, leading to stabilization of Degu~P protein. It is known, that this mutation leads to a multiple increase in the gene expression level, positively regulated by DegS-DegU system. Our data shows a 10-fold increase in metalloproteinase productivity in the recombinant strain. Thus, Deg-system participates in control of the proteinase synthesis but not only in the regulation of *mprBp* gene expression. The *mprBp* expression in the strain deficient in regulatory protein Spo0A remained at the level with expression in the strain with the complete *spo0A*. A similar pattern we observed in the study of *mprBp* gene expression in strains defective in other spore-specific regulatory proteins (Spo0B, Spo0F, Spo0K, Spo0J, SigF, SigH, SigK). These data indicate that *mprBp* gene expression is free of Spo-regulatory proteins. On this basis, we concluded that the expression of metalloproteinase gene is not correlated with the sporulation.

P-02.07.5-019
Paricalcitol attenuate activity and expression of matrix metalloproteinases in a rat model of renal ischemia-reperfusion injury

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Matrix metalloproteinases (MMPs) are endopeptidases involved in the degradation of extracellular matrix. They have been postulated to have a role in the pathogenesis of ischemia-reperfusion injury (IRI). In the present study, we investigated the effect of paricalcitol, a synthetic vitamin D analog, on MMPs in renal IRI. 21 wistar albino rats were divided into three groups: sham operated, ischemia-reperfusion, and paricalcitol-pretreated. IRI model was induced by bilateral clamping of renal arteries for 45 min followed by 24 h of reperfusion. The analysis of serum creatinine levels and activities/expressions of MMP-2 and -9 were performed after 24 h of IRI. The effects of paricalcitol on activities and expressions of MMP-2 and MMP-9 levels were investigated by gelatin zymography and immunohistochemistry, respectively. The pathological examinations were performed to score tubular damage by light microscopy. Creatinine levels increased significantly in the IRI group. Rats in the paricalcitol-pretreated group showed significant decrease in expressions and activities of MMP-2 and MMP-9 during IRI. Moreover, pathological examinations displayed significantly lower score of tubular damage in paricalcitol-pretreated group. In conclusion, Paricalcitol attenuated IRI by downregulating the expressions and activities of MMP-2 and -9.

P-02.07.5-020
The changes of matrix metalloproteinase 2, 9 activity and hyaluronic acid level in rat's heart and serum under cadmium influence

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The changes in the molecular mechanisms of the extracellular matrix degradation under toxic factors are not well known. The main goal of work was the investigation of the MMP2 and MMP9 activity and hyaluronic acid level in the heart and blood serum under cadmium influence at different doses.

The 18 Wister rats divided to 3 groups were used for the experiment. $CdCl_2x2.5H_2O$ in doses 0.1 μg/kg and 1 μg/kg was given to rats intragastrically in drinking water during 36 days. The rats were decapitated under Isoflurane anesthesia according to ethical rules; the heart was quickly removed. The relative activity [in arbitrary units (au)] of pro- and active forms of MMP9 and MMP2, total protein (TP) and hyaluronic acid levels were calculated.

It was shown that low doses of exogenous cadmium (0.1 μg/kg) lead to reduced activity of pro- and active forms of MMP9 in myocardium (7.3 ± 0.6 au/mgTP and 7.1 ± 0.6 au/mgTP compare to the 9.67 ± 0.4 au/mgTP and 9.7 ± 0.5 au/mgTP in the control rats accordingly) and in serum (0.95 ± 0.2 au/mgTP and 0.35 ± 0.05 au/mgTP compare to the 1.54 ± 0.05 au/mgTP and 1.49 ± 0.05 au/mgTP in the control rats accordingly), but pro-MMP2 activity in heart was increased (14.5 ± 1.6 au/mgTP compare to the 9.8 ± 0.6 au/mgTP in the control rats); level of HA was decreased in both tissues (0.69 ± 0.16 μg/ml and 3.63 ± 0.3 μg/ml compare to the 1.0 ± 0.13 μg/ml and 3.91 ± 0.3 μg/ml in the control rats accordingly). High doses of cadmium (1 μg/kg) caused a reliable increase of both gelatinase activity in the myocardium: MMP2 increased from 9.65 ± 0.4 au/mgTP to 14.1 ± 0.8 au/mgTP, proMMP9 – to 12.6 ± 1.5 au/mgTP, MMP9 – to 15.4 ± 1.6 au/mgTP. HA level was increased in serum (4.28 ± 0.1 μg/ml) and decreased in heart (0.49 ± 0.09 μg/ml).

The results indicate the dose-dependent and tissue-specific effect of cadmium on MMP-depended protein degradation and level of hyaluronic acid.

P-02.07.5-022
IL-33 stimulation down-regulated ADAMTS15 gene and protein in U118 glioblastoma cells

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A disintegrin-like and metalloproteinase domain with thrombospondin-1 repeats (ADAMTS) are a large family of proteoglycanase that show proteolytic activity towards proteoglycans like aggrecan, brevican, neurocan, and versican. Interleukin-33 (IL-33) is an IL-1 cytokine family member that uniquely plays a role as a cytokine and nuclear factor. It is released by necrotic epithelial cells and activated innate immune cells as an alarming danger signal. ADAMTS and IL-33 implicated in brain cancer pathogenesis. We aimed to seek the amount of ADAMTS15 in U118

glioblastoma cell line which was stimulated by IL-33. Western blot and Real-time PCR methods were used. IL-33 treatment decreased ADAMTS15 protein and mRNA amount significantly. Therefore, ADAMTS15 potentially has a role in glioblastoma pathobiology.

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Keywords: IL-33, Glioblastoma, ADAMTS15.

P-02.07.5-023
The effect of ficin, a non-specific plant protease, on staphylococcal biofilms disruption

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Proteolytic enzymes are able to speed up wound healing by removal of the necrotic tissues and fibrin. Several investigations have reported that proteases damage also the microbial biofilms formed by opportunistic bacteria including *Staphylococci* on surfaces of chronic and acute dermal wounds. Therefore, proteases are seemingly perspective enzymes for biofilm eradication by hydrolysis of both matrix proteins and adhesins, proteins providing cells attachment onto solid surface and other bacteria, as well as by the cleavage of signalling peptides of intercellular communication of gram-positive bacteria. Here we report that ficin, a plant protease, efficiently degrades the structural components of biofilm matrix formed by *S. aureus* and *S. epidermidis* at concentrations of 10 μg/ml while trypsin and chimotrypsin are used as 1–2 mg/ml solution. The spatial structure of the biofilm was analyzed by atomic force microscopy. After ficin treatment, the biofilm structure became porous, with reduced viscosity. The congo red staining of the treated biofilms confirmed the hydrolysis of the protein component of the matrix. Moreover, the biofilm treatment with ficin increased the antimicrobial efficiency of ciprofloxacin against biofilm-embedded cells of *S. aureus* and *S. epidermidis*. While 24 h antibiotic treatment did not lead to the increase of dead cells of neither *S. aureus* nor *S. epidermidis* embedded into the biofilm matrix, in the presence of ficin the fraction of viable cells decreased significantly. Accordingly, soluble ficin appears beneficial for outer wound treatment biofilm eradication and reduces the reinfection risk. The wound-healing activity of ficin requires further investigations.

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Tuesday 6 September 12:30–14:30

Structural biology: Membrane complexes and supercomplexes

P-03.03.3-001
Resveratrol modulates the lipid ordering in model membranes

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Resveratrol (Resv) is an antioxidant that belongs to the group of plant compounds, called polyphenols. Resv is defined as an antimicrobial substance that is produced by several plants (red grape skin, peanuts and berries) to protect them from rough environments like excessive ultraviolet light, infections and climate changes. As an antioxidant, this polyphenol protects the body against cardio-vascular and cancer diseases. Besides, Resv has anti-inflammatory, neuro-protective, anti-diabetic and other pharmacological effects. Although the positive pleiotropic effects of this polyphenol are well documented, there is a huge need to understand its influence on the biophysical properties of lipid bilayer. In the present work, the interaction of Resv with membranes composed of palmitoyl-docosahexaenoyl phosphatidylcholine (PDPC) or palmitoyl-oleoyl phosphatidylcholine (POPC), sphingomyelin (SM) and cholesterol (Ch) was investigated by means of fluorescence spectroscopy. Generalized polarization of the fluorescent probe Laurdan (GP) as a function of temperature was used to probe the changes in the fluidity of lipid bilayer induced by different Resv concentration. The obtained results showed decreased lipid ordering from 50 to 100 μmol Resv and opposite effect from 200 to 500 μmol in PDPC/SM/Ch mixtures as compared to the control without Resv. The interaction of Resv with POPC/SM/Ch mixtures caused only an increase in the lipid ordering as a function of Resv amount. POPC and PDPC have the same head group but different fatty acid chains at *sn-2*. Since Resv changes the physicochemical properties of lipid bilayer by different ways one might suggest that the interaction of polyphenol with the membrane depends on the level of fatty acid unsaturation. This specific effect of Resv on lipid organization could be related to its unique properties to prevent the cell from oxidative stress.

P-03.03.3-002
Effect of phloretin on the β-amyloid-induced permeability of model lipid membranes

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Neurodegeneration is the umbrella term for the deseases including progressive loss of structure or function up to death of neurons. Beta-amyliod peptide is proteolytic fragment of the amyloid protein. The spontaneously formation of selective, voltage-dependent, ion-permeable channels in the lipid bilayers was reported as one of amyliod peptide toxicity mechanisms.

The aim of our study was the investigation of the influence of the flavonoids (phloretin, phlorizin, quercetin and genistein) on the membrane activity of amyliod peptides.

Virtually solvent-free bilayer lipid membranes were prepared from mixtures of phospholipids in 0.1 M KCl (pH 7.4) using monolayer-opposition technique. Using spectrofluorimetry we estimated prepared from phospholipids by extrusion the liposomal membrane permeability for calcein.

We found that the addition of phloretin into membrane bathing solution led to an signicant increase in the channel forming activity of fragments 25–35 of amyloid peptide, fragment 25–35 of [Gly35]-amyloid peptide and 106–126 of human prion protein. Addition of other flavonoids did not cause any changes in the steady-state amyloid-induced current. It was found that the effect was caused by electrostatic interaction with the peptide. We found that time course of amyloid induced leakage calcein from liposome's is characterized by two components: the fast one is related to sorption of β-amyloid peptide on the membrane and the slow one is related to the oligomerization of the peptides on the surface of the lipid bilayer. Addition of the phloretin simultaneously with β-amyloid peptide to the suspension of liposomes caused significant reduction in time parameters characterizing fast and slow components. From this results we can proposed

that phloretin compensates the positive charge of the β-amyloid peptides and leads to the changes in their oligomerization status. The study was supported in part by RFS (14-14-00565) and SP-69.2015.4.

P-03.03.3-003
Ferritin nanocarriers: a focus on a metal-based drug encapsulated inside the protein cavity

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Ferritin is one of the main player involved in the iron metabolism. The biological role of ferritin is the storage of iron atoms inside the cavity preventing the uncontrolled accumulation of toxic species inside cells. Ferritins are polymers constituted by 24 subunits that self-assemble giving rise to an almost spherical nanocage. In vertebrates, ferritins are formed by two distinct subunits, the Heavy chain (H), with oxidoreductase activity, and the Light chain (L) without catalytic activity.

Ferritins are promising nanocarriers for the delivery of contrast agents for diagnosis and of drugs for therapeutic purpose. Their endogenous origin and the possibility to stabilize and protect the enclosed cargo inside the cavity, make ferritin a biocompatible vehicle. Moreover, there are specific receptors on cells that recognize and incorporate ferritin by endocytosis, prospecting a kind of targeted-delivery.

Following the increasing interest in nanotechnology, we studied the interaction between different isoforms of ferritin with an antimetastatic drug, called NAMI-A, which is the first ruthenium derived anti-cancer drug to have entered clinical evaluation. This molecule is a metal-based prodrug that can release the metal ion ligands.

Here, we describe NAMI-A hydrolysis in the presence of recombinant homopolymers of ferritin followed spectrophotometrically. Thanks to characteristic time dependent changes of spectral profile in the UV-visible region, we could detect differences in the hydrolysis process. The formation of a Ru-adduct with H-ferritin was established by UV-visible and circular dichroism spectroscopies, as well as by kinetics measurements that showed inhibition of the ferroxidase activity of H-ferritin. Crystallization trials are in progress to identify the binding site of Ru by solving the X-ray structure of the complex. Finally, we planned to test the cytotoxicity of ferritins pre-incubated with NAMI-A, using different cancer cell lines.

P-03.03.3-004
Bleomycin-induced trans lipid formation in liposomes

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Liposomes as biomimetic models of cell membranes were used for examining some novel aspects of drug–metal induced reactivity with unsaturated lipids under oxidative conditions. The chemical basis of cis to trans transformation was ascertained by liposome experiments, using bleomycin–iron complexes in the presence of thiol as a reducing agent that by incubation at 37 °C gave rise to the thiyl radical-catalysed double bond isomerisation of membrane phospholipids. The effect of oxygen and reagent concentrations on the reaction outcome was studied. As a chemical biology model for antitumoral strategies, liposomes highlight the role of cell membranes, which are not spectators but important targets of the drug effect, with synergic roles for chemotherapeutic effects. Indeed, fatty acid recruitment and membrane formation are attracting a lot of interest in cancer, and in this context the loss of the natural cis geometry and the oxidation-induced lipid remodelling are worthy of deeper studies in antitumoral strategies. Furthermore, the interaction between drugs and lipids can be suggestive of novel aspects of chemical reactivity for liposome carriers when circulating in vivo.

P-03.03.3-005
GPR17 (GPCR) expression in Sf9 cells. Evaluation of protein stability and surface expression

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GPR17 is a G-protein coupled receptor (GPCR), expressed in cells of brain, heart and kidney. It is related to the leukotriene and purinergic subfamilies of the rhodopsin-like Class A GPCRs.

GPR17 plays controversial role in the brain and spinal cord cells recovery after injuries. It is assumed that GPR17 is one of the cell death regulators immediately following an injury but at later stages it also takes part in tissue regeneration. There are also data implying some role of GPR17 in glucose homeostasis.

Drugs targeting GPR17 may help with treatments of multiple sclerosis and ischemia. The damage of rat's brain in artificially induced ischemia disease decreased after GPR17 inhibition. In addition, GPR17 takes part in myelin sheath formation, the lack of which is known to be the reason of multiple sclerosis.

To better understand functional role of GPR17 and enable design of more efficient ligands we initiated structural studies of this receptor. To improve receptor stability and facilitate crystallization we created a series of chimeric constructs using different fusion partners – small soluble proteins inserted into the native amino acid sequence. Preliminary experiments were carried out to evaluate the influence of different ligands on the receptor stability and cell surface expression in insect Sf9 cells. This work was supported by Russian Federal Target Program 14.587.21.0026 (RFMEFI58716X0026).

P-03.03.3-006
A calorimetric comparison of the effects of cholesterol and stigmasterol on zwitterionic dimyristoylphosphatidylcholine model membranes

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Sterols are significant for the structural and dynamical features of cell membranes. Among them, cholesterol is the major sterol in mammalian cell membranes whereas stigmasterol is the predominant sterol in plant membranes. Stigmasterol varies structurally from cholesterol in having both an ethyl group at carbon 24 and an additional trans double bond between carbons 22 and 23. Dimyristoylphosphatidylcholine (DMPC) is a widely studied

synthetic lipid, which has a neutral (zwitterionic) PC headgroup and two symmetrical 14-carbon fatty acyl chains. The studies on the interactions of cholesterol and stigmasterol with DMPC membranes at molecular level are very limited.

In the present study, a calorimetric comparison of the effects of the animal sterol cholesterol and the plant sterol stigmasterol on zwitterionic dimyristoylphosphatidylcholine (DMPC) multilamellar vesicles (MLVs) was investigated for the first time by using differential scanning calorimetry (DSC).

Our DSC studies indicate that with the inclusion of increasing cholesterol and stigmasterol concentrations from 5 to 40 mol% into pure DMPC MLVs, the pretransition disappears, the main phase transition shifts to lower temperatures and then disappears at cholesterol and stigmasterol concentration above 25 mol%. The main phase transition enthalpy (ΔH) is progressively reduced whereas full width at half maximum ($\Delta T_{½}$) gradually increases with increasing cholesterol and stigmasterol concentrations. Moreover, the main phase transition peak consists of overlapping sharp and broad components, indicating the hydrocarbon chain melting of sterol-poor and sterol-rich DMPC domains, respectively.

In conclusion, this study shows clearly that both cholesterol and stigmasterol interact effectively with DMPC membranes and cause changes in their structural and functional properties.

P-03.03.3-007
TRH receptor mobility in the plasma membrane is affected by its interaction with its cognate signaling molecules and by cholesterol depletion

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G protein-coupled receptors (GPCRs) play a fundamental role in transferring information from extracellular environment to the cell interior. Some GPCRs are supposed to form signaling complexes with their cognate G proteins and possibly other accessory proteins, which may facilitate the activation of G proteins and thus accelerate signal transmission.

Here we investigated the role of some components of thyrotropin-releasing hormone (TRH) receptor signaling in HEK293 cells stably expressing YFP-tagged TRH receptor using fluorescence recovery after photobleaching (FRAP). We observed significant changes in values of the diffusion coefficients if expression of β_2-arrestin or $G\beta_2$ subunit were suppressed by siRNA.

Results of our FRAP experiments indicated significant difference between control and TRH-treated cells as the diffusion coefficient markedly decreased after agonist stimulation. On the other hand, the same decline of the diffusion coefficient value was found after silencing with siRNA and subsequent treatment with TRH for most of the screened proteins. Treatment of cells with 10^{-5} M TRH led to fast internalization of TRH receptor, which was revealed by real-time confocal microscopy. It is known that cholesterol is an essential component of the cell membranes and it exerts modulatory effects on the functioning of various membrane proteins. Disruption of plasma membrane integrity by cholesterol depletion using β-cyclodextrin significantly reduced the apparent diffusion coefficient values. Interestingly, addition of TRH to cells treated with β-cyclodextrin did not further reduced TRH receptor mobility.

It can be concluded that stimulation with agonist and/or siRNA silencing of some components of the TRH receptor signaling cascade significantly affects the mobility of TRH receptor in the plasma membrane.

P-03.03.3-008
μ-Opioid receptor mobility in the plasma membrane is diversely affected by biased ligands

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Opioid receptors belong to the large family of G protein-coupled receptors (GPCRs), which are currently considered among the most important targets for pharmacological manipulations. During the past few years, a great deal of attention has been devoted to biased agonism. This phenomenon reflects the ability of different ligands to selectively affect the functioning of some GPCRs so they can display marked differences in their efficacies for G protein- or β-arrestin-mediated signaling.

Here we decided to investigate the effect of different agonists of the μ-opioid receptor (μ-OR) on the lateral mobility of this receptor in the plasma membrane of HEK293 cells which were stably transfected with μ-OR tagged with yellow fluorescent protein (μ-OR-YFP). It has been found previously that DAMGO stimulates G-protein-dependent signaling, endomorphine 2 stimulates arrestin-dependent signaling and morphine does not show any significant bias towards these two signaling pathways.

In our experiments, we used the fluorescence recovery after photobleaching (FRAP) method to estimate the diffusion coefficients of μ-OR-YFP in the resting state and after addition of the above mentioned specific agonists.

We observed that addition of DAMGO increased the value of the diffusion coefficient and addition of endomorphin 2 decreased the value of diffusion coefficient of μ-OR-YFP. Addition of morphine or the μ-OR antagonist naloxone did not change the value of the diffusion coefficient compared to the resting state.

These results indicate that different biased agonists of μ-OR may differently affect the mobility of this receptor in the plasma membrane. These findings provide new insights into the dynamics of μ-OR in the plasma membrane and contribute to better understanding of the mechanism of biased agonism at GPCRs, which is of central importance for receptor homeostasis and fine regulation of receptor activity.

P-03.03.3-009
Color tuning and adding potassium selectivity to a light-driven sodium pump

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Recently a light-driven sodium pump has been discovered, characterized and tested as an inhibitory optogenetic tool. Sodium pumping rhodopsin from *Dokdonia eikasta* KR2 has an absorption maximum at 523 nm at pH 7.5 and to create more red-shifted variants we analyzed available structures of the KR2 (PDB codes: 4XTL, 4XTN) and did the rational mutagenesis of residues in the retinal proximity region (i.e. M149, G153 and S254).

The mutants of KR2 under investigation were: M149A, G153V, M149A/G153V, S254A, S254F, S254G, S254L, S254M, S254N, S254T, S254V, S254Y. The protein mutants were expressed in *Escherichia coli* C41 strain, expression was induced by the addition of 1 mM isopropyl β-D-1-thiogalactopyranoside. The cells were

then washed trice with unbuffered 100 mM NaCl or KCl solution. Finally, the pH changes in cell suspensions (OD600 = 8.0) were monitored. pH changes upon the addition of 30 μM of protonophore carbonylcyanide m-chlorophenylhydrazone were also measured. The following mutants completely abolished the protein function and were not used for further characterization: S254F, S254L, S254M, S254N, S254V. The remaining mutants have shown sodium pumping activity and S254A mutant has gained an additional potassium pumping activity.

All functionally active mutants were purified using Ni-affinity chromatography and the absorption spectra were collected for them at pH 7.5 (50 mM Na/Na-Pi, 100 mM NaCl).

M149A mutant absorption maximum is blue-shifted to 519 nm. G153V and M149A/G153V – blue-shift to 470 nm. S254A, a potassium pumping variant, – red-shift to 545 nm. S254G, S254Y – red-shift to 545 nm. S254T – no change in absorption maximum position.

Based on structural analysis of KR2 we discovered another potassium pumping variant and provided the variants with absorption maximum blue-shift up to 53 nm and red-shift up to 22 nm.

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P-03.03.3-011
Pre-crystallization assays of an engineered human endothelin receptor type B

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Human endothelin receptors belong to the G-Protein coupled receptors (GPCRs) superfamily. This class is pharmacologically important, with more than 40% drugs targeting it. The human endothelin system, which includes endothelin receptors types A and B (ETB and ETA), plays a highly important role in the blood pressure regulation. Endothelium cells produce peptides, known as endothelins 1–4, which activate endothelin receptors and launch cascades of reactions that lead to vasoconstriction or vasodilatation depending on the receptor subtype and the tissue. Additionally, endothelin receptors take part in such processes as transmission of nerve impulses, development of neural crest, and regulation of acid-base-salt balance in kidneys.

In order to stabilize ETB receptor for crystallization trials we introduced a compact soluble protein, apocytochrome b564RIL (BRIL), is the third extracellular loop of the receptor. BRIL is known to be an effective crystallization driver for GPCRs. The engineered protein was expressed using baculovirus system in *Sf9* insect cells, purified and subject to variety of pre-crystallization assays. Localization of the overexpressed protein in insect cells was visualized via the confocal microscopy. Thermal stability of the protein in the presence and absence of ligands was measured by the Thermal Shift Assay. Finally, the mobility of the receptor in lipidic cubic phase (LCP) at many different conditions was probed by the LCP-FRAP (Fluorescence Recovery After Photobleaching) assay.

These tests showed that the obtained protein is thermally stable, functionally active and diffuses well in LCP at certain conditions, making it a suitable candidate for proceeding to in meso crystallization trials. This work was supported by the Russian Science Foundation (project no. 16-14-10273).

P-03.03.3-012
Mitochondrial respiratory chain deficiency in Turkey

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Mitochondrial oxidative phosphorylation is the key metabolic pathway for the production of ATP. Mitochondrial respiratory chain (RC) defects are some of the most commonly diagnosed inborn errors of metabolism with a diverse spectrum of clinical phenotypes. The aim of the study is to evaluate the RC enzyme activities and histopathological findings in muscle biopsies of patients with suspected mitochondrial disease.

Muscle biopsy samples were collected from 48 pediatric patients. The samples were homogenized in SETH buffer using a glass/glass homogenizer. The activities of citrate synthase (CS) and RC enzymes (Complex I, II-III, and IV) were measured in tissue homogenates by kinetic spectrophotometric assays by Schimadzu UV Spectrophotometer (UV-1800). Non-collagen protein was determined by the modified Lowry assay. Activities of complex (C) I, II-III and IV (COX) were normalized by CS. Histopathological investigations included H&E, modified Gomori trichrome, periodic acid Schiff, Oil-red-O, NADH, SDH, COX and ATPase stains.

Deficiency of RC complexes was detected in 39 biopsies (81%). C IV deficiency was most common (60%), followed by C I (40%) and C II-III (27%). Multiple complex deficiency was present in 40% and isolated deficiency was present in 42% of the biopsies (20% C I, 20% C II-III, 60% C IV). CS activity was elevated in 44% of the biopsies. Decreased C I/CS, C II-III/CS and C IV/CS ratio was observed in 44%, 42% and 52%, respectively. Comparing with histological data, biochemical analysis revealed additional findings in 50% of biopsies.

Complex IV deficiency, either isolated or accompanied by other complex deficiencies, is most common in our cohort. RC analysis may reveal additional findings to histopathological results and careful clinical investigation with correlation of clinical, biochemical and histopathological data is mandatory for the challenging diagnosis of mitochondrial disorders in childhood.

P-03.03.3-013
Investigation of adipocytokines, activity of GLUT and Na^+/K^+-ATPase in rats fed glucose, fructose, starch-based sugars

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Objective: All over the world, shows a significant increase in obesity and diabetes. Intake of foods that contain fructose, glucose and starch-based sugar is a potential risk for metabolic syndrome. Obesity and diabetes are important effects of high fructose corn syrup (HFCS). We aimed to research, activity of Na^+/K^+-ATPase in addition to glucose transporter (GLUT) 2, resistin, adiponectin and other biochemical markers in rats fed glucose, fructose and starch-based sugars.

Materials and Methods: Study was performed on rats and 3 groups were included in the study. Rats were fed with chows that were given either normal diet for control group (70%

carbohydrate, 20% protein and 10% fat), high fructose (70% carbohydrate (87% fructose and 13% starch), 20% protein and 10% fat), or high sucrose (70% carbohydrate (87% sucrose and 13% starch), 20% protein and 10% fat). Rats were fed with chows for 8 weeks. In this process, the weight of the rats were followed. At the end of the experiment, blood is taken in all groups. Level of HbA1c, glucose, resistin and adiponectin were studied. GLUT2 and Na^+/K^+-ATPase activity were studied in the liver tissue.

Results: A significant increase in adiponectin levels were determined in rats fed both HFCS and sucrose ($P < 0.001$). A significant decrease in level of Na^+/K^+-ATPase activity were determined in rats fed both HFCS and sucrose ($P < 0.001$). There was no significant differance level of HbA1c, glucose, resistin and GLUT2 in rats fed sucrose or HFCS ($P > 0.05$).

Conclusions: Fructose-rich diet has an effect on changes in the ATPase activity and is a major risk factor for obesity.

Keywords: Adiponectin, fructose, glucose, High-Fructose Corn Syrup, Na^+/K^+-ATPase, resistin.

P-03.03.3-014
Nucleic acid-biomembrane lipid self-assemblies: from primordial soup to novel genome organization model and cellular non-viral nanotherapies

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Turkey Nucleic acid-cell membrane complexes has attracted research interest as models in gene regulation, cell cycle and division, as biosensors designs, as well as in molecular evolution. Zwitterionic phospholipids, complexed with polyribonucleotides by divalent metal cations (Mg2+) are considered as genosome vehicles. Their formations are studied by spectroscopic, thermodynamic, interfacial and microscopic approaches to build thermodynamic and kinetic models of their structural transitions. DNA forms a Mg2+-driven ternary complexes with neutral liposomes both at the airwater interfaces and at vesicle surfaces. The described self-assemblies form relevant models for nuclear pore complexes and their further implications in gene expression and functions. Such membrane contacts could be considered also in prokaryotic nucleoids important in bacterial segregation, whereas in eukaryotes these complexes can be regarded as focal points for transcription-translationtranslocation processes.

P-03.03.3-015
The effects of ozone/oxygen mixture on citrate synthase and mitochondrial complex activities of striated muscle tissue of healthy mice

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We investigated the effects of ozone/oxygen mixture and oxygen on citrate synthase (CS) and succinate dehydrogenase (SDH) activities of striated muscle tissue of healthy mice.

Thirty-six mice were included the study. Firstly muscle samples were taken from all mice's left thigh muscle in under anesthesia (Group 0). Secondly mice were randomly divided in four groups as: Group 1: oxygen was given once at 3 days for 7 days, Group 2: ozone/oxygen was given once at 3 days for 7 days, Group 3: oxygen was given once at 3 days for 30 days, Group 4: ozone/oxygen was given once at 3 days for 30 days. Ozone/oxygen mixture and oxygen were given at a dose of 1 mg/kg groups (1–4). After treatment animals were sacrificed, and muscle samples were taken and stored in −80 °C for until the analyses. Muscle tissues were homogenized in 0.05 M Tris-HCI and 0.15 M KCI. CS and SDH activities were measured with spectrophotometer. CS and SDH activities were expressed as μmol/min/g tissue.

CS and SDH activity results were given as mean ± SD. CS activity has been found in group 0 (37.8 ± 11.7), group 1 (46.9 ± 10.8), group 2 (34.7 ± 6.8), group 3 (32.9 ± 7.5) and group 4 (33.9 ± 8.5). SDH activity has been found in group 0 (2.50 ± 1.03), group 1 (2.37 ± 0.77), group 2 (2.52 ± 1.45), group 3 (2.24 ± 1.06) and group 4 (2.59 ± 1.25). There was no statistically significant difference among all groups in terms of CS ($P > 0.130$) and SDH activities ($P > 0.997$). There was no difference between all groups in terms of inflammation, muscle fiber size, regeneration or necrosis. Vascular structures, connective tissues, lipid and glycogen content of fibers were normal. Cytochrome oxidase activity was also normal. Ratio of ragged blue fibers of all groups were less than 0.3%, so they were scored as 1. There was no difference among groups for ragged red fiber content.

We have not found a significant effect of ozone/oxygen mixture and oxygen on CS and SDH activities of striated muscle tissue of healthy mice.

P-03.03.3-016
Reversible optical control on lipidic cubic phases

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Lipidic cubic phases (LCPs) consist of bicontinuous lipid bilayers and water channels. LPCs are widely used for membrane proteins crystallization and further determination of their spatial structures by means of X-ray crystallography. This approach was successfully used for studying G-protein coupled receptors structures. Usually crystallization initiates by adding the precipitants (buffers with high salt concentrations). Here we propose to use photo-switchable analogs of 1-monoolein to change lattice parameter of LPC.

We synthetized a number of novel diazo-analogs of 1-monoolein. Their structures were confirmed by NMR-spectroscopy and mass-spectrometry. Being incorporated in phospholipid vesicles or detergent micelles they subjected to trans- to cis-isomerization under the light exposure at 365 nm. Also we characterized the LCP's structures and properties by small-angle X-ray scattering on the RIGAKU instrument.

One of the synthetized compounds, 3-(4-{-[4-(octyloxy) phenyl] diazenyl} phenoxy) propane-1,2-diol (1% mol), was incorporated into the 1-monoolein cubic phase. According to small-angle X-ray scattering data the structure of the monoolein cubic-Pn3 m phase with lattice parameter 106.3 angstrem was not affected by insertion of this photo-switchable monoolein's analog. After the light exposure at 365 nm we observed trans-cis-isomerization. In the same time the cubic-Pn3 m phase was not changed but the lattice parameter reduced to 98.8 angstrem. This effect on monoolein LPC is similar to the addition of a precipitant to initiate

protein crystallization process. The spontaneous return to the initial lattice parameter was completed after 3 days in dark.

Thus, we demonstrated the possible controlling of the monoolein cubic phase lattice parameters by adding the photo-switchable diazo-derivatives of monoglyceride analogs, which can be used for crystallization of membrane proteins.

P-03.03.3-017
Evaluation of certain protein and phosphoprotein expression levels by using western blot technique in head and neck squamous cell carcinoma

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Introduction: Head and neck squamous cell carcinoma (HNSCC) is a primer tumor type in head and neck cancers, characterized by aggressiveness, early recurrence and metastasis. While alcohol and smoking play an important role at pathogenesis of disease, deregulation of some signaling pathways, genes and protein levels related to these pathways are considered as important at contribution of development of HNSCC.

Materials and Methods: In this study, protein and phosphoprotein expression levels of the frequently phosphorylated sites (EGFR, pEGFR, IGF-IR, pIGF-IR, PDGFRB, pPGDFRB, PTEN, pPTEN, AKT and pAKT) were investigated by using a western blot to confirm the expression of mRNA in the context of protein levels at normal-tumor tissues of HNSCC and SCCL-MT1 that is a HNSCC tumor cell line and HEK-293 that is a normal cell line.

Results: As a result of western blot analysis EGFR, PDGFRB and IGF-1R were detected as highly overexpressed cell surface receptors in tumor tissues of HNSCC.

Discussion and Conclusion: The findings of this study revealed the overexpression of other cell surface receptors as well as EGFR in HNSCC. In conclution, potential pathways were identified by determining the cell surface receptors overexpressed in HNSCC, these data support each other and may be important in pathogenesis of HNSCC.

Tuesday 6 September 12:30–14:30

Biochemical mechanisms in tolerance and autoimmunity

P-04.03.3-001
Assessment of the levels of lipid peroxidation and antioxidant protection in giardiosis before and after the treatment

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Introduction: The investigation of final products of lipid peroxidation is considered as the main mechanism involved in development of pathogenesis, diagnostics and prognosis of various parasitic illnesses.

Materials and Methods: The concentration of LP-AP in the blood was determined in the study group considered of 129 women (65%), and 69 men (35%).

Results: Before antiparasitic treatment, women infected with *G. intestinalis* showed a statistically significant 1.5 times increase of GPx activity levels; and 1.7 times ADA level increase compared to the control group. After the treatment, the CAT activity showed a sharp increase, whereas the ADA activity decreased by 1.4 times, compared to the average level before the treatment.

The results of the blood samples of the infected men with giardiasis, show the statistically significant increase in the level of all the studied parameters of lipid peroxidation, except the total primary production (TPP). The exception was the MDA level, remaining significantly increased, in contrast to the control group and to the condition after antiparasitic treatment. In infected men, the level of CAT activity was more than 5.8 times higher than that noted in control group. After treatment the levels of ADA activity and GPx returned to the values of the control group, while the level of CAT activity remained elevated.

Conclusion: An accumulation of primary and secondary metabolites in the course of giardiasis seems to confirm its involvement in the induction of oxidative-antioxidative homeostasis.

Antiparasitic treatment in giardiasis leads to normalization of the AP parameters studied in women and men, except the MDA content in the blood of men. Therefore, additional antioxidant treatment is advised for the antiparasitic therapy of men.

P-04.03.3-002
In vitro effects of ethanol on rat brain synaptosome and dose-dependent antioxidative role of boric acid

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Ethanol is a psychoactive drug that is very large and used frequently today. It has suppressive effects brain's comminication pathway. Depending on its acute or chronic use and the dose, ethanol increase membrane fluidity and it causes oxidative stress.

This study deals with the in vitro effects of ethanol toxicity (200 mM) and potential protective effect of different doses of boric acid (BA) (5, 10 and 25 mM) on rat brain synaptosomes. With this aim, five male Spraque Dawley rats are killed by decapitation under anesthesia. After the frontal cortexes of the rats are taken out, each of them is divided into four pieces. These pieces were used as a sample in five groups (control, ethanol, ethanol+5 mM BA, ethanol+10 mM BA, ethanol+25 mM BA) which include six samples. The synaptosomal fractions are prepared by the homogenization of the frontal cortex pieces and centrifugation for each samples.

As markers of ethanol-induced oxidative stress in the synaptosome of the rats, malondialdehyde (MDA), nitric oxide (NO) and catalase (CAT) levels were measured. MDA levels in the ethahol group were a quantity increased compared with those in the control group but it unchanged significantly as statistically ($P < 0.05$). However the MDA level in the ethanol+ boric acid (25 mM) group was shown to be significantly decreased compared with that in the ethanol group ($P < 0.05$). The CAT activity of the ethanol group was significantly higher than that in the control group and CAT activity of the BA (5 mM,25 mM) groups were close compared with control groups ($P < 0.01$). NO levels in ethanol groups were decreased but unchanged compared with control groups as statistically. Neverthless, NO levels in ethanol+ boric acid (25 mM) groups were increased ($P < 0.05$).

These results demonstrate that ethanol (200 mM) is capable of triggering damage to rat brain synaptosome and BA could be influential in antioxidant mechanisms against oxidative stress resulting from ethanol exposure.

P-04.03.3-003
Vitamin D levels in patients with rheumatoid arthritis

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Background: Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by systemic features and joint involvement which affects 1% of the world's adults. It can give rise to important morbidity and mortality. Vitamin D might be one of the environmental factors relevant with RA. Vitamin D is a steroid hormone precursor that undergoes chemical conversion in the liver and kidney: the first reaction produces 25OHD3, an objective indicator of vitamin D status, and the second produces the main bioactive form, 1,25-dihydroxyvitamin D (1,25(OH)2D). The aim of this study was to investigate the serum vitamin D levels in patients with rheumatoid arthritis.
Materials and Methods: This is a retrospective observational study; evaluating the vitamin D values of 47 healthy control and 109 patients with rheumatoid arthritis. The mean age for controls and patients were 50, 89 ± 9.22 and 53.87 ± 13.44, respectively. Mass spectrometric analyses were performed using an Shimadzu LC-20-AD (Kyoto, Japan) coupled with a ABSCIEX API 3200 triple quadrupole mass spectrometer (USA) equipped with an atmospheric pressure chemical ionisation (APCI) operating in positive mode for determination of vitamin D. Statistical analysis was performed with SPSS v16.
Results: The mean of vitamin D values in patients with RA (15.44 ± 8.04) were significantly lower compared to control group (21.18 ± 12.89) ($P < 0.05$).
Conclusions: Vitamin D plays an immunomodulatory role in inflammatory arthritis. Our results suggest that Vitamin D supplementatiton is recommended to patients with RA.

P-04.03.3-004
Anti-carbonic anhydrase antibodies in patients with acute myeloid leukemia

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Acute myeloid leukemia (AML) is the most common form of acute leukemia in adults and its incidence increases with age. Carbonic anhydrases (CAs) are zinc-containing enzymes. These enzymes catalyze a very simple physiological reaction, the inter conversion between carbon dioxide and the bicarbonate ion, and are thus involved in crucial physiological processes connected with respiration and transport of CO_2/bicarbonate between metabolizing tissues and lungs, pH and CO_2 homeostasis, electrolyte secretion in a variety of tissues/organs, and biosynthetic reactions and many other physiologic or pathologic processes including reproductive tract.

Investigation of autoantibodies in AML patients have been popular research area in recent years. The aim of the current study was to investigate carbonic anhydrase I and II (CA I and II) autoantibodies in the serum of subjects with AML based on the information and considerations of autoimmune relation of acute myeloid leukemia. Anti-CA I and II antibody levels were investigated by Enzyme-Linked Immunosorbent Assay method (ELISA) in serum samples of thirty patients with AML and thirty healthy peers.

Anti-CA I and II antibody titers of AML group were significantly higher compared with the control group ($P = 0.000$), ($P = 0.034$), respectively. We found significant positive correlation between anti-CA I antibody and anti-CA II antibody titers in patients with AML ($r = 0.613$, $P = 0.000$). We found significant positive correlation between anti-CA I antibody and anti-CA II antibody titers in women and the men ($r = 0.851$, $P = 0.000$), ($r = 0.503$, $P = 0.080$), respectively. At an anti-CA I cut-off point of 0.123 ABSU, sensitivity was 80% and specificity 100%. At an anti-CA II cut-off point of 0.097 ABSU, sensitivity was 60% and specificity 77%.

The CA I and CA II autoantibody levels in patients with AML were found higher compared to control group and the results suggest that CA I and CA II autoantibodies may be involved in the pathogenesis of AML.

P-04.03.3-005
Platelet/lymphocyte ratio in patients with Behçet's disease

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Aim: Behçet's disease (BD) is a systemic autoimmune disease. Recurrent oral and genital mucosal ulcers, uveitis, and skin lesions are characteristic findings for BD. Platelet-lymphocyte ratio reflects a novel marker for romatological diseases. The aim of this study was to investigate the platelet/lymphocyte ratio in Behçet's Disease.
Methods: Whole blood samples were collected from 50 healthy control and 21 patients with Behçet's disease. The mean age for controls and patients were 40 ± 1.0 and 37 ± 13, respectively ($P = 0.639$). Patients with chronic disease and inflammatory disorders were excluded. Thrombocyte and lymphocyte counts were analyzed with Abbott Cell Dyne heamotolgy analyzer. Statistical analysis was performed with IBM SPSS v20.
Results: Platelet counts were higher but not statistically significant in patients with Behçet's disease compared to control group (289 ± 100 vs. 257 ± 57) ($P = 0.088$). Lymphocyte counts were lower in patients with Behçet's disease compared to control group (2.56 ± 0.58 vs. 2.69 ± 0.85) ($P = 0.513$). Platelet/Lymphocyte ratio was higher but not statistically elevated in patients with Behçet's disease compared to control group (118 ± 41 vs. 106 ± 49) ($P = 0.344$)
Conclusions: Platelet/lymphocyte ratio (NLR) and mean platelet volume (MPV) as inflammatory markers recently became popular because of their simplicity, cost effectivity and their advantages to predict clinical prognosis of specific diseases. According to this study's results, platelet/lymphocyte ratio must be analyzed in vast scale patient populations to identify the disease.

P-04.03.3-006
Neutrophil/lymphocite ratio values in patients with systemic lupus erythematosus (SLE)

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Objectives: Systemic lupus erythematosus (SLE) is a chronic relapsing autoimmune disease characterized by production of autoantibodies against a series of nuclear antigens and by chronic inflammation. In recent years, neutrophil-lymphocyte ratio (NLR) was determined to be a good indicator of inflammatory

status. NLR can be easily calculated from a whole blood count. The aim of this study was to discover NLR values in SLE.

Materials and Methods: Whole blood samples were collected from 30 healthy control subjects and 52 patients with SLE. The mean age for controls and patients were 44.9 ± 3.8 and 40.8 ± 10.6 years, respectively. Patients with chronic disease and inflammatory disorders were excluded. NLR levels were calculated with Abbott Cell Dyne heamotolgy analyzer. Statistical analysis was performed with SPSS v16.

Results: The NLR values in patients with SLE disease [2.05 ± 0.80] were significantly higher compared to control group [1.76 ± 0.42] ($P < 0.05$).

Conclusions: Our analyses confirmed previous studies; Although it has not been considered as a spesific marker, NLR might be a useful and valuable inflammatuary marker in patients with SLE.

P-04.03.3-007
Could KIR2DS3 and KIR2DL3 genes be useful to predict the prognosis of neuroblastoma patients

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Introduction: Neuroblastoma, an embryonal malignancy, is the most common extracranial solid tumor of childhood. Untreated neuroblastoma tumors and cell lines are reported to have reduced HLA class I expression, rendering them potentially susceptible to natural killer cell killing due to lack of engagement of HLA class I–specific inhibitory killer cell immunoglobulin-like receptors (KIRs). The aim of this study was to investigate whether KIR genes could influence the risk of neuroblastoma and prognosis of the patients.

Materials and Methods: Study group consisted of 50 neuroblastoma patients (15 male, 21 female, median age: 36 months) followed at the Pediatric Oncology Clinic of Çukurova University Medical Faculty. Control group consisted of 100 healthy children. 14 patients had stage 1, 2, 3 or 4S disease, 36 patients had stage 4 disease. 16 different KIR genes were analysed by sequence specific oligonucleotide probe (SSOP) analyses. Statistical analysis were done using Fisher's Exact test.

Results: Compared to the control group, neuroblastoma patients had lower expression of activating KIR gene, KIR2DS3 ($P = 0.005$), and higher expression of inhibitory KIR gene 2DL3 ($P = 0.038$). Additionally KIR2DS3 genes were more common in patients with early stages (stage 1, 2, 3 or 4S) ($P = 0.023$) and KIR2DL3 genes were more common in patients with stage 4 ($P = 0.044$). Furthermore, there were no statistically significant differences between the rate of AA and Bx genotypes and their centromeric/ telomeric regions of patients and controls.

Discussion: KIR2DL3 gene can have a triggering effect in neuroblastoma pathogenesis; whereas KIR2DS3 can have a protective role. Investigating NK cell infiltration and KIR receptors in neuroblastoma tissue samples will give more insight to the pathogenesis

P-04.03.3-008
Neuroprotective and immunomodulatory effects of *Urtica urens*

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Urtica urens (Small Stinging Nettle) has been used for medical purposes in Turkey as an alternative therapy. It has been used in the treatment of inflammation that is early, non-specific immune reaction to tissue damage or pathogen invasion, plays an important role in the initiation of neurodegenerative disorders such as multiple sclerosis. However, there are limited studies that investigate anti-inflammatory activity of urtica. Therefore, aim of this study is to find out theanti-inflammatory effect of chloroform extract in Caco-2 cell line. For this purpose, firstly, chloroform extract of urtica leaves was prepared. Chemical composition of extract was determined by LC-MS. The effect of chloroform extract on selected pro-inflammatory and inflammatory proteins such as Tumor necrosis factor-α (TNFα), nuclear factor kappa B (NF-κB), C-X-C motif chemokine 10 (CXCL10), and 11 (CXCL11) were determined. Whole genome transcriptome analysis was performed by using Human HT-12 V4 BeadChip. Extract treatment caused 35% and 40% increases in protein and mRNA levels of NF-κB, respectively. On the other hand, TNF-α protein and mRNA levels decreased significantly (24% and 12%, respectively). Similarly, CXCL10 and CXCL11 mRNA levels decreased 45% and 38%. Transcriptome analysis showed that 233 probes were significantly changed ($P < 0.05$). Pathway analysis revealed that the extract altered a group of genes involved in immune response, calcium ion homeostasis and transport, potassium channel complex, G-protein coupled receptor protein signalling pathway, etc. It is well established that calcium is very critical for brain cell death and formation of many brain disease including multiple sclerosis. These observations suggests that urtica maybe used in neurodegenerative diseases. In order to further test this hypothesis experimental autoimmune encephalomyelitis experimentsand activity guided fractionations have been still continuing. This work is supported by TUBITAK 111T515.

P-04.03.3-009
Linear low molecular weight α-1,6-glucan from *Bifidobacterium bifidum* BIM B-733D is implicated in pathogenesis of celiac disease

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The bifidobacteria are recognized as human commensals and widely used as probiotics. Earlier, we have found (Kiseleva et al., Benef. Microbes, 2013, 4(4): 375–391) that *Bifidobacterium bifidum* BIM B-733D contains low molecular mass (5–7 kDa) α-1,6-glucans ($G_{anti\text{-}TPO}$ and $G_{anti\text{-}Tg}$) that interact selectively with human autoantibodies to thyroid peroxidase (anti-TPO) and thyroglobulin (anti-Tg), recognized markers of autoimmune thyroid disease (ATD).

The aim of the study was isolation and identification of *B. bifidum* BIM B-733D biopolymers (BPs) interacting selectively with autoantibodies to tissue transglutaminase (anti-tTG) and antibodies to gliadins (anti-Gl), recognized markers of celiac disease (CD).

We used affinity chromatography with anti-Gl, size exclusion chromatography, ^{1}H and ^{13}C NMR spectroscopy, ELISA with immobilized BPs, tissue transglutaminase (tTG) and gliadins (Gl) as positive controls.

The BP isolated by affinity chromatography with anti-Gl (as more available marker of CD) and size exclusion chromatography was identified by two-dimensional NMR spectroscopy as 5–7 kDa linear α-1,6-glucan identical to $G_{anti\text{-}TPO}$ and $G_{anti\text{-}Tg}$. The functional activity of the BP named $G_{anti\text{-}Gl}$, *viz*., ability to interact selectively with anti-tTG and/or anti-Gl was proven by ELISA with (*i*) serum samples of CD patients containing either both anti-tTG and anti-Gl without anti-TPO and anti-Tg or anti-Gl without anti-tTG, anti-TPO and anti-Tg *vs*. serum samples of healthy donors without four types of antibodies and (*ii*) pure anti-Gl *vs*. pure total IgG (without anti-tTG, anti-Gl, anti-TPO, anti-Tg). Since (*i*) serum samples of CD patients do not contain anti-tTG without anti-Gl and (*ii*) pure anti-Gl isolated by affinity chromatography with gliadins (Gl) cross reacts with tissue transglutaminase (tTG), additional studies with pure anti-tTG are necessary to find out which of the two antibodies, anti-tTG and anti-Gl, bind $G_{anti\text{-}Gl}$.

In conclusion, we proved that *B. bifidum* BIM B-733D cells contain linear low molecular mass α-1,6-glucan, $G_{anti\text{-}Gl}$, that interacts selectively with anti-tTG and/or anti-Gl. Since $G_{anti\text{-}Gl}$ is identical to earlier found $G_{anti\text{-}TPO}$ and $G_{anti\text{-}Tg}$, we hypothesize that the α-1,6-glucan is implicated in pathogenesis of both autoimmune diseases, CD and ATD.

P-04.03.3-010
Evaluation of vitamin D with hematologic parameters: OBESITY vs. NIDDM

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Influences of elevated serum ferritin levels on insulin resistance and non-insulin-dependent diabetes mellitus (NIDDM) have predicted either because of increased body iron stores or influenced by several inflammatory diseases. Low serum 25 hydroxyvitamin D is known to perturb cellular function in many tissues, including the endocrine pancreas, which are involved in obesity and NIDDM. We planned to investigate the association between 25-hydroxyvitamin D with hematologic parameteres and iron status in obesity vs. diabetic patients.

Study groups consist of control, non-diabetic obese, obese-diabetic and lean-diabetic groups. Serum triglycerides, total cholesterol, LDL-C, HDL-C, fasting glucose, HbA1c, uric acid, creatinine, GGT,25-hydroxyvitamin D, insulin, CRP, ESR, total blood count and iron status.

Apart from the three parameters, there were no significant difference ($P > 0.05$) between groups. Serum ferritin and MCHC levels were significantly higher in lean-diabetic patients ($P < 0.05$). On the other hand, RDW are determined to be significantly lower ($P < 0.001$) in the non-diabetic obese group. No difference was detected in 25-hydroxyvitamin D levels between the control and the study groups ($P > 0.05$). Non-diabetic obese patients had significantly ($P < 0.05$) higher levels of TG and lower levels of HDL compared to obese-diabetics. Insulin levels were higher in non-diabetic obese and obese-diabetics than lean-diabetics ($P < 0.05$).

This study provides evidence that lean diabetic patients show higher ferritin and MCHC levels than obese patients. The increase in serum ferritin and MCHC levels is related with altered iron metabolism at cellular level.

Keywords: 25-hydroxyvitamin D, Ferritin, Obesity, NIDDM.

Tuesday 6 September 12:30–14:30

Stem cells and cancer

P-05.03.3-001
The roles of FYCO1 and midbody degradation in regulating cancer and stem cell maintenance and differentiation

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At late mitosis, the mother cell divides, leaving two daughter cells connected by a thin intercellular bridge (ICB). During abscission of the ICB, the ingression of the cleavage furrow is formed, and the central spindle microtubules are compacted into the structure known as midbody (MB). The MB is situated within the ICB, with the abscission usually occurring at one side of the MB. As a result, only one daughter cell inherits the post-mitotic MB. These MBs can then either accumulate in the cytoplasm or be degraded.

Recent studies have identified MBs as novel signaling platforms regulating stem cell fate and proliferation. Indeed, stem cells as well as cancer cells were shown to accumulate post-mitotic MBs, resulting in reprogramming of the cell fate and conversion to highly-proliferative, stem cell-like phenotypes. It has been proposed that regulated macroautophagy may be playing a key role in mediating pots-mitotic MB degradation. Therefore, the experimental approach involved studying the dynamics and function of a protein known as FYCO1, which associates with post-mitotic MBs and may regulate their degradation.

In this study we identified FYCO1 as a protein, which associates with post-mitotic MBs and may regulate their degradation. Interestingly, FYCO1 is also known to be present on autophagosomes, and overexpression of FYCO1 can induce the formation of enlarged LC3-containing autophagocytic structures. Here we demonstrate that FYCO1 knock-down leads to defects in autophagic MB degradation, and that FYCO1 functions by targeting endocytic membranes to the autophagic phagophore during early stages of MB degradation. Additionally, we showed that FYCO1 depletion leads to increased proliferation and cell growth in soft agar.

Based on all these data, we hypothesize that FYCO1 mediates selective MBs degradation via endosome-dependent extension of the phagophore around the post-mitotic MBs, and that MBs may be the regulators of cancer proliferation and progression.

P-05.03.3-002
Proliferative effect of hypericine on human skin fibroblast cells and identification of the mechanism of action in molecular level

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Hypericine is traditionally used as a wound healing agent. Therefore it is a potential promoter for reprogramming induced pluripotent stem cells (IPSCs) which posses several problems due

to drawbacks associated with efficiency and viral genome integration. In order to improve reprogramming efficiency and compensate for viral transduction, new chemicals have been explored through IPSC research. The aim of this study was to investigate the proliferative effect of hypericine on human skin fibroblast cells (SF) in-vitro, and to identify the mechanism of action in molecular level.

The proliferation was measured using the Clonogenic and Dimethylthiazol Diphenyltetrazolium Bromide (MTT) assays. Real-time quantitative polymerase chain reaction (qRT-PCR) was performed to detect the mRNA levels of cyclins (D1 and B1) and cell cycle controller genes (p53 and p21).

SF cells were treated with different doses (1 nM–100 μM) of hypericine for 24 h and 48 h. A significant cell proliferation was observed in moderate concentrations (0.1–15 μM; %110–%134), but at high concentrations (25–50 μM) cytotoxic effects emerged in SF cells (IC50 = 23.62 M, $R^2 = 0.915$). qRT-PCR results revealed that the most proliferative dose of hypericine (15 μM) stimulates cyclin D1. The anti-proliferative activity of hypericine was accompanied by inhibition of cyclin B1 mRNA, whereas it induced expression of p53 and p21 genes, and thus apoptosis was observed by DNA laddering at the same dose (50 μM).

Overall results suggested that hypericine can compensate for viral transduction and improve reprogramming efficiency of IPSCs by enforcing them in G1 phase. Hence we report that hypericine can be a good candidate component for cocktails produced to trigger IPSC proliferation.

P-05.03.3-003
Nucleolin overexpression in glioblastoma stem-like cells enables targeted intracellular delivery and improves cytotoxicity

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Glioblastoma (GBM) is the deadliest brain tumor. The mean survival time of GBM patients is approximately 12 months, increasing to 14 months after treatment with temozolomide, which is the *gold standard* chemotherapy. The resistance of GBM to chemotherapy seems to be associated with the blood-brain barrier (BBB) that limits the delivery of chemotherapeutics, and the presence of a population of cells that expresses stem cell-like properties, which are known to be chemo- and radioresistant,5 the glioblastoma stem cells (GSCs). The difficulties imposed by these two factors could be reduced by the use of a targeted drug-delivery liposome-based strategy that allows BBB passage and reduces the side effects of chemotherapeutics.

The present study evaluated the ability of the F3 peptide-targeted pH-sensitive lipid-based nanoparticle containing doxorubicin (DXR) to target GSCs and non-GSCs. We evaluated the expression of cell-surface nucleolin by flow cytometry, as well as of stem cell-like markers, in two GBM cell lines. We also determined the ability of GBM cell lines to specifically uptake the F3 peptide-targeted pH-sensitive lipid-based nanoparticles, by flow cytometry, and correlated it with the expression of stem cell-like markers. Moreover, to ascertain the impact of intracellular delivery of chemotherapeutic drugs into GBM cell lines, cytotoxicity was further assessed by the MTT assay.

Our results showed that the F3 peptide-targeted pH-sensitive lipid-based nanoparticles successfully targeted glioblastoma cells and particularly GSCs. In addition, the results also provided evidence of the nucleolin overexpression-dependency of this strategy, emphasizing the need to adapt the therapeutic strategy to the individual patient.

This study showed that F3-targeted pH-sensitive liposomes may constitute an appropriate strategy to overcome the chemoresistance associated with glioblastoma cells.

P-05.03.3-004
Leukemic cell plasticiy as a resistance mechanism towards tyrosine kinase inhibitors

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Chronic myelogenous leukemia (CML) is a hematopoietic stem cell disease characterized by the *t*(9;22)(q34;q11) translocation, which encodes the chimeric tyrosine kinase onco-protein, Bcr-Abl. The tyrosine kinase inhibitor (TKI) imatinib is the first-line treatment for patients with CML. Unfortunately drug resistance is one of the main problems observed. While secondary resistance is associated with Bcr-Abl kinase domain mutations, oncogene amplification and mechanisms interfering with intra-cellular drug concentrations; primary resistance mechanisms haven't been elucidated. We generated high dose imatinib-resistant K562 subclones (K562-Ir) by clonal selection to study primary resistance mechanisms in vitro. Drug resistance was shown by caspase 3 and annexin V/PI assays. We also showed cellular uptake and function of imatinib with Western blot technics. K562-Ir cells are not only resistant to imatinib but also to 2nd, 3rd generation tyrosine kinase inhibitors. We demonstrated that K562-Ir cells have a highly adherent character, proliferate slowly and are resistant to drug-induced senescence. Microarray analysis revealed that K562-Ir cells differentially express tissue/organ developement and differentiation genes at high levels. We showed that K562-Ir cells forms intact tumor spheroids in 3D cell culture conditions which is a marker of tumor initiating potential. Cell surface maker analyses and protein analyses of K562-Ir cell population, points towards an epithelial-mesenchymal plastic cell capable of adopting different morphologies. We hypotizied that imatinib and other tyrosine kinase inhibitors may cause the gain of phenotypic plasticity potential in leukemic cells, by interfering with signalling pathways; which in itself may lead to therapy resistance.

P-05.03.3-005
In vitro hypoxia effect on cancer stem cells

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Hypoxia has multiple effects on cancer cells, which are critically involved in tumor progression. Hypoxia leads to changes in tumor cell metabolism and can promote cancer cell survival, invasion and metastasis by its critically important role on maintenance of cancer stem cell (CSC) phenotype.

In this research, human $CD133^+$ CSCs isolated from human osteosarcoma cell line SaOs-2 using MACS magnetic separation technique were characterized, and their stemness properties under hypoxic (1% O_2) and normoxic (21% O_2) conditions were compared in two and three dimensional culture conditions. Two different 3D culture techniques (nanofibrous bacterial cellulose scaffolds and scaffold free microtissues) were used to evaluate effects of hypoxia on CSC behavior, and the results were compared with the cell behavior in classical 2D culture systems. The morphologies of cells were examined by scanning electron microscopy (SEM); RT-PCR and immunocytochemistry staining were used to examine the cancer stem cell phenotype maintenance under hypoxic and normoxic conditions.

It is shown that hypoxia supports the expression of stemness markers such as *OCT3/4*, *Nanog* and *SOX2* compared to normoxic conditions in 3D cultures. Although similar effects of hypoxia were observed in 2D cultured CSCs, the expression levels of stem cell phenotype – indicative markers were significantly lower on 2D compared to 3D culture systems.

This study is seen as an introduction to develop a more relevant 3D hypoxic cancer stem cell based tumor model to study CSC behavior and tumor genesis *in vitro* for testing of novel cancer stem cell therapeutics and to understand signal transduction in cancer stem cells.

P-05.03.3-006
The flavonoid apigenin reduces survival and migration of CD44+ prostate cancer stem cells

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Prostate cancer (PCa) is the second most frequent cause of cancer-specific mortality in the world. Cancer stem cells (CSCs) are a subpopulation of cells that involved in drug resistance, metastasis and recurrence of cancers. The efficacy of natural flavanone apigenin on cell survival, apoptosis and migration of CSCs were evaluated. $CD44^+$ CSCs were isolated from human PCa PC3 cells using a magnetic-activated cell sorting system. PC3 and CSCs were treated with different concentrations of apigenin, docetaxel and combinations of the two agents for 48 h. Apigenin dose dependently inhibited CSCs and PC3 cell viability, and this was accompanied with a significant increase of the cell cycle inhibitors p21 and p27 (KIP1). The flavonoid significantly induced apoptosis via an extrinsic caspase-dependent pathway by upregulating the mRNA expressions of caspases-8, -3 and TNF-α, but failed to regulate the intrinsic pathway as determined by the Bax, cytochrome c and APAF-1 in CSCs. In contrast to CSCs, apigenin induced intrinsic apoptosis pathway as evidenced by the induction of Bax, cytochrome c and caspase-3 while caspase-8, TNF-α and Bcl-2 levels remained unchanged in PC3 cells. The ability of apigenin to inhibit the proliferation of CSCs through apoptosis was confirmed by Tali image-based cytometer. The flavanone strongly suppressed the migration rate of CSCs compared to untreated cells. Significant downregulation of MMP-2 and -9 exhibits the ability of apigenin treatment to suppress invasion. The expressions of PI3K/Akt and NF-κB p105/p50 were significantly decreased after 48 h apigenin treatment. Taken together, these data demonstrated that flavonoid apigenin is an invaluable chemopreventive compound that inhibits proliferation, invasion and the stemness properties of CSCs.

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P-05.03.3-007
Anti-proliferative and inducing apoptosis of the hydro alcholic Achelia. wilhelmsii extract on human breast adenocarcinoma cell lines MCF-7 and MDA-MB-468

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Introduction: Achelia Wilhelmsii containing different components such as Flavonoid, the previous study showed flavonoid has anti-cancer property. The aim of present study was to determine the anti-proliferative and inducing apoptosis potential of the hydroalcholic Achelia. wilhelmsii extract (HAWE) on MCF-7 and MDA-MB-468 human breast carcinoma cell lines.
Method and Materials: The anti-proliferative activity of the HAWE was evaluated by MTT, Flowcytometery by annexin V/PI double staining and caspase-3 activity.
Results: The results of MTT showed that ED50 of MCF-7 and MDA-MB-468 are 25 μg/ml of the HWAE after 48 h was treated. Flowcytometery by annexin V/PI showed that the HAWE induced late apoptosis in MCF-7 and early apoptosis in MDA-MB-468. In addition, the caspase-3 colorimetric method showed that caspase-3 had increased in the MDA-MB-468 after treatment by the HAWE.
Conclusion: This study found that the hydroalcholic extract of Achelia Wilhelmsii induced apoptosis in both of the MCF-7 and MDA-MB-468 human breast carcinoma cell lines.
Keywords: Acheli wilhelmsii, Breast Cancer, MTT assay, Apoptosis, Caspase-3.

P-05.03.3-008
Cellular modelling of Type IA PI3K dynamics in health and disease

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Mosaic activating mutations in PIK3CA, the gene encoding the catalytic p110a subunit of class IA phosphoinositide 3-kinase

(PI3K), are frequently found in patients with severe early-onset segmental overgrowth. Whilst differences in timing and location of the founder mutation are likely to explain part of the observed disease heterogeneity, it is less clear whether and how quantitative differences in the strength and timing of PI3K activity contribute to phenotypic variability.

Our aim is to characterise PIK3CA mutant-specific signalling as well as to explore the effects of varying the strength and/or temporal pattern of PI3K activation on downstream output specificity in the cell. We are currently employing CRISPR/Cas9-mediated gene editing in human induced pluripotent stem cells to generate isogenic disease models of three such activating PIK3CA mutations. These cells will be used for signalome profiling by reverse-phase protein arrays (RPPA) to compare and contrast mutant-dependent alterations to candidate signalling networks. In parallel, ongoing efforts focus on developing an endogenously expressed optogenetic p110a, allowing precise spatiotemporal control over PI3K signaling to unravel the extent to which PI3K-dependent phenotypes are determined by strength of activation and/or dynamic encoding.

Ultimately, the outcome of this research will yield novel insight into fundamental aspects of PI3K signalling and potentially aid the development of targeted therapies for human diseases of PI3K hyperactivation.

P-05.03.3-009
Resemblance of human cancer stem cells based on genome-wide expression profiles

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Cancer stem cells (CSC) have been proposed to be the cancer initiating cells. Because of their highly tumorigenic and drug resistant properties, CSCs offer significant potential for developing novel anticancer drugs and therapeutic strategies.

In the present study we analysed eight gene expression datasets for breast, ovarian, lung cancer and glioblastoma by comparing gene expression levels between stem cells and tumor cells and integrating them with genome scale biological networks. Consequently, mutual molecular signatures (i.e: differential expressed genes, transcription factor, miRNA) and biological characteristics were determined via integrative analyses, which might be feasible to uncover the mutual biological mechanism insights behind the CSCs.

It was identified twenty mutual differential expressed genes in four cancer types; JUN and KLF6 as transcription factors, EGFR and CDK14 as receptors come into prominence as mutual signatures. Molecules and pathways that were related to MAPK, WNT, p53 signaling and pathways in cancer were the common indicators in CSC types.

Our results provided similarities in gene expression profiles of various CSCs and gave clues about the seed of tumorigenesis.

This study proposed signatures and pathways that could be considered as effective therapeutic approaches in further experimental and clinical applications to eliminate subpopulation of CSC.

P-05.03.3-010
The detection of circulating tumor cells (CTCs) in patients with colorectal cancer by flow cytometry

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Colorectal cancer (CRC) is one of the leading causes of mortality worldwide. Metastasis is associated with the presence of circulating tumor cells (CTCs) in the peripheral blood of cancer patients. CTC cut-off values have been shown to predict for poorer overall survival in metastatic breast (≥5), prostate (≥5), and colorectal (≥3) cancer based on assessment of 7.5 ml of blood. In our study, CTCs were detected in blood samples of colorectal cancer patients, using with our modified convenient method for the strategies of CTC enrichment and detection. 7.5 mL peripheral blood samples were firstly collected and peripheral blood mononuclear cells (PBMCs) were isolated from the fresh blood samples by ficoll gradient separation. Next, the leukocytes in PBMCs were removed by magnetic microbeads conjugated with CD45 for a negative selection. Finally, the retained cells were labeled with anti-epithelial cell adhesion molecule (anti-EpCAM), cytokeratins (CK8, CK19) and the leukocyte-specific marker as anti-CD45. All samples were analyzed by BD FACS Aria III flow cytometry. In total, 10 patients and 7 healthy people were included in this study. The results showed that CTCs were not detected in the blood samples of healhty volunteers, but 3-13 CTCs were detected with CK14, 15, 16, 19-based gating strategy in the blood samples of colorectal cancer patients. It is accepted that the cut off value is 3 CTCs for colorectal cancer and CTC is negative if it is below this value or CTC is considered as a positive, if it is equal to or above this value, which might be an indication for poor prognosis. Thus CTC's detection may serve a representative surrogate tumor biomarker for real-time monitoring of disease status and tailoring personalized therapy.

Keywords: Circulating Tumor Cells, Colorectal Cancer, Flow Cytometry.

P-05.03.3-011
Effects of various electromagnetic field applications on the aggressiveness of breast cancer line

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Treatment of breast cancer is problematic disease and it can be lethal when untreated. Low frequency electromagnetic fields (EMF) are used as an adjuvant treatment by producing small electrical currents on biological tissues. They are a new technique in this sense and it is believed that they have inhibitory action on cancer cells. In this study we have applied two different signal types which are Pulsed Electromagnetic Fields (PEMF) and Pulsed Radiofrequency Energy (PRFE) to MCF-7 breast cancer

cell line and compared with MDA-MB-231 cell line which has aggressive character.

Cells were grown in culture flasks in a humidified incubator at 37 °C with 5% CO_2 and were used at the proliferation and confluent stages. Cultured cells were exposed to the PEMF and PRFE. The proliferations of the cells are measured by MTT assay for the effect of EMF on the cancer cells. On the other hand the wound healing was investigated by closure of the wound by the cell proliferation with cell morphology using inverted microscope images.

The proliferation decreased significantly by the effect of PEMF on the semi confluent MCF-7 and MDA-MB-231 cells. This effect was observed more prominent on MCF-7. Considering PRFE therapy this effect is much more pronounced especially for MDA-MB-231 comparing with PEMF. The phase contrast observations of these results were consistent with MTT analyses. Similarly, this effect was seen less for PEMF but the proliferation was more suppressed with PRFE on the wound models.

It was considered that the EMF applications could be effective in cancer cells, but this effect has not been studied how it occurs in invasive cancers. In our cell culture study, the appropriate EMF applications were found to be effective though the inhibition of proliferation of cancer cells even in invasive cancer but with lower effect. This means that EMF applications may support the existing treatment methods of cancer patients and even people who suffer from invasive cancer.

P-05.03.3-012
Circulating tumor cells (CTCs) in peripheral blood: liquid biopsy for cancer patients

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Metastasis is the one of the most known causes of death in patients diagnosed with cancer. Circulating tumor cells (CTCs) are shed from primary tumors and circulating in the bloodstream, and thought to play a key role in metastasis. A hypothesis that CTCs may contribute to metastasis was first introduced in the mid 19th century by Thomas Ashworth, an Austrilian pathologist. In today's research, identification and molecular characterization of CTCs are thought to be a novel target for treatment of cancer and a key factor to understand the metastatic process. Existing methods of CTC capture based on the Cell Search system, flow cytometers, laser scanning cytometers instruments, fiber-optic array scanning technology (FAST), isolation by size of epithelial tumor cells (ISET), and definition fluorescence scanning microscopy. CTCs are increasingly considered as a 'liquid biopsy' and when liquid biopsy is compared to tumor tissue biopsy, liquid biopsy for CTCs detection can be carried out routinely in patients due to accessibility and ease of blood collection. Also, primary tumor sampling may not reflect the actual metastatic conditions, CTCs are thought to be a novel tumor biomarker for real-time monitoring of disease status and tailoring personalized therapy. With futher works, CTCs may be used as liquid biopsies and it might provide better understanding metastatic process, new approaches in cancer diagnostics and treatment.

Keywords: Circulating Tumor Cells, Cancer, Flow Cytometry.

P-05.03.3-013
Glucose transporter proteins in the human amnion derived mesenchymal stem cells

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Mesenchymal stem cells (MSCs) are distributed all over the organism as a source of tissue formation and regeneration. Glucose is vital for the proliferation and differentiation of MSCs. Glucose uptake is mediated by specific glucose transporters of two families, the Na-coupled glucose transporters (SGLT) and glucose transporter facilitators (GLUT). The presence and function of GLUT proteins in human placental amnion derived MSCs (hAMSCs) is unknown. We aimed to investigate the presence of GLUT1, GLUT3, GLUT4 proteins and genes in hAMSCs isolated from term placentas.

MSCs were isolated from human term placenta amniotic membrane, the characterization of cells were provided by flow cytometry. MSCs were used to assess their chondrogenic, osteogenic and adipogenic differentiation potential. The expression of GLUT1, GLUT3 and GLUT4 proteins was detected in hAMSCs by immunofluorescence. GLUT1, GLUT3, GLUT4 protein and gene expression in these cells were investigated by Western blot and real-time PCR, respectively.

Flow cytometry analysis results of isolated cells showed that they were positive for CD44, CD90, CD73, CD105 (mesenchymal stem cell markers) and hematopoietic markers CD34, CD11b, CD19, CD44 and HLA-DR were negative. The presence of GLUT1, GLUT3, GLUT4 proteins and genes were identified in hAMSCs.

In this study, for the first time in literature, GLUT1, GLUT3 and GLUT4 gene and protein presence was determined in hAMSCs. Therefore, GLUTs could mediate glucose transport in human amniotic membrane MSCs. Proliferation and differentiation of MSCs in vitro are still not optimized. Further studies are required to clarify the complex mechanisms regulating the relationship between glucose and mesenchymal stem cells. Disclosure of this relationship may provide a better understanding of glucose-related pathologies such as diabetes.

P-05.03.3-014
Trans-dichloridoplatinum (II) complex induces apoptosis in cancer stem-cell enriched mammospheres

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Tumors have hierarchically organized heterogeneous cell populations and a small subpopulation of cells, termed cancer stem cells (CSCs), is responsible for tumor initiation, maintenance as well as drug resistance. Therefore, killing the CSCs along with the other cancer cells is gaining an importance. In the present study, it was aimed to evaluate the cytotoxic and apoptotic activity of a novel Platin (Pt) (II) complex $[Pt(Hepy)_2Cl_2]$ on mammospheres obtained from MCF-7 human breast cancer line.

Elevated expression of stemness markers were determined by western blotting. Cytotoxicity was assessed using the ATP viability assay. Effect of the Pt (II) complex on the formation and development of mammospheres was analyzed with sphere formation (SFA) assay. Apoptosis was determined via cytofluorimetric analysis (caspase 3/7 activity, annexin-V-FITC and Bcl-2 activity) as well as gene expression analysis. Cytotoxicity was confirmed with the ATP viability assay after the treatment with zVAD-fmk (an apoptosis inhibitor) and necrostatin (a necroptosis inhibitor). In addition, alterations in mitochondrial membrane potential were evaluated by JC-1 staining.

Mammospheres exhibited increased Oct-4 and Sox2 (stemness markers) expressions compared to parental MCF-7 cells. Cytotoxicity by Pt (II) complex was evident in a dose-dependent fashion (1.56–100 μM). Pt (II) complex significantly prevented mammosphere formation and disrupted mammosphere structure in a dose-dependent manner. Pt (II)-induced apoptosis was determined based on the presence of caspase 3/7 activity, annexin-V-FITC positivity and Bcl-2 inactivation. Apoptosis was also confirmed with increased TNFRSF10A and HRK gene expressions. In addition to apoptosis, necroptosis was also present as evidenced with increased MLKL expression. Mitochondrial membrane was depolarized.

In conclusion, the Pt (II) complex seems to be a powerful apoptosis-inducing compound on cancer stem cells, thereby warrants further in vivo experiments.

P-05.03.3-015
Circulating tumor cells as liquid biopsy in larynx cancer

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Cancer is a disease which arises from destruction of growth and proliferation mechanisms in cells and is the second leading cause of death worldwide [1]. In the development of primary cancers, the head and neck cancer is accounting for approximately 550.000 new cases annually around the world [2]. Laryngeal cancer is a type of head and neck cancer in which malignant cells arise from the mucosal tissues of the larynx [3]. Cancer might spread from primary tumor by getting into the lymph and blood vessel system and forms secondary tumor. Greater than 90% of deaths in cancer patients are attributed to metastasis [4]. Circulating tumor cells (CTC's) provide an opportunity to understand the metastatic process of cancer patients. Identification and molecular characterization of CTC's in the peripheral blood of cancer patients is a promising research area in the field of biomarker development and novel treatment targeting in today's cancer research [5]. The detection of CTC methods include Cell Search system, flow cytometry, high-definition fluorescence scanning microscopy, fiber-optic array scanning technology, isolation by size of epithelial tumor cells, and laser scanning cytometers [6]. In our study, 7.5 ml of peripheral blood samples were collected from larynx cancer patients and healthy volunteers and the samples were analyzed by BD FACS Aria III flow cytometry via biomarkers EpCAM, CK19, CK8 for positive selection and CD45 for negative selection [7]. According to the results of our study; CTCs were detected in larynx cancer patients by our newly modified method whereas there was no CTC's detection in the samples of controls. Thus, this study may provide us monitoring of the treatment process of larynx cancer and this method might be used as diagnostic, prognostic, and predictive biomarkers in cancer therapy as a liquid biopsy.

Keywords: Circulating Tumor Cells, Larynx Cancer, Flow Cytometry.

P-05.03.3-016
The detection of circulating tumor cells in the peripheral blood samples of prostate cancer patients by flow cytometry before and after surgery

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Prostate cancer is the second most common cancer and the fifth leading cause of death from cancer in men[1]. Circulating tumor cells (CTCs) present in the peripheral circulation of cancer patients with different solid malignancies including prostate cancer and have a potential as a liquid biopsy to monitor disease progression and response to therapies at cell and molecular level[2]. One of the general methods in CTC detection is flow cytometry[3]. *Radical prostatectomy* is the most frequently applied procedure in the surgical management of localized prostate cancer. In this surgical operation, the surgeon removes the entire prostate gland with the seminal vesicles. A radical prostatectomy procedure can be done using the *da Vinci* robotic system (Intuitive Surgical, Sunnyvale, CA, USA)[4]. Robotic surgery has been suggested to have fewer complications, lower risk of infections and shorter recovery period following robotic radical prostatectomy[5,6]. In this study, our aim was to detect CTCs before and after robotic radical prostatectomy in clinical localized prostate cancer patients. The CTC detection study was performed with our modified method in which 7.5 ml of peripheral blood samples were collected from each prostate cancer patient and healthy individual; the samples, using with biomarkers EpCAM, CK19, CK8 for positive selection and CD45 for negative selection, were analyzed by BD FACS Aria III flow cytometry[7]. According to our results, we detected CTCs in the peripheral blood samples of prostate cancer patients before robotic radical prostatectomy. However, following this surgical procedure no CTC or decreased number of CTSs was detected. Our study might contribute to understand disease progression after robotic radical prostatectomy in clinically localized prostate cancer patients that warrants further research.

Keywords: Circulating Tumor Cells, Prostate Cancer, Flow Cytometry, Robotic Radical Prostatectomy.

P-05.03.3-017
Determination of effect cytotoxic, apoptotic, caspace-3 activity and mRNA expression levels of apoptototic related genes of vulpinic acid on breast cancer cell lines

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Breast cancer is the most common cancer types in women. Several drugs used to treat breast cancer patients are developing resistance to the treatment for this reason success rate falls. Therefore the discovery of alternative therapeutic agent and molecular detection of anticarcinogen effect because of treatment for cancer patients may be a source of hope for the contributions.

In this study, different concentrations (1.562, 3.125, 6.25, 12.5, 25, 50, 100 μM) vulpinic acid (VA) lichen seconder metabolite was determined to cytotoxic, apoptotic effect and caspase-3 activity in breast cancer cells (MDA-MB-231, MCF7, BT-474, SK-BR3) and normal cell (MCF12A). In addition to the quantitative real-time PCR (qRT-PCR) using apoptose specific primers (TP-53, Bcl-2, Bax, BIRC-5, GAPDH, Caspase-3, Caspase-7, Caspase-8, Caspase-9) and SYBR green dye were performed to determine expression patterns of transcript level in cancer cell lines, using *GAPDH* as a reference gene. The antiproliferative characterization of VA effects identification of the gene set at molecular level and we determination role of VA on apoptotic pathway.

According to our study, VA is demonstrated significantly ($P < 0.05$) effect cytotoxic, apoptotic, caspase-3 activity. Beside this, dose dependent expression patterns decreased apoptose spesific genes (except of Bcl-2) mRNA levels from six to eleven fold change more than untreated VA cell lines.

VA will be used as candidate molecule for effective treatment on breast cancer in the future.

P-05.03.3-018
Role of paucimannosidic glycoepitopes in tumorigenic processes

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Glycosylation largely determines the variety and functions of proteins. Paucimannose, a mannosidic N-glycoepitope has long been thought to be specific for plants and invertebrates. Recently, it has also been detected in mammals – in physiological conditions (stem cells) and in pathophysiological conditions (inflammation and cancer). In glioblastoma cells, paucimannose also seems to play a role in cell proliferation.

Glioblastoma is the most frequent brain tumor in adults with poor prognosis due to a lack of suitable treatments. We hypothesize that paucimannose could be a promising new biomarker as it is otherwise rarely found in mammals. Therefore, paucimannose levels were investigated in different glioblastoma cell lines differing in their proliferation rate and tumorigenicity. The highest paucimannose levels were detected in low proliferating, non-tumorigenic cells. Furthermore, we found that modulation of paucimannose function by application of a specific antibody regulated cell proliferation and the capability of cells to form colonies in soft agar. These data support a functional role of paucimannosidic epitopes in tumorigenic processes.

P-05.03.3-019
TNFRSF21 and TNFSF7 are possible markers to target CD133+ GBM stem cells

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Glioblastoma Multiforme (GBM) is the most lethal type of malignant brain tumors. Recently, GBM stem cells (GSCs) have been studied in great deal and accepted that they have a legitimate role in tumor formation, development, chemo-resistance and recurrence. In this study, it is aimed to investigate new therapeutic targets within apoptosis related molecules to select and eliminate CD133+ GSCs effectively.

Ten primary GBM cells were isolated from GBM tissue samples and they were cultured among with the 4 GBM cell lines (U87, U138, U118 and T98). CD133+ and CD133− cells were seperated by MACS method via anti-CD133 (AC133) antibody from cultured cells and cell lines. RNA isolation from CD133+ and (−) cells, cDNA synthesis was performed. Finally, by performing PCR array, mRNA expression levels of 88 genes were detected. Proper results were collected and analysed statistically.

According to the results of PCR array; it has been found that CD133+ cells express approximately 223 fold TNFRSF21 and 20 fold TNFSF7 when they are compared with control cells.

TNFSF7 binds to CD27 that is expressed on the surface of T-cells. CD27 does not have a death domain, instead it has a cytoplasmic tail which binds to TRAFs. TRAFs act as adaptor molecules that are related with JNK and NF-κB signalling pathways.

TNFRSF21 (DR6) is a death receptor which are known for transmitting the pro-apoptotic signals from outside to the inside of the cell. It negatively regulates T-cell activation and the release of few cytokines.

As a conclusion, TNFSF7 and TNFRSF21 both are found on immune system cells, mostly on T-cells, which may mean that GBM stem cells act as a immune system cells to avoid the elimination by the immune system. To conclude, acting as an immune system cell and promoting survival via TNFSF7 and TNFRSF21, these molecules may be essential markers to target CD133+ GBM stem cells.

P-05.03.3-020
The effect of docetaxel on P53, Sin3A and MDM2 gene expression in MCF-7 breast cell line

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Docetaxel is a cytotoxin effective in treating breast cancer. It stabilizes microtubules and causes catastrophic cell cycle arrest in G2/M. It also initiate signaling through cell death pathways that result in programmed cell death. In this study, it was aimed to investigate apoptotic and cytotoxic effects of docetaxel has on the MCF-7 breast cancer cells line. In this study, MCF-7 breast cell line was applied different doses docetaxel (10 nM, 100 nM, 1 μM, 10 μM, 100 μM) as 24 h and 48 h. MTT analysis was performed to the MCF-7 breast cancer line in control group and groups of docetaxel. Afterwards, evaluation of apoptosis by

TUNEL and levels of P53, sin3A and MDM2 gene expression by Real-Time PCR were determined in an order. It was observed cell variable was significant lower in docetaxel groups compared to control group ($P < 0.05$) in 24 h as MTT analysis. The lowest cell viabilty was determinated in group applied 100 μM docetaxel. While the lowest positive cell density was determinated in control group, it was observed apoptotic cell density gradually increased with increasing docetaxel concentration in groups treated docetaxel ($P < 0.05$). The highest P53, Si3A and MDM2 expressions were apperared in 100 nM docetaxel group compared to control group.

P-05.03.3-021
Influence of the surface charge on intracellular trafficking pathways and cytotoxic effects of alpha-fetoprotein receptor binding domain conjugate with PAMAM dendrimers in tumor cells

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Human alpha-fetoprotein (AFP) and AFP receptor binding domain (AFPRbd) are able to bind and internalize effectively by wide range of human tumor cells and tissues. As other vector molecules AFPRbd has insufficient quantity of chemical groups which can be conjugated with drugs or diagnostic agents. Conjugation of vector molecules with macromolecular polymer carriers like dendrimers aims to solve this problem. Our study describes influence of AFPRbd-dendrimer-doxorubicin conjugate surface charge on intracellular trafficking routes and toxicity. The amine-terminated (G2) and acetyl-terminated ($G2_{12}$) 2nd generation PAMAM dendrimers carrying doxorubicin (Dox) were used to synthesize conjugates with AFPRbd. Unmodified by AFPRbd G2 and $G2_{12}$ dendrimer derivates labeled with Dox were absorbed by the cells at 37 °C with different efficiency. $G2_{12}$-Dox derivate characterized much slower internalization rate than non-acetylated G2-Dox. Only $G2_{12}$-Dox shown partial colocalization with lysosomal marker LAMP2 after 4 h of incubation. Internalization of AFPRbd-G2-Dox and AFPRbd-$G2_{12}$-Dox did not show significant difference. At the same time, both conjugates contained AFPRbd wyкy almost fully associated with LAMP2 already after 30 min of incubation. Cytotoxicity results revealed that IC50 levels of $G2_{12}$-Dox and AFPRbd-$G2_{12}$-Dox coincided and demonstrated a bit higher activity against sensitive to Dox SKOV3 and resistant SKVLB cells than AFPRbd-G2-Dox conjugate after 72 h of incubation. At the same time, after 1 h of incubation AFPRbd-G2-Dox and AFPRbd-$G2_{12}$-Dox were much more than $G2_{12}$-Dox and G2-Dox. We may conclude that there is significant difference in ways of dendrimers internalization by tumor cells depending on nature of surface chemical groups. On the other hand, chemical modification of dendrimer conjugated with does not AFPRbd influence dramatically on the protein trafficking and resulting cytotoxic effect. Russian Scientific Foundation supported this study (No. 15-15-10013).

P-05.03.3-022
A new biosensor for rapid determining of breast cancer

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Introduction: Pyruvate kinase (EC 2.7.1.40), a key enzyme in glycolysis, catalyzes conversion of phosphoenolpyruvate (PEP) into pyruvate with regeneration of adenosine triphosphate (ATP). The key regulator of the metabolic alterations found in tumor cells is the glycolytic isoenzyme pyruvate kinase type M2 that is generally expressed in all proliferating cells and overexpressed in all tumor cells investigated to date. During carcinogenesis a shift in the pyruvate kinase isoenzyme equipment always takes place, such that the tissue-specific isoenzymes disappear, and M2-PK is expressed.

Breast carcinoma, the third most common cancer worldwide, accounts for the highest morbidity and mortality. Breast cancer tissue analysis confirmed the upregulation of M2-PK in breast cancer, and high M2-PK levels were associated with poor prognosis of breast cancer patients.

Materials and Methods: Poly HEMA (MAC) nanoploymers were immobilized by binding covalently with sulphur atoms on the gold electrod's surface. Pyruvate immobilization was actualysed with cross linking reagent glutaraldehyde. Biosensor was developed by preparing pottasiumferrociyanide, selected as a mediator.

Results: Cyclic voltammograms have been carried out at between ~ 0.4 and 0.6 V potentials vs. Ag/AgCI. M2-PK activty was detected by using differential pulse method at between 0.3 and −0.25 V potentials by observing the differentiations in the current values. In the optimization studies, some parameters such as optimum pH, temperature, concentration of glutaraldehyde and p-HEMA-MAC, were investigated.

Discussion and Conclusion: The method developed for the measurement of the Tumor M2-PK activity by using biosensor. We found that more advantageous in comparison to other methods reported in the literature so far; it was determined that the method is sensitive, economic, practical and less time-consuming. Piruvat kinase Tumor M2-PK activity determination at low concentrations is possible with this method.

P-05.03.3-023
Tie2/TEK: a potential biomarker for targeting glioblastoma stem cells

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Glioblastoma multiforme (GBM) is the most prevalent and most malignant of the glial tumors. GBM is still incurable despite the current therapies. Recently cancer stem cells in charge of tumor aggressiveness and resistance to chemotherapy in various cancers including GBM have been tried to target considered one of the key determinants tumor relapse, metastasis, angiogenesis. Angiopoietin-1 receptor also known as CD202B is encoded by the TIE2/TEK. Tyrosine-protein kinase is cell-surface receptor for ANGPT1, ANGPT2 and ANGPT4. TIE2/TEK plays a key

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role in angiogenesis, endothelial cell survival, proliferation, migration and adhesion. Therefore, Tie2/TEK could be a potential target for therapeutic strategies directed against Glioblastoma stem cells and their microenvironment.

In this study, we investigated the gene expression levels of TIE2/TEK in both CD133+ GSCs and CD133− GBM cells.

GBM primary cells were freshly isolated from glioblastoma tissue samples and cultured in DMEM supplemented with 10% fetal calf serum and 1% penicillin-streptomycin at 37 °C in 5% CO_2-humidified incubator. We isolated CD133+ and CD133− cells from 10 GBM primary cells using MACS system. Following RNA isolation from healthy brain tissues, CD133− and CD133+ cells, cDNA synthesis was performed. Finally, according to microarray protocol, cell surface marker panel array was applied. Expression levels were analyzed using the delta delta Ct method. Statistical analysis was performed using SPSS software for windows version 13.0.

Tie2/TEK gene expression was determined as 50.07 fold higher in CD133+ GSCs than normal brain tissue ($P < 0.05$). Morever it was determined 7.52 fold higher compared to normal brain tissue in CD133− ($P < 0.05$). According to our results Tie2/TEK expression was higher in GSCs, indicating that Tie2/TEK may be a potential marker for targeting cancer stem cells in GBM.

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P-05.03.3-024
Adenosine inhibited the breast cancer stem-like cell population through ERK1/2 pathway

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Cancer Stem Cells (CSCs) are immortal tumor-initiating cells that can self-renew and drive tumorigenesis in various cancers, including breast cancer and others solid cancers. In a study indicated that Extracellular ATP reduces tumor sphere growth and cancer stem cell population. But At present, there are no reports available in literature on the effect of adenosine on breast cancer stem cells. In this study we evaluated the effect of adenosine inhibition and its mechanism of action in breast cancer stem cells isolated from breast cancer cell lines. Our result showed that adenosine significant reduces breast cancer stem cell population. Reduction of ERK1/2 protein levels was also observed after treatment cancer cells with adenosine. In conclusion, our results indicate that adenosine decreases the breast cancer stem-like cell population through ERK1/2 pathway.

P-05.03.3-026
Evaluation of taxane-resistance in prostate cancer stem cells

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Taxanes are commonly used for the treatment of many cancers as chemotherapeutic drugs that resistance to these agents has become a major clinical obstacle. Taxane based chemotherapy drugs such as paclitaxel, docetaxel and cabazitaxel bind microtubules and inhibit to microtubule polymerization appear to stimulate programmed cell death. Taxane-resistance to cancer has not been clearly in progression and development of drug resistance. Multiple mechanisms are involved in the drug efflux proteins as multidrug resistance protein, differences in amino acid sequences among the β-tubulin isotypes. We investigated taxane resistance with different doses of paclitaxel, docetaxel and cabazitaxel in prostate cancer stem cells.

We compared the expression level of apoptotic proteins, and its functional role in resistance mechanisms in $CD44^+/CD133^+$ prostate cancer cell lines. Taxane drugs were categorized as concentration-dependent or time-dependent. Cabazitaxel caused a time-dependent and dose-dependent reduction in cell viability in all tested cell lines. Resistance activity was consistently higher in docetaxel in prostate cancer cells compared with the other drugs. There are many different response of clonogenic formation $CD44^+/CD133^+$ cells with resistance to docetaxel, paclitaxel and cabazitaxel in prostate cancer stem cells.

The innate of prostate cancer resistances are important characterization steps and critically limits treatment outcomes therefore novel drugs must be focus on antiresistance and molecular based combinations.

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P-05.03.3-027
Proliferation in the human amnion derived mesenchymal stem cells under hyperglycemic conditions

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Mesenchymal Stem Cells (MSCs) are self-renewing cells with ability to differentiate into organized, functional network of cells. MSCs isolated from various tissues including adipose tissues, bone marrow, umbilical cord, placenta and pancreas have different differentiation and proliferation potential. Good knowledge of the metabolism and proliferation mechanisms of stem cells is required for stem cell therapies. Glucose is an important molecule in the culture of stem cells. Glucose concentration affects the differentiation and proliferation potential of stem cells. The aim of the study was to investigate the proliferation status by identifying the proliferating cell nuclear antigen (PCNA) expression under normoglycemic and hyperglycemic conditions in MSCs.

MSCs were isolated from human term placenta amniotic membrane. Characterization of the isolated cells was performed using flow cytometry. Chondrogenic, osteogenic and adipogenic differentiation potential of these cells were investigated. Characterized cells were cultured in normoglycemic and hyperglycemic conditions for 24 and 48 h and the expression of PCNA protein expression in these cells were investigated by Western blot.

Flow cytometry analysis showed that isolated cells were positive with mesenchymal stem cell markers CD44, CD90, CD73, CD105 and negative with hematopoietic markers CD34, CD11b, CD19, CD44 and HLA-DR. Western blot result of PCNA protein expression statistically significantly increased in human amniotic membrane MSCs under hyperglycemic conditions for 24 and 48 h culture.

The glucose content of stem cell medium is important because glucose is an effective molecule of the proliferation of stem cells. Proliferation of MSCs in vitro are still not optimized. When the relationship between glucose and stem cells be understood, it will provide a better understanding for the glucose-related pathologies such as diabetes during pregnancy.

P-05.03.3-028
A preliminary biosensor system for sarcosine determination in urine

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Prostate cancer (PCa) is the second most common type of cancer among men in the World. It is revealed that some gene, protein and metabolite sets control the PCa, however the whole metabolomics changes are not completely understood yet. PCa is common among older men, and this is an important health problem in developed countries.

Sarcosine is the N-methyl derivative of the glycine amino acid. Glycine N-methyl transferase produces sarcosine from glycine. Besides, it is metabolized to glycine by sarcosine dehydrogenase. In 2009, high level of sarcosine in urine was associated with PCa by Sreekumar et al. They identified sarcosine as a PCa biomarker that was significantly increased in urine during prostate cancer progression to metastasis. Following this study, several studies have been published indicating sarcosine as a PCa biomarker.

In our study, a preliminary biosensor system was fabricated for determination of sarcosine in urine by using sarcosine oxidase. Sarcosine oxidase was immobilized on Au electrode surface using gelatin as an immobilization matrix. Glutaraldehyde was used as a cross-linking agent to avoid the loss of the enzyme-gelatin mixture. Optimization and characterization studies were carried out. Sarcosine concentrations were detected carefully with the developed biosensor system. The fabricated preliminary biosensor is a promising system that can allow lower detection limits after surface modifications.

P-05.03.3-029
6-Phosphofructo-2-kinase/fructose 2,6-bisphosphatase-3 regulates the epithelial-mesenchymal transition in tumor cells

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Activation of the epithelial-mesenchymal transition (EMT) program in tumor cells is associated with invasiveness and stemness. Recent studies implicate EMT-inducing molecules in reprogramming energy metabolism. The 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 (PFKFB3) regulates glycolysis by producing fructose 2,6-bisphosphate (F2,6BP). Given that PFKFB3 is induced by several established EMT-inducers in tumor cells, e.g. HIF-1α and Ras, we hypothesized that PFKFB3 may be involved in regulation of the EMT in tumor cells.

Silencing of PFKFB3 in pancreatic adenocarcinoma cell lines PANC1 and S2VP10 was achieved using specific siRNA molecules. mRNA and protein expressions of the CDH1 gene (encoding E-cadherin, an established epithelial marker), as well as ZEB1 and SNAI1 genes, by Real-time quantitative (q)-PCR and Western blot, respectively. Immunfluorescence analysis was performed to visualize E-cadherin protein expression on plasma membrane. In order to test the effect of PFKFB3 on the invasive ability of the cells, a Matrigel invasion assay was performed. Ectopic expression of ZEB1 was achived by transfecting cells with a plasmid carrying ZEB1 cDNA.

Cells that were depleted of PFKFB3 exhibited markedly increased CDH1 mRNA and E-cadherin protein expressions and reduced SNAI1 and ZEB1 mRNA expressions. Immunfluorescence analysis confirmed the upregulation of the E-cadherin protein on plasma membrane. Silencing of PFKFB3 caused approximately 40% reduction in matrigel invasion, compared to non-targeting siRNA. Inhibition of the matrigel invasion caused by PFKFB3 depletion does not appear to be associated with reduced ZEB1 expression, as ectopic expression of ZEB1 did not reverse the effect of PFKFB3 silencing on invasion.

Taken together, these data suggest that PFKFB3 may be required for the maintenance of the mesenchymal phenotype and associated traits in pancreatic adenocarcinoma cell lines.

P-05.03.3-030
Haematological status of patients suffering from chronic leucosis

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Introduction: Leukemias are neoplasms that arise from hematopoietic cells initially proliferate in the bone marrow, and then disseminated in the peripheral blood, spleen, lymph nodes and eventually to other tissues. Lymphomas occur primarily in the lymph nodes, but can be extended in peripheral blood and bone marrow infiltrate.
Aim: To determine the values of haematological parameters the control and test groups. To determine the prevalence of types of chronic leukemia in relation to the experimental group. Compare haematological parameters in relation to the type of chronic leukemia.
Materials and Methods: A prospective-retrospective study included subjects who were made laboratory hematology in OJ Clinical chemistry and biochemistry UKCS. Blood tests conducted on the hematology analyzer Siemens ADVIA 2120 Hematology System and Abbott Cell Dyn 3700 and microscopic analysis of the peripheral blood smear.
Results and Discussion: According to the age of respondents test group was established mild form of anemia, a red blood cell count is totaled $3.69 \pm 1.13x10^{12}$, which is signifycantly lower compared to the control group. The average number of leukocytes was significantly higher in subjects studied groups and amounted to $136.91x10^9$, with a maximum value of 580 $x10^9$. In the peripheral blood of patients with chronic leucosis has established a significantly higher number of cells compared to the control group ($P = 0.001$), while the number of monocytes was a significantly smaller. In the group of patients with chronic leukosis largest number had chronic lymphocytic leukemia (70%), and chronic myeloid leukemia had 30% of respondents.
Conclusion: Subjects with CLL were statistically older than patients with CML, and as regards the gender structure, men have dominated in CLL and CML in women. White bloodline was found that the number of leukocytes in both forms of chronic leukemia high above the reference value.

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P-05.03.3-032
Effect of enzymatic and non-enzymatic isolation methods of endometrial stem cells on their cell proliferative potential and mesenchymal stem cell characteristics

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Human endometrial stem cells (hESCs) are responsible for the monthly renewal of the basal layer of the human endometrium by facilitating stromal and vascular regeneration. In this study, hESCs were isolated with three different isolation methods including non-enzymatic and enzymatic digestion using trypsin and collagenase type 1. The effect of these three isolation methods on the acquisition of mesenchymal stem cells (MSC) and on hESC proliferative potential was evaluated through flow cytometric analysis of CD surface markers and WST-1 tetrazolium salt assay.

Our findings indicate that hESCs isolated with these three methods have statistically similar cell proliferation rate at 24 h time point. However, at 48 h time point, hESCs isolated with the non-enzymatic and collagenase type 1 method displayed a higher expansion in cell number when compared to the hESCs isolated with trypsin. The late passage of hESCs isolated with non-enzymatic and trypsin methods showed the highest proliferation rate in comparison to the hESCs obtained via collagenase type 1 isolation method at 24 h, 48 h and 72 h. The three isolation methods for the early passages of hESCs had a resemblance in their MSC profile with no significant difference. On the other hand, late passage hESCs isolated using trypsin non-enzymatic method showed a higher CD31and lower CD44 profile. Moreover, late passage of hESCs isolated with non-enzymatic method displayed a significant reduction in their cell surface CD90, CD73, and CD105 surface expression levels. Only hESCs isolated with collagenase type 1 did not present a significant shift in their mesenchymal CD marker profile from early to late passages,

Taken together results from this study suggest that the long-term maintenance of mesenchymal markers can only be achieved in cell isolation with collagenase type 1, while non-enzymatic method is more suitable to obtain higher MSC cell yield for immediate use.

P-05.03.3-033
HGF/c-met signaling controls cell proliferation, invasion and branching morhphogenesis in EpCAM+/CD133+ hepatic stem cells in HCC cell line, HuH-7

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Hepatocellular carcinoma (HCC) abundantly arises on the viral and/or chemical-induced cirrhosis in liver. Cirrhosis is defined as one of the premalignant stage HCC in which microenvironmental changes occurred such as uncontrolled production of collagen type I and activation of hepatocyte growth factor (HGF)/c-met signaling. It has been shown that EpCAM+/CD133+ subpopulation of cells isolated from HCC tissue can initiate tumor at very low concentration in xenograft model and behaves as hepatic cancer stem cells. However, the molecular mechanisms supporting hepatic stem cell activation are not well understood and knowledge about the role of HGF/c-Met pathway in this process is not clear. In this study, we aimed to define effect of collagen type I and HGF induction on the cell behaviours of EpCAM+/CD133.

EpCAM+/CD133+ cells were sorted by magnetic separation from HuH-7 cells. Then proliferation and invasion of cells were analyzed under the HGF induction as well as branching morphogenesis in vitro. After HGF stimulation, phosphorylation level of c-Met increased in EpCAM+/CD133+ subpopulation. Moreover, presence of collagen type I enhanced significantly effect of HGF stimulation in the invasion of EpCAM+/CD133+ cells. We also have showed that HGF stimulation increased branching tubulogenesis capacity of EpCAM+/CD133+ subpopulation while it did not effect proliferation of cells. These effects of HGF reverted by c-Met inhibitor, SU11274, in vitro. All these findings showed that HGF and collagen type I regulates aggressive phenotype as microenvironmental changes via induction of invasiveness of EpCAM+/CD133+ subpopulation of HuH-7.

In conclusion, we showed that HGF/c-Met signaling causes to get more metastatic phenotype based on invasion and tubulogenesis in EpCAM+/CD133+ hepatic cancer stem cells in HCC and it might be possible to use c-Met inhibitors to target hepatic cancer stem cells during hepatocarcinogenesis.

P-05.03.3-034
Does tissue transglutaminase have a role in the development of endometriosis?

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Endometriosis is defined by the migration of endometrial mesenchymal stem cells (eMSCs) into the peritoneal cavity or other site of body rather than uterus in a retrograde fashion. Its previously known intracellular crosslinking enzyme called tissue transglutaminase (TG2) was shown to play important roles in the extracellular matrix (ECM) modelling, fibrosis, cell adhesion and migration. We have hypothesized that TG2 might be expressed in eMSCs and take part in the formation of endometriosis.

The difference in the proliferation capacity of eMSC isolated from endometrial tissue with/without endometriosis was determined using WST-1 assay and TG2 activity and expression levels were analysed by BTC assay and RT-PCR. The biosynthesis and activity for MMP-2 and -9 were investigated with zymography and RT-PCR, respectively.

Although TG2 activity was found to be 50% less in eMSCs isolated from endometriotic tissue, these cells showed 9 times higher TG2 protein expression than those isolated from the control tissue without endometriosis. eMSCs from endometriotic tissue have 2.3 times higher TG2 and 10.3 fold higher ITGB1 mRNA levels when compared to the cells of healthy group. Similar results were observed in SDC-4 gene expression with a 2.5-fold increase. Endometriotic eMSCs demonstrated an average of 11.5-fold increase in the MMP-2 activity while a onefold increase was evident in MMP-9 activity when compared to the healthy eMSCs. eMSCs from patient group possessed a higher proliferative ability in comparison to that of healthy subjects within 96 h.

The fact that eMSCs from the control tissue showed lower TG2 protein levels with a high enzyme activity suggested that TG2 might be important in the development of endometriosis not only by destabilizing ECM but also enhancing the cell migration. In this context, the upregulation of TG2 along with ITGB1

and SDC4 was evident in eMSCs of endometriosis which was possibly associated with the increase in the activity of MMP-2 and -9.

P-05.03.3-035
Characterization of Wharton Jelly Mesenchymal stem cells from the human umbilical cord and investigation of the role of cellular metabolism on their *in vitro* differentiation

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Recent studies have indicated that pluripotent stem cells and some stromal stem cells such as mesenchymal stem cells (MSC) are metabolically different from their differentiated counterparts. In this study, the cellular mechanisms controlling metabolic changes in stem cells was investigated using Wharton jelly Mesenchymal/stromal stem cells (WJ-MSSC).

WJ-MSSCs were isolated by the explant method and cultured in DMEM-F12 with 10% FBS. Endothelial differentiation was induced by the addition of VEGF, EGF, insulin and hydrocortisone for 6 days. Neuronal differentiation was achieved by using commercial neuronal differentiation medium (Millipore) for 3 days. In parallel experiments, cellular metabolic activity such as lactate production was measured.

The MSC characterization was performed by flow cytometry using antibodies against CD90, CD105, CD73 and CD44 (BD Human MSC Analysis Kit). The differentiation process was followed by measuring the expression of CD31, CD34 for endothelial and GFAP, neu and tyrozine hydroxylase proteins for neuronal cells by immunofluorescence. For gene expression, Nanog, CD34 and GAPDH genes were analyzed by RT-PCR.

Differentiation stimuli to endothelial or neuronal cells resulted in a significant decrease in MSC marker proteins. Expression of stem cell markers other than CD73 were decreased to 2–20%. Differentiation induced the expression of CD31, CD34 for endothelial and GFAP and neu proteins for neuronal cells. In vitro lactate production was decreased following differentiation in both lineages. Neuronal differentiation increased glucose consumption by ~ 48% and the extracellular calcium concentration of these cells was significantly lower than synchronous undifferentiated cells.

Glycolytic activity is decreased during in vitro differentiation of WJ-MSSCs. Metabolic reprogramming and glucose uptake of cells may be an early indicator of the differentiation process in WJ-MSSCs, supporting the view on their metabolic plasticity.

P-05.03.3-036
Effects of STIM1 and Orai1 proteins on the differentiation of hepatocellular cancer stem cell-like cells

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Store-operated Ca^{2+} entry (SOCE) activated by depletion of intracellular Ca^{+2} stores has been shown to control intracellular Ca^{2+} homeostasis in many physiological and pathological events. Stromal interactive protein, STIM1, as endoplasmic reticulum (ER) Ca^{+2} sensor and Orai protein as pore-forming subunit of SOC channels play crucial roles in the activation of SOCE channels. STIM1 and Orai were reported to have pathophysiological roles especially in hepatocellular carcinoma (HCC). Anticancer chemotherapy frequently falls back because of these tumor-initiating subpopulations, tentatively called 'cancer stem cells'. The purpose of this study was to investigate the roles of STIM1 and Orai on SOCE in differentiation of Huh-7 HCCs expressing EpCAM and CD133 surface adhesion molecules ($EpCAM^+CD133^+$). $EpCAM^+CD133^+$ subpopulations in Huh-7 cells were separated via flow cytometry and transfected with STIM1 and Orai-1 over-expressing (OE) plasmids. Expression levels were confirmed by RT-PCR. Changes in intracellular Ca^{2+} concentration were monitored via dual wavelength spectrofluorimeter in fura 2-loaded cells. In $EpCAM^+CD133^+$ cells, ER Ca^{2+} release increased without any change in SOCE compared to that of $EpCAM^-CD133^-$ cells. Similar results were observed in STIM1-OE $EpCAM^+CD133^+$ cells. On the other hand, increase in Orai1 has no effect on either parameter. Although not statistically significant, ER Ca^{2+} release and SOCE increased in STIM1- and Orai1-OE cells. STIM1 overexpression may facilitate more Ca^{+2} stored inside the ER due to its Ca^{+2} binding property. Based on the data obtained from double OE cells, enhanced ER Ca^{2+} release and SOCE confirm the mutual requirement of STIM1 and Orai in Ca^{2+} homeostasis provided by ER. Briefly, SOCE dynamics may reveal the basics of carcinogenesis in terms of proliferation, metastasis, invasion and drug resistance. This study was supported by The Scientific and Technological Research Council of Turkey (TUBITAK, 113S399 to MT).

P-05.03.3-037
An immunoevasive mechanism for cancer stem cells: increased expressions of HLA-G and HLA-E

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Cancer is globally one of the most death causes. Recently, huge improvements occurred in the cancer diagnosis and treatment due to advanced technology, however recurrence occurs almost 30–40% of patients and their survival times decreases. In this study, we aimed to investigate of relationship between the cancer stem cells which are strongly associated with chemotherapy and radiotherapy resistance and recurrence with the non-classical MHC I antigens which have immunosuppressive properties. For this purpose, we immunohistochemically evaluated the expression patterns of CD133, CD44, Nanog, Oct3/4, HLA-G and HLA-E in the advanced stage colorectal, gastric and breast cancer and also non malign biopsy samples. We detected that the cancer stem cell markers CD133, CD44, Nanog and Oct3/4 significantly increased in the advanced stage cancer tissues. However, the immunosuppressive HLA-G and HLA-E expressions increased only in the colorectal and gastric tumor tissue. In addition to the presence of cancer stem cell like cells in the tumor tissues, increased expressions of HLA-G and HLA-E may indicate an immune evasive adaptation of tumor cells. According to our findings, the HLA-G

and HLA-E may be potential therapy targets to elimination of cancer stem cells of colorectal and gastric cancers. However, more detailed studies are needed to support our findings and also to determinate of clinical values of these markers.

P-05.03.3-038
Endocannabinoids increase SDF-1 release from human mesenchymal stem cells

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Lipid-structured endocannabinoids are endogenous morphine ligands and present widespread receptor-mediated effects at physiological and pathological levels on the nervous system as well as many other systems. These effects are partially realized through mechanisms affecting cell growth, differentiation, apoptosis and migration at the molecular level. The hematopoietic progenitor cells (HPCs) and mesenchymal stem cells (MSCs) form a distinct niche in bone marrow where they interact with each other in harmony. The stromal cell-derived factor 1 (SDF-1/CXCL12) is a chemotactic factor in bone marrow and is released from MSCs and their receptor CXCR4 is found in HPCs. With these rationale in mind, we asked if HPCs and MSC interaction mediates SDF-1 release via endocannabinoidal system. Bone marrow MSCs obtained from healthy donors and passage 3 MSCs were induced with 200 ng/ml lipopolysaccaride (LPS) for 4 h. Antagonists for CB1 (AM281) and CB2 (AM630) receptors were added to cultures for 4 days. After incubation with antagonists MSC culture supernatants collected and processed with human SDF-1 beta in ELISA medium. Analyses demonstrated direct decreasing effect of endocannabinoid receptor antagonists on SDF-1 beta release from bone marrow MSCs. In conclusion, endocannabinoidal system regulates SDF-1 release on MSCs and directly act on HPCs mobilization in bone marrow microenvironment (niche). This may have a clinical implication on therapeutic mobilization strategies for HSCs in hematology clinical applications.

P-05.03.3-039
Effects of stem cells applications on growth factors during implantation

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Implantation is invasion of the embryo into the endometrium and occurs in three stages apposition, adhesion and invasion, via the complex cellular and molecular mechanisms. During these stages, both of maternal endometrium and embryo should be appropriate for the implantation which is the beginning of pregnancy. Receptivity of uterine consists in the existence of growth factors such as TGFbeta-1, IGFR1, VEGF. It is indicated that damages of factors relesead from endometrium and blastocyst prevent implantation. Recently, stem cells can be obtained from many sources to use for therapeutic purposes and mesenchymal stem cells derived from bone marrow are the most studied. In our study, it was aimed to investigate molecules play a role in blastocyst implantation after bone marrow derived mesenchymal stem cell application into the rat endometriyum. Female rats were divided into three groups which were saline (SF, *n*:7), media (M, *n*:7), stem cell in media (M+BMSC, *n*:7). After vaginal smear technique, female rats in estrous cylcle were injected into the uterine and periton 200 μL saline, 200 μL culture media and 1×10^6 cell/200 μL culture media. The pregnant female rats on the 7 day were sacrified and uterine samples removed and were stained with heamatoxylin-eosin histochemically and anti-TGFbeta-1, anti-IGFR1, anti-VEGF and anti-PCNA immunohistochemically and obseved under light microscope. H-score results were determined using One-Way ANOVA test statistically. It was found that intraperitoneal administration of stem cells with media, was increased TGFbeta-1, IGFR1, VEGF and PCNA parameters when compared with the intrauterine administration of stem cells. In this study, it was revealed that distribution of molecules play role in implantation were changed due to stem cell application. It is supposed that stem cell treatments can be cured the molecules caused infertility.

Tuesday 6 September 12:30–14:30

Cardiac regeneration: Programming human heart cells

P-06.02.5-001
Slow-releasing H2S donors: growth-stimulating molecules for cardiac tissue repair

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Many unconventional biochemical factors remain to be investigated for their potential effects on stem cells. Among others, endogenous *gasotransmitter* H_2S, generated from L-cysteine and organosulfur-compounds (OSCs) metabolisms, plays very important roles in the central nervous, respiratory and cardiovascular system. Slow-releasing H_2S donors are viewed as powerful tools for basic studies and innovative pharmaco-therapeutic agents for cardiovascular and neurodegenerative diseases. Exogenous H_2S administration is able to promptly scavenge ROS, activate myocardial K_{ATP} channels and increase pro-cell survival signaling, very likely activating ERK and phosphatidylinositol 3-kinase (PI3K)/AKT pathways. The effects of H_2S-releasing agents on the growth of stem cells are not yet widely investigated. Therefore, stem cell therapy combined with H_2S may have great clinical relevance in cell-based therapy for regenerative medicine.

The effects of slow-releasing H_2S agents on the *in vitro* cell growth and differentiation of human Lin^- $Sca1^+$ cardiac progenitor cells (hCPC) were here studied. In particular, the effects of H_2S-releasing agents, such as Na_2S, GYY4137 and water-soluble GSH-Garlic conjugates (GSGaWs), on the cell viability and differentiation of hCPC were here investigated by colorimetric assay, immune-fluorescence microscopy and western-blotting analysis. The treatment with slow-releasing H_2S donors increased the cell proliferation in a concentration dependent manner respect to the control. Moreover, the treatment with GSGaWs led to an up-regulation of the expression of proteins involved in the cell adhesion and differentiation processes. These preliminary results highlight on the effects of this *gasotransmitter* on the stem/progenitor cells and on the possibility to develop functional 3D-systems for cardiac tissue repair, that take into account the relevant biological role of H_2S in the cardiovascular system.

P-06.02.5-002
Investigation of the protective effect of boric acid and omega-3 fatty acid in model of acute myocardial infarction changes in myocardial rats

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Ischemic heart disease being the most common cause of the mortality and morbidity in worldwide commonly results from the occlusion or narrowing of the coronary arteries by atheromatous plaque and thus is named as coronary artery disease.

Male Sprague Dawley rats were used in the present study. Rats were divided into 5 groups with 10 rats in each: control, MI, MI+Boric acid, MI+Omega-3 and MI+Boric acid+Omega-3 groups. Control rats were treated with 2 mL/day saline, boric acid-treated rats received 100 mg/kg/day Boric acid and Omega-3-treated rats received 800 mg/kg/day for 28 days by oral gavage. For the experimental MI model, 200 mg/kg izoproterenol-HCl (ISO) was administered subcutaneously two times with a 24-h interval in the last 2 days of the Boric acid and/or Omega-3 treatments. Twelve hours after the second dose of ISO, general anesthesia was induced. Under general anesthesia and spontaneous respiration, ECG recordings were obtained by using a computerized data recording and analysis system (MP100, Biopar) and D-II recordings were used in the analysis.

Compared to the control group, serum CK-MB, BNP and TNF-α levels were higher in MI group ($P < 0.001$, $P < 0.001$ and $P < 0.01$ respectively). In the heart tissue homogenate, biochemically measured Calpain activation and MDA were increased ($P < 0.01$ and $P < 0.001$, respectively) and PON1 levels were decreased ($P < 0.05$). According to the ECG recordings, ST wave and heart rate were found to be decreased ($P < 0.001$ and $P < 0.001$, respectively). On the other hand, all above mentioned parameters were found to be improved in rats treated with boric acid and/or Omega-3 after induction of MI. Moreover, histological analysis including light microscopy and TEM revealed a significant histological improvement in rats treated with boric acid and/or Omega-3 after induction of MI.

Results of the present study suggest that Omega-3 and/or boric acid treatment significantly decreases the cellular damage in MI.

P-06.02.5-003
The role of heart-type fatty acid-binding protein in identifying early cardiac ischemia

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This is study is aimed at measuring the level of serum heart-type fatty acid binding protein (h-FABP) in patients presenting with diabetic ketoacidosis (DKA) and diabetic ketosis (DK) and to determine its role in identifying early period cardiac ischemia by comparing this level with the level of a control group at a comparable age

This study was planned to be a prospective study and it included 35 patients diagnosed with DKA, 20 patients diagnosed with DK and 20 voluntary pediatric and adolescent healthy control subjects. The h-FABP, creatine kinase-MB (CK-MB) and troponin-I levels were studied in patients with DKA and DK as well as in the control group at the time of presentation. For DKA patients, their h-FABP values were measured once again after acidosis correction and compared with the values they had at the time of presentation

There were no differences among groups in terms of sex, age, height and weight. No statistically significant differences were found among groups with respect to troponin-I values (0.06 ± 0.08, 0.07 ± 0.04 0.04 ± 0.04; $P = 0.457$). No statistically significant differences were found among groups with respect to CK-MB values (1.48 ± 0.91, 1.55 ± 0.9, 2.09 ± 1.37; $P = 0.229$). The h-FABP values of DKA patients at the time of presentation were found to be statistically significantly higher than those of DK patients and control group (1.17 ± 0.79; 0.79 ± 0.5 0.69 ± 0.36; $P = 0.006$). The h-FABP value of the DKA group at the time of presentation was found to be statistically significantly higher than the value at Hour 36 after acidosis correction (1.17 ± 0.79; 0.55 ± 0.28; $P = 0.0001$)

The fact that h-FABP levels were found to be high in pediatric patients diagnosed with DKA at the time of presentation suggested that myocardial ischemia had been triggered. In diabetic patients, every ketoacidosis attack may lead to cardiac ischemia, thereby accelerating progress to necrosis. In conclusion, we would like to propose h-FABP as a potential marker for indicating myocardial ischemia.

P-06.02.5-004
Genome-wide analysis of hypoxic stress response in human cardiomyocytes

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Gene therapy is an emerging modality for the treatment of ischemic heart disease and heart failure. Genes related to hypoxic

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stress in human cardiomyocytes on a genome-wide scale remains poorly understood. This study aimed to identify the gene expression patterns of adaptive response of the human cardiomyocytes (hCM) to hypoxic stress. *In vitro* experimental models of hypoxia mimicking in-vivo coronary ischemia, are useful tools to identify molecular pathways involved in myocardial ischemia.

In the current study, we cultured AC16-hCMs in DMEM/F12 with 10%FBS. To simulate hypoxia model, cardiomyocytes were plated in hypoxia chamber (1%O_2, 5%CO_2, 94%N_2) for 3, 6, 12, 24 h and the control group was incubated in normal conditions (5%CO_2, 95%O_2). Cell viability was determined using MTT-assay. Annexin-V assay was used to monitor apoptosis. Gene expression profiling was analysed with Affymetrix-HG-U133-Plus-2 arrays. Following bioinformatic and statistical analyses differentially expressed genes (DEG) were classified according to gene ontology using DAVID and KEGG pathway analysis tools.

According to MTT, Annexin-V and HIF gene expression results, hypoxia time was determined as 3 h. We identified 649 genes (279 down-regulated and 370 up-regulated) ($P < 0.001$, Fold change ≥ 1.5) were differentially expressed in hypoxic-AC16 vs. AC16. DEGs were mainly clustered in cell proliferation, regulation of cell death, cell adhesion and response to stress. Furthermore, transcriptome analyses revealed that 'Metabolic, cytokine-cytokine receptor interaction, HIF-1 signaling, TGF-beta, cell cycle and apoptosis' pathways were involved in the hypoxic stress response of human cardiomyocytes.

This study provides molecular information regarding gene expression reprogramming of human myocardial hypoxia. The pathways identified in this study may pave the road for translational medicine. This study was supported by TUBİTAK project number 111S189.

P-06.02.5-005
Angiogenic potential of endothelial progenitor cells derived from induced pluripotent stem cells

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Autologous iPS cells after reprogrammed into endothelial progenitor cells (EPCs) may offer several advantages in the treatment of cardiovascular disorders because of their cardiogenic and vasculogenic differentiation potential. To reach that purpose, we differentiated and characterized mouse iPS cells into $Flk1^+$, a well-recognized EPC marker. Further maturation of EPC was characterized by the expression of CD31 and CD133 markers.

Purified iPS cells were differentiated into $Flk1^+$ cells with the use of differentiation medium on type IV collagen-coated dishes in the absence of LIF. We then analyzed Flk1 gene expression and protein levels with qRT-PCR, Western blot and immunocytochemical methods on days 2.5 to 7.5. $Flk1^+$ cells isolated with MACS system and then recultured these cells in differentiation medium with VEGF to induce EPC cells. Following induction, CD31 and CD133 gene expression and protein levels were analyzed with genomic and proteomic methods. After isolating these cells and aggregate overnight, we cultured cells in Three-dimensional condition in collagen type I and used differentiated medium including VEGF and EGF.

We found that Flk1 expressing cell number reached to a peak level (24%) on day 5.5 followed by a progressive decline subsequently. In the second step, CD31 and CD133 positive cells were generated and enriched during day 4 of induction. We showed optimal time for harvesting $Flk1^+$ cells is day 5.5 of initial differentiation. Following isolation of $Flk1^+$ progenitor cells they were further matured into functional EPCs by VEGF within 4 days of induction. Additionally for evaluation of angiogenic potantial differentiated cells, we monitored EPCs behavior along vascular formation in 3D culture.

Our work demonstrates that EPCs could be successfully derived from iPS cells and these cells have vascular formation and angiogenic potential in 3D culture. EPC drived iPS cells play important role in the treatment of cardiovascular disease.

P-06.02.5-006
Electrophysiological, biochemical and genotoxic effects of Luna Experience on heart tissue in rat model

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Pesticides are widely used for the control of agricultural, industrial and domestic pests. However, the uncontrolled use of pesticides has diverse effects on ecological system and public health. Fungicides are one of the pesticide type used to kill fungi or fungal spores. In this study, the effect of different doses of Luna Experience, a fungicide, on the cardiac electrophysiology and genotoxicity in rats were investigated. Among five groups (5 mg, 10 mg, 20 mg, control and positive control for comet assay) treatment groups received by gavage doses of Luna Experience for 30 days. Electrical activity of heart were recorded using electrophysiological recording techniques. Tissue activities of paraoxanase (PON) and arylesterase (ARE) and level of malondialdehyde (MDA) were measured using biochemical methods. Comet assay was performed on heart tissue. We calculated genetic damage index (GDI) and damaged cell percent (DCP) from comet assay. It was observed that there is a significant decrease in heart rate in all treated groups as compared with control group ($P < 0.05$). Amplitude of P wave and QRS complex did not change ($P > 0.05$). In all treated groups, statistically significant differences were found for values of PON, ARE, MDA, GDI and DCP when compared to control group ($P < 0.05$). According to our results, exposure to different doses Luna Experience have a probable hazard potential for the cardiac system.

Tuesday 6 September 12:30–14:30

Developments in biomaterials and tissue engineering

P-07.01.3-001
Effect of the substituents isomery in functionalized clathrochelates on their interaction with proteins

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The macrocyclic cage complexes iron (II) clathrochelates are of the interest due to their bioactivity; they are able to inhibit T-7 RNA polymerase, possess toxicity to leukemia cells HL-60 and suppress amyloid fibril formation. Their binding to serum albumins was reported; the extreme binding affinity to albumins is observed for the compounds bearing carboxy groups. Upon this interaction, clathrochelates quench protein intrinsic fluorescence and gain optical activity inducing circular dichroism (CD) signal in 350–600 nm region.

Here we examine the effect of spatial arrangement (isomery of substituents) of clathrochelates on their binding to globular proteins. We study 6 bis-substituted clathrochelates bearing two same or different isomers (ortho-/meta-/para-) of carboxyphenylsulfid groups. Their interaction with bovine (BSA) and human serum albumins, β-lactoglobulin and lysozyme are explored by CD and protein fluorescence quenching method.

The binding of compounds to albumins evoked the CD bands of the same shape, but their intensities vary up to 45 times depending on substituents isomery. In the presence of β-lactoglobulin, the intensities, shape, and positions of the induced CD-bands differ for the compounds with different isomer groups. The CD bands induced by the lysozyme in the case of di-para substituted clathrochelate are shifted relatively to the bands of other isomeric compounds. The pronounced quenching of protein fluorescence by clathrochelates was observed only in the case of BSA, its intensity depends on the geometry of substituents (9–17 times).

The different spatial arrangement (isomery) of carboxyphenylsulfid substituents in clathrochelates causes the distinctions in both their CD-signal induced by interaction with proteins and their effect on the protein fluorescence. The geometry of ribbed substituents is important for their binding to biomolecules (particularly proteins) and is suggested to determine the structure of the formed guest-host complex.

P-07.01.3-002
3D Bioprinting of human stem cells to engineer vascular tissue

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3D Bioprinting is a new technology that revolutionized the field of tissue engineering and regenerative medicine, allowing reconstruction of living tissue and organs preferably using the patient's own cells. Using a 3D printer we can design biological structures by controlling exact deposition of cells, growth factors and extracellular molecules in a spatially-controlled manner. The aim of this study was to evaluate the differentiation of human amniotic fluid stem cells (AFSC) into endothelial progenitor cells using a BioInk® hydrogel photopolymerized in a 3D network resembling vascular tissue. Characterization of AFSC was performed by flow cytometry, followed by sorting of the $CD177^+$ stem cell subpopulation. $CD117^+$ stem cells were stained with cell tracker Red CMTPX and then mixed with BioInk® hydrogel. Printing was done using a 150 μm diameter needle, under 1 bar pressure, and 150 mm/min speed. The network models with define distance apart were printed and analyzed by fluorescent microscopy. MTT test was used to evaluate the viability of the $CD117^+$ stem cells. Our results showed that AFSC remained viable as shown by MTT assay. The fluorescent microscopy images confirm the viability biochemical test showing that the $CD117^+$ cells viability is maintained after 7 days of cultured in BioInk® hydrogel. Furthermore, histological section of hydrogel showed that cells have a relatively uniform distribution forming network interactions between cells. Flow cytometry assay showed that CD117+ cells expressed endothelial markers such as CD31, CD105, CD133, CD144 and VEGFR2. In conclusion 3D printers are useful tools for creating three-dimensional scaffolds that mimics the cell microenvironment where different types of cells could proliferate, differentiate and crosslink with each other forming tissue-like structures.

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P-07.01.3-003
Biomaterials based on functional magnetite and natural compounds with improved biocompatibility, biodistribution and low immunomodulatory effects

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This study aims to reveal the biocompatibility, biodistribution and immunomodulatory impact on the production of inflammatory citokines of magnetite (Fe3O4) nanoparticles functionalized with natural compounds with proved antimicrobial and immunomodulatory effects. Co-precipitation synthesized Fe3O4 were functionalized with plant-derived compounds: eucalyptol, carvone, limonene and β-pinene. Characterization was done by IR, SEM and HR TEM, while *in vitro* biocompatibility was tested using endothelial human cells (fluorescence microscopy and proliferation assay). *In vivo* biodistribution was tested in a *balbC* mouse model at 2 and 7 days post-intraperitoneal injection, followed by experimental organ removal. Tissue sections obtained from vital organs were stained with hematoxylin-eosin. Production of inflammatory cytokines was assessed by ELISA. Results demonstrated that, at concentrations of 500 μg/mL, all prepared nanosystems have a good biocompatibility *in vitro* and *in vivo*, allowing the development of cultured cells and also not affecting any visible behavior and organ morphology of the mice. Microscopy evaluation of the organs sections revealed that nanoparticles are not present in vital organs such as brain, heart, kidney and liver, but aggregates were visible in the lungs and spleen. At 7 days post-injection no visible aggregated were found in the lungs, few dark-brown nanoparticles clusters being visible in the red pulp of spleen. ELISA results revealed that Fe3O4 functionalized with carvone and limonene significantly stimulated

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the production of IL-2, IL-10 and IL-6, while reducing the production of TNFα. Other nanosystems din not impact significantly on the cytokine production. Functional Fe3O4 nanoparticles are efficient drug delivery shuttles, able to stabilize pharmacological compounds, such as plant-derived bioactives, and their biocompatibility, specific biodistribution and limited immunomodulatory effects recommend their use in pharmacological formulations.

P-07.01.3-004
New approach for cell imaging with fluorescent carbon nanoparticles

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In the nanotechnology field, much interest was focused on the new carbon nanomaterials for cell imaging. Recently discovered inorganic carbon nanoparticles ('C-dots') due to their excellent fluorescence characteristics and biocompatibility have ample opportunities for their use in imaging and functional transformations in living cells. Their distinctive features, such as high brightness, small sizes, high biocompatibility, small negative charge on the surface and very easy methods of their preparation present a good alternative to other nanoscale materials. The focus of our research was to determine the possibility of using C-dots as the easily available probes for apoptotic cells detection.

The carbon nanoparticles were prepared from alanine, citric acid, urea, etc by hydrothermal treatment at 180 C. The studies were performed with adherent epithelial Vero and HeLa cell lines (ATCC).

With these tools we demonstrate that both native and apoptotic cells can be easily visualized. The CDots uptake occurs probably by endocytosis, which allows for much larger their number to accumulate in apoptotic cells. Using the different methods of sample preparation, they show the ability for labeling various structural compartments of the cell. For living cells there are the intracellular vesicles and lysosomes. In contrast, in fixed cells the nucleus is labeled preferentially.

The fact that apoptotic cells accumulate strongly increased amount of CDots can be efficiently used in flow cytometry for characterizing the cell populations regarding the relative amount of apoptotic cells in different experimental conditions. The application of such cheap and easily accessible nanoparticles provides more opportunities to simplify the popular methods of cell labeling and detection. Previously, our studies showed the possibility of using these nanoscale fluorophores for super resolution method SOFI.

P-07.01.3-005
Simultaneous determination of epinephrine and dopamine by using *Candida tropicalis* yeast cells immobilized in a carbon paste electrode (CPE) modified with single wall carbon nanotube (SWCNT)

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A new electrochemical microbial biosensor for the fast detecting of dopamine and epinephrine based on *Candida tropicalis* immobilized in a carbon paste electrode (CPE) modified with single wall carbon nanotube (SWCNT) was described in this paper. The immobilized cells were used as a source of polyphenol oxidase (PPO) to develop voltammetric epinephrine and dopamine biosensor. Voltammetric determination of phenolic compounds like epinephrine and dopamine is a simple technique available. Direct oxidation of phenols can be used, but the oxidation potentials of this compounds are similar and they can not be detected distinctively. Another possibility is the use of biosensors based on the polyphenol oxidase (tyrosinase) enzyme that oxidises the phenolic compounds into their corresponding quinones. By this way phenolic compounds that epinephrine and dopamine that used in this study were detected at the different potential. The effect of varying the amounts of SWCNT and microorganism on the response to epinephrine was investigated to find the optimum composition of the sensor. The effects of pH and temperature were also examined. Increases in biosensor responses were linearly related to dopamine concentrations between 0.1 and 1.0 mM and epinephrine concentrations between 0.01 and 1.0 mM. Limits of detection of the biosensor for dopamine and epinephrine were calculated to be 0.021 and 0.0029 mM, respectively. Finally, proposed systems were applied to epinephrine and dopamine analysis in pharmaceutical drugs.

Keywords: Microbial Biosensor, *Candida tropicalis*, dopamine, epinephrine, SWCNT.

P-07.01.3-006
Is artificial human blood produced *in vitro*? Cataliyic hemoglobine molecules

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Objective: It has started a long time ago to search for a material that can replace blood. This material does not require special storage conditions, independently of the recipient's blood group and can be applied to all individual. Milk, casein derivatives, starch, saline and Ringer were used for this aim in the past. The determination of toxic effect of natural hemoglobin (Hb) on human, researchers have focused on development modified blood. In this work, the development of an artificial biomaterial alternative of blood for using as preoperative and operative aims was aimed.

Material and Methods: In our study, ultrapure Hb molecules are immobilized on triethanolamine coated magnetic nanoparticles using various techniques. Prepared nanoparticles were characterized by FT-IR, cTEM, XRD and cyclic voltammetry (CV). The cytotoxic effects of artificial blood were tried on MTT cell proliferation.

Results: The characteristic peaks of hemoglobin were obtained from FT-IR spectra differently from support. Particles size is concluded by using Debye-Scherrer equation as > 80 nm from XRD spectra. SEM and cTEM images supported XRD result. CV results showed that HB molecule has −0.418 V cathodic potential against Ag / AgCl standard electrode. Significant differences were not observed in the MTT results ($P < 0.01$).

Conclusion: The nanoparticles were obtained in accordance with the intended desired method. It is determined that the hemoglobin molecules give the same potential with natural blood even after 3 weeks of immobilization and carrying oxygen as natural blood. There are statistical differences between results of MTT tests due to used concentration. But, It is considered that decantation advantage of the artificial blood minimized cytotoxic effects.

P-07.01.3-007
Development of a biomimetic affinity column coupled to tandem mass spectrometry by molecular imprinting for specific glycosaminoglycan separation from urine

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Proteoglycans are among the most abundant molecules of the inter-cellular structure and they are present in extracellular matrices of connective tissues. These glycosylated proteins contain one or more (GAG) chains that are covalently attached to the core protein and their hydrodynamic function is mainly due to the physicochemical characteristics of this GAG component which provides hydration and swelling pressure to the tissue. GAG levels excreted via urine are used as a marker to monitor different diseases (chronic renal disease, renal fibrosis, glomerular filtration abnormalities, bladder stones, breast and lung cancers, hypertension and diabetes, etc.) besides the well known mucopolysaccharidoses. However, their detection by using chromatographic methods is hard, because of the high polarity of negative charges and different functional groups such as acetyl sulfates that generate microheterogenity. In this study, we developed molecularly imprinted chromatographic HPLC columns for specific Heparan Sulfate (HSA), Chondroitin Sulfate (CS) and Dermatan Sulfate (DS) detection in urine. Positively charged acrylamide monomers were first polymerized by precipitation polymerization, to produce polymers which will show specific recognition for GAG's via electrostatic interactions and hydrogen bond formation. These GAG selective polymers were then filled in the steel HPLC columns and columns eluents were chemically degraded. Degradation products of GAG's were examined off-line column coupled with tandem mass spectrometry. The results showed that our imprinted columns separated GAG's specifically and sensitively. Thus, urine GAG's can be specifically determined by using a GAG specific molecularly imprinted column. In this study internal standart weren't used because the matrix effect was lower than 5% for each urine samples. %CV of DS, CS and HSA was calculated as; 1.41, 3.40 and 4.96 respectively.

P-07.01.3-008
Analysis of the stability of supported lipid bilayers for cell culture studies

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Supported lipid bilayers (SLB) were started to be used for cell culture studies to focus on cell adhesion, cell signaling etc. Testing the stability of SLBs is essential to utilize them as cell culture platforms. In this study, the stability of *phosphatidylcholine* (PC) lipid bilayers on glass was investigated under Milli-Q water, phosphate buffer saline (PBS) and Dulbecco's modified Eagle medium culture (DME) medium supplemented with/without serum. The stability was also checked by enriching SLB with different lipids.

PC-liposomes were prepared by hydrating the dried thin lipid film with PBS and then by extruding the suspension through a polycarbonate membrane. A negatively charged phospholipid, *phosphatidylserine* (PS, 25%); a positively charged phospholipid, DOTAP (50%) and cholesterol (25%) were also used for liposome preparation. Liposomes were fluorescently labelled and series of SLB imaging were taken for a week.

In all experiments in Milli-Q water and PBS, the stability was conserved for 7 days. PC bilayers in medium supplemented with serum showed hole formations on the second day and their number and size increased rapidly in time. When the bilayers were prepared in medium without serum, disruption was lowered but not completely removed as a result of other factors in medium.

Cholesterol providing an increased rigidity to the membrane caused higher stability. Positively charged bilayer structures also showed increased stability. This can be explained by decreased mobility of bilayer as a result of electrostatic interaction between positively charged molecules and negatively charged glass surfaces. Decreased mobility decreases the interactions within the medium. Lastly, negatively charged bilayers did not show high stability. Strong repulsive forces between the negatively charged surface and bilayer probably prevented the integrity of the bilayer and increased the deformation.

P-07.01.3-009
Covalent immobilization of LL-37 onto lignin caprolactone based polymer and investigation of its antimicrobial activities

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In recent years the use of biopolymers has gained priority in tissue engineering and biotechnology, both as dressing material and for enhancing treatment efficiency. There is a demand for new biopolymers designed with protease inhibitors and antimicrobials. LL-37 is an important antimicrobial peptide in human skin and exhibits a broad spectrum of antimicrobial activity against bacteria, fungi, and viral pathogens. Using lignin which is an abundant carbohydrate polymer in nature and a polyacrylic acid, we prepared a polymer film by plastifying caprolactone and polyacyrlic acid. Films were actified to immobilize LL-37. The structure was elucidated in terms of its functional groups by fourier transform infrared spectroscopy (FTIR), and the morphology of the film was characterized by scanning electron microscopy (SEM) before and after the immobilization process. The amount of LL-37 immobilized was determined by ELISA method. 99.9% of LL-37 peptide was successfully immobilized onto the films. Antimicrobial activity was determined in the film samples by quantitative antimicrobial activity method. According to the results, LL-37 immobilized film samples were effective on test organisms; Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli*. In bio-compatibility assays, the ability to support tissue cell integration was detected by using 3T3 mouse fibroblasts. Samples were examined under transverse microscope, non-immobilized sample showed a huge cellular death, whereas LL-37 immobilized film had identical cellular growth with the control group. This dual functional film with enhanced antibacterial properties and increased tissue cell compatibility may be used to design new materials for various types of biological applications.

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P-07.01.3-010
In vitro modulation of the cross-talk between macrophages and osteoblasts by titania nanotube-modified Ti surfaces

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Bone remodeling is a dynamic process that maintains a fine balance between bone formation and resorption, and is highly influenced by the inflammatory state of the local microenvironment. Therefore, a proper modulation in the cellular interactions and cytokine expression is a promising approach to achieve enhanced bone healing. As the biomaterials surface has a major impact on cellular behavior, the goal of the current study was to investigate the influence of TiO_2 nanotube-modified Ti surfaces (Ti/TiO_2) on the cross-talk between RAW 264.7 macrophages (MF) and MC3T3-E1 preosteoblasts (OB) in mono- and co-culture systems in comparison with flat Ti (cpTi).

RAW 264.7 and MC3T3-E1 cells were seeded on the test surfaces and grown in standard culture conditions for various periods of time. For co-culture studies, the cells were cultivated using a Transwell system. Inflammatory mediators released by RAW 264.7 cells were measured using ELISA technique, while the OB capacity to produce calcified bone matrix was evaluated by Alizarin Red staining.

In co-cultures, LPS-stimulated TNF-α, IL-6 and MCP-1 release was significantly increased at 24 h, while after 7 days only IL-6 exhibited higher amounts when compared with MF cultures alone. Moreover, the secretion of these mediators by cells exposed to Ti/TiO_2 was diminished, especially in LPS evoked conditions. Also, Alizarin Red staining demonstrated the presence of calcium deposits when OB were co-cultured with MF for 24 h and 7 days, whereas the presence of the MF for 4 weeks significantly inhibited mineralization. On Ti/TiO_2 surface elevated calcified matrix was observed, as compared with cpTi.

This study reveals that the overall effect of inflammation suppression induced by Ti/TiO_2 may contribute to the enhanced mineralization. Also, chronic inflammation may inhibit or delay the regeneration process.

Therefore, an adequate modulation of MF and OB interactions is vital for the biomaterials success in stimulating bone regeneration.

P-07.01.3-011
Synthesis and characterization of the branched magnetic polymer for drug delivery systems

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Magnetic nanoparticles (MNP) have gained a lot of attention in biomedical and industrial applications due to their biocompatibility, easy of surface modification and magnetic properties. Magnetic nanoparticles can be utilized in versatile ways, very similar to those of nanoparticles in general. However, the magnetic properties of these particles add a new dimension where they can be manipulated upon application of an external magnetic field. This property opens up new applications where drugs that are attached to a magnetic particle to be targeted in the body using a magnetic field. Often, targeting is achieved by attaching a molecule that recognizes another molecule that is specific to the desired target area.

In recent years, the development of the systems in which drug is delivered magnetically to the target is drawing considerable attention since it is a current issue. It is possible to eliminate the most of the problems caused by high doses of chemotherapy by using the magnetic drug delivery systems. Therefore, it is important to design delivery systems with high drug loading capacity. It is necessary to increase the number of reactive groups on the surface of nanoparticles in order to increase drug loading capacity.

In this study, we synthesized a novel magnetic surface for drug delivery systems. Magnetic dextran-NTA (MD-NTA) was synthesized by using magnetic O-carboxymethyl dextran (OCMD) and NαNα-Bis (carboxymethyl) -L-lysine hydrate (NTA) in order to increase the number of reactive carboxyl groups on the surface of biocompatible and biodegradable magnetic dextran. Magnetic material (MD-NTA) which was prepared and characterized by the analysis of Transmission Electron Microscopy (TEM), scanning electron microscope (SEM), Vibrating Sample Magnetometer (VSM), Fourier Transform Infrared Spectroscopy (FTIR) and X-Ray Photoelectron Spectroscopy (XPS).

P-07.01.3-012
Preparation of TGF-β3 loaded PLGA nanoparticles and film formulations for wound healing

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There are three subtypes of the TGF-β protein that has been reported to be involved in tissue repair process; scar tissue formation has been reported on tissues that has been affected by TGF-β1 and 2 due to high collagen synthesis. On the other hand the other isoform TGF-β3, suppresses the dense collagen production caused by TGF-β1 and prevents the scar formation. To be able to use these growth factors local or iv route, new drug transport systems are needed to protect the bioactivity during the treatment and controlled release. For this purpose poly(lactic-co-glycolic) acid polymer which is widely used in controlled release systems was chosen as the matrix material. Aim of the project was to design, formulate, prepare and optimize TGF-β3 loaded PLGA nanoparticular and/or PLGA polymeric film drug delivery systems and to test their effect on cell proliferation.

TGF-β3 loaded nanoparticles was prepared with emulsion–solvent evaporation method; whereas polymeric film systems was prepared with film casting – solvent evaporation method. Following the preparation TGF-β3 loaded drug delivery systems was characterized. Quantification and in vitro release of the growth factor TGF-β3 was studied with ELISA. HepG2 cell line was used on MTT cell proliferation assay for both TGF-β3 loaded nanoparticles and films on a time course study.

Nanoparticles and films were prepared and loading efficiency of the nanoparticles were found to be 42.42%. Particle size, zeta index and polydispersity index for this formulation were determined as 204.9 ± 10.3 nm, 0.0381 mW and 0.380, respectively. Thickness of the prepared films were 286 ± 20.16 nm. Additionaly prepared nanoparticles and films were found non-toxic.

TGF-β3 nanoparticles and films which were prepared in this study are planned to be used as an effective treatment strategy for wound healing after injury.

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P-07.01.3-014
Preparation, characterization and some biological properties of PVP/*Ganoderma lucidum* composites

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Polyvinylpyrrolidone (PVP) is a biodegradable material and natural polymeric biomaterial in such studies. *Ganoderma lucidum* is a natural material containing triterpenes, polysaccharides, adenosine, polypeptides, and amino acids. These constituents have been shown to exhibit anti-cancer properties, enhance and regulate immunity, resist oxidation and ageing, and promote metabolism and cell proliferation.

Composites of polyvinylpyrrolidone (PVP) have been prepared by solution intercalation method using *Ganoderma lucidum* at different loading amounts. The characterization of PVP/ *Ganoderma lucidum* composites was made by X-ray diffraction (XRD) and scanning electron microscopy (SEM); the interactions between *Ganoderma lucidum* and PVP was determined by FTIR-ATR; the thermal stability was determined by simultaneous TG/DTA. Hemocompatibility of the prepared composite samples were investigated by a 96-well plate spectrophotometer. In addition, contact angles and antimicrobial activity of biomaterials were also determined.

FTIR-ATR confirms interactions formed between *Ganoderma lucidum* and PVP. XRD and SEM results give evidence that *Ganoderma lucidum* was well dispersed and homogenously in the PVP matrix. Thermogravimetric analysis indicated that introduction of clay to the polymer network resulted in an increase in thermal stability. The results of in vitro hemocompatibility test were showed that PVP/ *Ganoderma lucidum* composites are used as biomaterial. The development of synthetic materials, textured polymers and metals and their increasing use in medicine make research of biomaterials' hemocompatibility very relevant.

P-07.01.3-015
PEG/*Ganoderma lucidum* biocomposites

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Composite material is a multi-phase system consisted of matrix material and reinforcing material. Matrix material is a continuous phase and reinforcing material is a dispersed phase. The main two bioactive components of *Ganoderma lucidum* can be broadly grouped into triterpenes and polysaccharides. Despite triterpenes and polysaccharides being widely known as the major active ingredients at anti-cancer effect.

This study describes the synthesis and characterisation of biocomposites of different molecular weight of PEG (polyethylene glycol) as matrix with *Ganoderma lucidum* as a filling material at different loading (%1, %2.5, %5 wt). The composites have been prepared by solution intercalation method using ground and sieved *Ganoderma lucidum at 25 micron scale*. The characterization of composites was made by X-ray diffraction (XRD), scanning electron microscopy (SEM) and Fourier transform infrared attenuated total reflectance (FTIR-ATR) Also in this study the hemocompatibility and antibactarial properties of composite investigated.

When XRD and FTIR-ATR results discussed, all of the composites using the different loading amunt of *Ganoderma lucidum* (%1, %2.5 and %5 wt) were shown a homogen distribution in the matrix (PEG). And an interaction have occured between matrix and filling material. The SEM photos have confirmed these results. PEG and composites have been detected as hemocompatible. These results showed that they can be used as biomaterials.

P-07.01.3-016
Evaluation of the genotoxic potential of some nanocomposites by comet assay

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Due to its similar nature to the bone, nanohydroxyapatite is a biocompatible particle and poly(methyl methacrylate) (PMMA) is a polymer that has been used in dentistry and orthopedic applications for years. In this study, genotoxic potential of PMMA/Nanohydroxyapatite nanocomposite films composed of polymers having different molecular weights and nanohydroxyapatite fillers in different concentrations (1, 2.5 and 5%) were investigated by comet assay which is a kind of gel electrophoresis that can be used to measure DNA damage in individual cells. If the DNA is damaged we expect broken ends to migrate apart from the head. At the end of the assay performed after incubation with lymphocytes of healthy humans, we measured the DNA Damage Index (DDI) and Percentage of Damaged Cells (PDC). In addition, to prove the morphological properties of the nanocomposites scanning electron microscope was used and an interaction between the matrix and nanoparticles with a homogeneous dispersion was observed.

P-07.01.3-017
Protein adsorption on stimuli-responsive mixed PDMAEMA/PEO polymer brushes

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Smart polymer brushes are made of macromolecules that are sensitive to stimuli from the external environment, including pH, ionic strength, temperature, etc. When stimuli-responsive polymer brushes are introduced onto material surfaces, their properties can be adjusted by tuning the environmental stimuli. These brushes can find promising applications across many areas of research, including surface science, nanotechnology, and biotechnology. In our work, the adsorption of human serum albumin (HSA, molecular weight of 66.5 kDa, isoelectric point Ip at pH 4.7) and lysozyme (Lys, molecular weight of 14.3 kDa, Ip ~ 11) was studied on polymer brushes composed of poly(ethylene oxide) (PEO) and poly(2-(dimethylamine)ethyl methacrylate) (PDMAEMA). PEO is a protein-repellent polymer and PDMAEMA is a polyelectrolyte bearing a variable density of positive charges depending on pH. A gold substrate was modified by these thiolated polymers according to the 'grafting to' method. The obtained polymer brushes were characterized by X-ray Photoelectron Spectroscopy, static Contact Angle Measurements and Atomic Force Microscopy. Polymer brush formation and protein adsorption were monitored by Quartz Crystal Microbalance. Surface characterization of the mixed brushes

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revealed the presence of both polymers at the surface. Conformational changes of PDMAEMA/PEO brushes were experimentally evidenced, and the results indicated that the brushes collapse at pH 9.00 (PDMAEMA is neutral in such conditions) and were swollen at pH 3.5 (PDMAEMA is positively charged). Protein adsorption was performed at different pH values (3.5–9.0) and salt concentrations (0.001–0.15M). It was shown that PDMAEMA has a high affinity to HSA at pH above its isoelectric point. However, the adsorption of positively charged lysozyme in a wide range of pH was not observed. These results indicate that PDMAEMA/PEO brushes are promising candidates for selective adsorption from a mixture of proteins.

P-07.01.3-022
Synthesis and characterization of modified kaolinite/poly(vinyl chloride) nanocomposites via solvent blending method

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Clay-polymer nanocomposites (CPN) developed in recent years as a new type of inorganic–organic hybrid materials that were conceived for medical uses such as tissue engineering or drug delivery [1],[2]. The understanding of the structure and physico-chemical properties of CPN is a first step in the investigation of biomaterials, but their potential in this respect is determined by their interaction with living tissue components. In this study, pure kaolinite was intercalated with dimethyl sulfoxide (DMSO) and then intercalated kaolinite was modified pyridine, 2-amino pyridine and 2,6-diamino pyridine to expand the interlayer basal spacing. Modified kaolinite samples as filler and poly(vinyl chloride) (PVC) polymer as matrix were used in the nanocomposite synthesis. Nanocomposites of PVC have been prepared by solvent blending method using THF as a solvent. The material characterizations were carried out by XRD, AFM, FTIR-ATR, DTA/TG and DSC. The XRD results reveal the formation of intercalation/exfoliation of modified kaolinite in the PVC matrix. FTIR and AFM results confirm the presence of nanomaterial in kaolinite/PVC nanocomposites. TGA data show that the modified kaolinit/PVC nanocomposites have significant enhanced thermal stability. The glass transition temperature (*Tg*) of PVC nanocomposites is higher than that of pure PVC. In addition, the antimicrobial activity of clay-polymer composites were also determined.

P-07.01.3-023
Utilisation of poly(3-hydroxybutyrate) from *Bacillus* marmarensis GMBE 72T (DSM21297) as scaffold

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Introduction: Polyhydroxyalkanoates (PHAs) are biocompatible and biodegradable materials obtained from microorganisms. They are produced in the cytoplasm of several bacteria as energy reserve. The physical properties of poly(3-hydroxybutyrate) (PHB), which is from the group of PHAs, make it a competitive source to petrochemical plastics. PHB has potential in order to be used in a variety of application fields such as packaging industry, printing materials, agriculture and food industry. Furthermore, PHB meets expectations for tissue engineering applications, since it is biocompatible, biodegradable, non-toxic and has good mechanical properties. Although its many advantages, blending approach could be needed in order to fulfill all expectations of a material. Due to its flexibility, polycaprolactone (PCL) is a promising candidate to be blended with PHB.

The aim of this study is to construct a scaffold by using PHB produced by extreme alkaliphilic *B. marmarensis* GMBE 72 ^{T} (DSM 21297) and commercial PCL as components and investigate its properties.

Materials and Methods: Electrospinning method was used in order to construct scaffolds from blend polymer solution containing PHB from *B. marmarensis* and commercial PCL.

Results: Nanofiber structures were observed on Scanning Electron Microscope (SEM) images and Fourier Transform Infrared Resonance (FTIR) analyses have shown characteristic peaks for both PHB and PCL.

Discussion and Conclusion: PHB could be blended with other polymers in order to enrich its properties. In addition, nanofiber structure of electrospun PHB-PCL blend makes it a rewarding material as scaffold for several tissue engineering applications.

P-07.01.3-024
Lipopolysaccharide-poly (N-vinyl-2-pyrrolidone-co-acrylic acid) complex synthesis for development of diagnostic kit for Q fever

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Q Fever is a zoonotic disease that is encountered widely around the world, the most common acute form of Q Fever shows the following symptoms; a sudden fever, shivering, lassitude, headache, anorexia. Because this disease does not show specific symptoms its diagnosis is possible with laboratory tests. Current diagnostic kits lack effectiveness; this is why the main goal of our studies is to come up with a new diagnostic kit that does not have disadvantages that current diagnostic kits show.

With this goal, Nine Mile I strain (RSA 493), S serologic virulent phase I, were obtained from Slovak Science Academy, Virology Institute for Rickettsia Reference and Research from WHO Co-operation Centre. These cells were purified and lipopolysaccharide (LPS) isolation from *Coxiella burnetii* was performed. The polymeric carrier, poly (N-vinyl-2-pyrrolidone-co-acrylic acid) [P(VP-co-AA)] was synthesized and characterized. Physical complexes of obtained LPS and P(VP-co-AA) with varying ratios. Ternary complexes of LPS-Cu^{2+}-P(VP-co-AA) were also synthesized with copper metal mediation. Structure and interaction of Lipopolysaccharide-P(VP-co-AA) complexes were investigated with Zeta-sizer device using zeta potential analysis and FTIR spectrophotometry according to the ratios of components, reaction environment conditions and chemical structure of the polymer.

The best complex ratio according to analysis results will be used in the future studies for obtaining monoclonal antibodies which will be an important step for obtaining more effective and stable diagnostic kits that can be used for Q Fever.

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Application Purpose of Vaccine Prototype and Diagnostic Kit' with the project number of 113Z938.

P-07.01.3-025
Synthesis of water soluble conjugates of anionic polyelectrolytes with peptides

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Development of water soluble polymer-biomolecule conjugate synthesis is one of the remaining issues for designing new biomedical preparations and development of more effective new generation vaccines.

In this study; new types of water soluble polymer-biomolecule conjugates were synthesized using covalent bonding techniques between polymers and co-polymers (varying monomers of polyacrylic acid and acrylic acid) with peptides. Different compositions of polymers, varying ratios of biomolecule/polymer and different molecular weight of polymer has yielded new types of bioconjugates. Conjugation mechanism, composition and structure were investigated with various physicochemical methods (UV, FTIR, HPLC, GPC, etc.). The peptide used in this study was the antigenic peptide epitope of sheep-goat pox disease (EAKSSIAKHFSLWKSYADADIKNSENK). Whether this peptide series was bound to polymers or whether it was bound to polymer-protein carrier; peptide-specific immunogens that were capable of producing antibodies were synthesised. It is thought that using polymer-peptide bioconjugates that contain just peptide will yield a higher peptide-specific immunogenicity compared to traditional adjuvants.

In vitro and *in vivo* investigations of bioconjugates effectivity is planned to be done in the future studies.

P-07.01.3-026
Bioinert fluorinated ethylene-propylene copolymer modified for keratinocyte adhesion

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Surface properties are crucial when adhesion of a cell to a polymeric material is required for a biomedical application. One of the methods for polymer surface tailoring is argon plasma treatment. This simple and reproducible method alters the surface properties such as roughness and wettability without affecting the bulk properties of the material.

For the modification of the bioinert fluorinated ethylene-propylene (FEP), related to Teflon®, we employed argon plasma treatment with the powers of 3 and 8 W for 20–240 s. The human keratinocytes of the HaCaT cell line served as a model cell line for biocompatibility testing. We studied adhesion, proliferation and morphology of the cells on modified FEP matrices as well as controls (pristine FEP and standard polystyrene tissue culture dish) by means of fluorescence microscopy. Further morphological details were acquired by scanning electron microscopy. Furthermore, fluorescence microscopy with immunochemical labelling was used to determine the size and distribution of focal adhesions in cells grown on the modified matrices. The overall effect of the matrices on metabolic activity of cultured HaCaT cells was also evaluated using the WST-1 reagent.

The Ar plasma treatment of FEP matrices improved significantly cell adhesion and proliferation and promoted spreading of the HaCaT cells compared to the pristine FEP, on which cells were not able to spread properly. Also, increased metabolic activity rates for cells cultured on modified matrices were found in comparison to the pristine FEP.

Altogether, we found that Ar plasma treatment improved the surface properties of FEP to such extent, that it allows cultivation of adherent cells on its surface. We therefore propose that Ar plasma treatment is a useful method for FEP surface modification.

P-07.01.3-027
Graphene oxide enriched biomaterials display potential for tissue engineering applications

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Tissue engineering (TE) requires more efficient systems that favor local tissue regeneration with minimum cytotoxicity. Materials based on natural compounds ensure biocompatibility and have better effects for regeneration. Graphene oxide (GO) has been shown to enhance cell adhesion and to improve the rate of cell differentiation. In this context, the aim of this study was to evaluate if the addition of different concentrations (0.25–1 wt.%) of graphene oxide (GO) improves the properties of cellulose acetate (CA) materials for biocompatibility and cell differentiation, in the prospective of using these films for TE applications.

GO-containing CA films were tested for cytocompatibility by quantitative and fluorescence microscopy assays, and compared to the CA control. Cell cytoskeleton architecture in contact with biomaterials was revealed by confocal microscopy. Furthermore, bioconstructs were exposed to in vitro osteogenic and adipogenic induction and monitored for 21 days. Histological stainings were performed to validate differentiation. Osteogenic and adipogenic markers gene expressions were assessed via qPCR.

CA/GO 1 wt.% revealed best biocompatibility among all tested scaffolds. Adhesion proved to be dependent on the percentage of GO in material's composition. Cells cultivated on CA/GO 1 wt.% expressed adipogenic and osteogenic markers earlier than cells cultivated on materials with lower GO content. Differentiation markers displayed an increasing profile of gene expression from 7 to 21 days post induction, with higher levels registered for materials with high GO content as compared to films with low GO content and to the control.

GO added to CA materials positively influenced cell survival, proliferation and cell differentiation. CA/GO films represent potential candidates for TE applications.

P-07.01.3-028
3D composite hydrogel scaffolds for driving the fate of human cardiac progenitor cells

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The design of appropriate scaffolds remains one of the most important challenges for TE. Current idea is that the cell-scaffold interaction could drive cell differentiation and be linked to gene expression and protein organization. Therefore, their quality is essential and should favour cell attachment, growth, migration, *in situ* vascularization and release of biochemical and physical factors able to address the cell fate. Moreover, for an ideal scaffolding material an adequate and interconnected porosity is relevant for facilitating cell spreading and colonization of the inner layers.

A combination of optimal mechanical and biochemical properties were here utilized to design a 3D Composite Hydrogel Scaffold (3D-CHS) in order to favour cell-scaffold interactions and promote a functional differentiation of human Lin^- $Sca1^+$ cardiac progenitor cells (hCPC). The biocompatible PEG-diacrylate (PEGDa) was used to prepare hybrid protein-PEGDa hydrogels with embedded albumin-microspheres (MS) as protein component. MS were able to modulate the mechanical and biological behavior of the scaffold acting as air-reservoir, porogen agents and potential carriers of biomolecules. An increase of the hCPC viability in the MS-concentration dependent manner was observed. Moreover, the microarchitecture of the 3D scaffold also plays a key role in the stability and functionality of cellular-composite systems. Therefore, PEGDa-honeycomb structures were fabricated using microstereolithography process and the hCPC viability and adhesion to the microstructures were assessed. 3D-CHSs were synthesized embedding honeycomb-structures into MS-PEGDa hydrogel and the effects on cell proliferation, cell-cell interactions and cellular alignment were investigated.

These results could be of relevant interest for expanding the knowledge on cell-scaffold interaction processes and to promote the development and the application of 3D-CHS for tissue regeneration using the emerging bioprinting technologies

P-07.01.3-029
Gene expressions of mesenchymal stem cells after osteogenic induction on ceraform bone substitute

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Ceraform®, is a synthetic calcium phosphate ceramic with the chemical composition of hydroxyapatite 65% and tricalcium phosphate 35%, with 60–85% pore volume and 100–400 μm pore diameter. In this study adipose tissue derived mesenchymal stem cells were differentiated into osteoblast cells and loaded on Ceraform®. In order to improve cell adherence, Ceraform® was covered with fibronectin. The cells were cultivated for 28-day period by osteogenic induction medium. Days 1, 7, 14, 21 and 28 were selected as specific intervals for incubations. Total RNA was isolated and cDNA was synthesized. Differences in the expression of runt-related transcription factor 2 (Runx2), bone morphogenetic protein-2 (BMP-2), and osteocalcin (OCN) were determined by qPCR. The peptidylprolyl isomerase A (PPIA) gene was used as an internal control. According to the qPCR results, OCN gene expression was observed on the day 14th, continued to increase in day 28. BMP-2 gene expression was increased in 14, 21, 28 day compared to 7 day. On the other hand, Runx2 gene expression was increased only on days 21 and 28. These findings pointed out that the osteogenic induction was successfully activated on FN coated bone material. Therefore, these results can be used in bone injury treatment and related disorders.

P-07.01.3-030
On the in vitro cytotoxicity of graphene oxide nanomaterials

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During the last decade, Graphene and its derivates have proven unique physicochemical properties, several applications being continuously developed. Among them, electronic, catalytic, mechanical, optical, and magnetic properties have attracted huge interests. However, the merging of graphene and graphene oxide (GO) with biotechnology is still in its infancy, many challenges remaining unexplored. Potential applications are related to biosensors, drug delivery or gene therapy and cells imaging.

In order to use GOs as drug release matrices for cancer cells targeting, it is necessary to ensure that these molecules do not affect normal cells within tissues. It was shown that the cytotoxicity of graphene nanomaterials is highly dependent on surface functionalization. Studies suggest that pristine and reduced GO with fewer surface functional groups tend to be more toxic than GO. In striking contrast, it has been reported that functionalized graphenes, can significantly reduce the cytotoxicity even at relatively high concentrations.

In this study, we report on the comparison between GO and protein functionalized GO when submitted to *in vitro* cytotoxicity tests. Bovine Serum Albumin (BSA) was used for the noncovalent GO surface conjugation. Three case-studies were investigated: aqueous nano-colloids consisting of serial dilutions of both GO and GO-BSA conjugates, dropcasted thin films and laser-assembled thin films on glass substrates. Safe concentration windows were identified by live/dead staining and MTS assays for different human melanoma cell lines, while melanocytes and human dermal fibroblasts were used as normality controls. The predominant melanoma subtype is represented by cells bearing BRAF (V600E) activating mutation. With a view to target this specific melanoma subpopulation, we embedded BRAF inhibitors into GO laser-deposited scaffolds and tested their anti-tumoral effect. Our results evidence the high potential of these nanomaterials for biomedical applications.

P-07.01.3-031
Use of hydroxyapatite specific elastin-like polypeptide in targeting of PEI-siRNA complex loaded PLGA nanocapsules to bone tissue

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Osteoporosis is a skeletal disorder associated with low bone mass and increase in bone fragility due to increased osteoclastic activity. Binding of RANK ligand to its receptor on osteoclast precursor cells results in the osteoclast differentiation. siRNA is a dsRNA, used to inhibit the translation of the target gene. The aim of the study is to develop an injectable siRNA-delivery system targeted to the bone for osteoporosis treatment. PEI (polyethyleneimine) and RANK complex was loaded into poly(lactic acid-co-glycolic acid) (PLGA) nanocapsules which are bound to hydroxyapatite (HAP)-specific elastin-like protein (ELP) for targeting to bone tissue.

PLGA nanocapsules were prepared by w/o/w double emulsion technique. Affinity of ELP to HAP was determined by FTIR. ELP was coated on the nanocapsules by using the transition temperature of ELP. ELP on PLGA nanocapsules were crosslinked by genipin and binding of ELP on PLGA nanocapsules were studied by XPS and TEM. The optimum ratio of N (PEI) to P (siRNA) in the complexes to be loaded into PLGA nanocapsules were studied by EtBr staining and zeta potential measurements with varying N/P ratios and finally PEI-DNAoligo encapsulation efficiency of the capsules was determined by PicoGreen Reagent.

XPS results of ELP treated PLGA (ELP-PLGA) nanoparticles indicated the presence of nitrogen atom (11.7%) in the sample which appeared as a fuzzy halo in TEM micrographs.

N/P ratios up to 5 show negatively charged particles. When N/P was 5, the zeta potential of complex was neutralized which also resulted in larger particles compared to the others. Zeta potential moved to positive values when N/P was higher than 5.

The migration of polyplexes with different N/P ratios (1–10) was analyzed by gel electrophoresis. DNAoligo complexes show the same patterns of complexation wih that of siRNA and thus were used in the encapsulation efficiency studies instead of siRNA. The encapsulation efficiency of PEI-DNAoligo in PLGA nanocapsules was 29%.

Tuesday 6 September 12:30–14:30

Aging

P-09.03.3-001
Novel benzenesulfonamides exhibit low toxicity on zebrafish embryonic development and selectively inhibit carbonic anhydrase IX with nanomolar affinity in *Xenopus* oocytes

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Introduction: The toxic effects of two recently discovered inhibitors (VD12-09 and VD11-4-2) that selectively and with extraordinary strong, picomolar affinity bind to human carbonic anhydrase (CA) IX, an anticancer target, were investigated on zebrafish embryonic development and in *Xenopus laevis* oocytes. Zebrafish has emerged as a promising animal model to evaluate the toxicity of the drug candidates. *Xenopus* oocytes do not natively possess any CA activity and thus became a convenient *in vivo* model system to study the pH effects and the selectivity of synthetic CA inhibitors.
Materials and Methods: Morphological changes in zebrafish treated with the compounds were studied by light-field microscopy and histological analysis. CA activity in *Xenopus* oocytes was monitored by measuring pH in the cytosol and at the outer membrane surface and confirmed by mass spectrometry of lysed oocytes.
Results: The toxicity studies showed LC50 values to be 13 μM for VD12-09, 120 μM for VD11-4-2 and 9 μM for ethoxzolamide (EZA), a non-selective CA inhibitor commonly used in clinic. The zebrafish exposed to LC50 doses of VD12-09 and VD11-4-2 showed fewer phenotypic abnormalities and less morphological changes compared to the zebrafish treated with the corresponding dose of EZA. VD11-4-2 exhibited 10–25 nM IC50 for both intracellularly and extracellularly expressed CA IX in *Xenopus* oocytes while exhibiting strong selectivity over CA II, CA IV and CA XII.
Discussion: Interestingly, the compounds exhibited 10-fold lower toxicity, induced fewer side effects in zebrafish than EZA and the amount of VD11-4-2 needed to cause complete inhibition of CA IX enzymatic activity in *Xenopus* oocytes was 30-fold lower than EZA.
Conclusions: VD compounds did not lead to deleterious effects on the zebrafish embryonic development and reached the IC50 of 10 nM for CA IX in *Xenopus* oocytes. The compounds could be further developed as anticancer drugs.

P-09.03.3-002
Interplay between CacyBP/SIP phosphatase and factors involved in the immune response

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CacyBP/SIP is present in various cells and tissues, both normal and pathological. In normal tissues, e.g. stomach or colon,

CacyBP/SIP is weakly or barely detected whereas in gastric or colon cancer this protein is expressed at a high level. There are also data indicating that the level of CacyBP/SIP expression correlates with tumor metastatic potency and multidrug resistance. Taking into consideration data that suggest association of CacyBP/SIP with many vital cellular processes, in this work we decided to investigate the possible mechanism involved in regulation of CacyBP/SIP gene expression, mainly by transcription factors and, on the other hand, the influence of CacyBP/SIP on the expression of other genes. We have shown that NFAT (Nuclear Factor of Activated T cells) influences the CacyBP/SIP gene expression and that overexpression of CacyBP/SIP has an effect on the level of AP-1 and on the activity of NFAT and AP-1 transcription factors. By analyzing the *CacyBP/SIP* gene promoter sequence we also found potential binding sites for transcription factors from the STAT family, which are involved in interferon signaling. Microarray data indicate that indeed overexpression of CacyBP/SIP affects levels of the STAT proteins as well as of some interferons and interleukins. Based on functional analysis we have found many genes the products of which are involved in immune response. To analyze in more detail the influence of an altered level of CacyBP/SIP on interferon signaling pathways as well as on factors involved in expression of interleukins, including NFkappaB, we plan to apply methods such as luciferase assay, real-time PCR or immunocytochemistry.

P-09.03.3-003
The extracellular Aβ levels are reduced by the cholinesterase inhibitor toluidine blue O in an Alzheimer's disease-like cellular model

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One of the pathological hallmarks of Alzheimer's disease (AD) is the neuritic plaques occurred as a result of the extracellular accumulation of Aß peptides formed from amyloid precursor protein (APP) via the ß-amyloidogenic pathway. Aß42 is more prone to aggregation to form plaques and more toxic to neurons than Aß40. In addition to change in APP metabolism, the decline in levels of neurotransmitter acetylcholine and cholinergic dysfunction are also observed in AD. Thus, current strategies for AD treatment focus on compounds with inhibitory effect on cholinesterases as well as preventive effect on Aß aggregation.

In our earlier studies, toluidine blue O (TBO), a phenothiazine dye, was shown to be a highly effective inhibitor of cholinesterases with K_i values in nM range. We also found that intracellular APP and Aß42 levels are reduced in human neuroblastoma cells after treatment with TBO. Additionally, an earlier study revealed that TBO has a selective inhibitory effect on tau aggregation, the other pathological characteristic of AD. The aim of this study was to investigate whether TBO may effectively lower the level of extracellular Aß40/42 in an AD-like cellular model.

Chinese hamster ovary cells that express human wild type APP and presenilin 1, namely PS70, were treated with a dose range of TBO (0–15 μM) or vehicle control for 24 h. After treatment, Aß40/42 levels in cell culture media were assayed by separate sandwich-based ELISAs and normalized to total protein levels, determined by BCA protein assay. Besides, biocompatibility of TBO was evaluated in the PS70 cells using cell viability assay for flow cytometry.

Strikingly, all dose ranges of TBO inhibited both Aß40 and Aß42 secreted into the cell culture media. Significant reduction for both Aß species was evident at 5 μM ($P < 0.05$), 10 μM ($P < 0.001$), and 15 μM ($P < 0.001$) of TBO vs. vehicle control. In conclusion, these results support the idea that TBO may be used as a therapeutic in AD.

P-09.03.3-004
Monitoring the changes of key molecules participating in the osmo-regulatory response of nucleus pulposus intervertebral disc cells during stress-induced senescence

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Introduction: Intervertebral disc cells are faced with a harsh extracellular milieu characterized by hyperosmotic conditions, nutrient and oxygen deficiency because of the absence of vascularization and oxidative stress due to the accumulation of their metabolism's by-products. We have previously shown that high osmolality is anti-proliferative for disc cells through the activation of the G1 and G2 cell cycle checkpoints by p53 and p38 MAPK, respectively. In addition, we have shown the participation of nine solute transporters, with the α1 subunit of Na^+/K^+-ATPase being central in this response. Here we assessed the changes in the expression of these key osmo-regulatory molecules during *in vitro* stress-induced senescence.

Materials and Methods: Changes in cell cycle progression were assessed using flow cytometry; overall transcriptional alterations were assessed by whole-genome arrays; differences in expression at the mRNA and protein level were revealed by quantitative RT-PCR and western blotting, respectively; knocking-down of selected proteins was performed by siRNA.

Results: High osmolality led to the differential expression of > 200 genes, including nine genes encoding transporters. p38 MAPK and p53 were demonstrated to differently participate in the regulation of the aforementioned transporters, while knocking-down of three selected transporters had a distinct outcome on the overall cellular response towards hyperosmotic stress. These molecules were found to show differences in their expression in senescent cells.

Discussion: Given that the presence of senescent cells has been demonstrated in the intervertebral disc *in vivo* and could most probably attributed to the prevailing stressful conditions, here we showed differences in the expression profile of known key molecules for osmo-adaptation during senescence.

Conclusion: Understanding disc cells' physiology is of outmost importance when designing cell-based therapies for disc degenerative disorders.

P-09.03.3-005
SMAD specific E3 ubiquitin protein ligase 2 (Smurf2) and its potential effects on inhibitory transmission in aging

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SMAD Specific E3 Ubiquitin Protein Ligase 2 (Smurf2) is part of the TGF-β signaling pathway associated with cellular proliferation, differentiation, genomic stability and senescence.

Moreover, Smurf2, via its downstream partners, may regulate inhibitory synaptic transmission. Our research group previously found that the Smurf2 transcript is significantly higher in old zebrafish brains. Thus, Smurf2 may alter inhibitory synaptic transmission in aged animals. The focus of this study was to examine age-related changes in Smurf2 protein levels and related key inhibitory synaptic proteins; gephyrin (GEP), a scaffolding protein for GABA receptors, and $GABA_A$, an ionotropic GABA receptor subtype. Additionally, the levels of those proteins were studied in a mutant zebrafish line, which lacks acetylcholinesterase (ACHE) and is suggested to be a delayed aging model.

Whole brain tissues were isolated from young, middle-aged and old male and female zebrafish brains (AB/wildtype strain), as well as from old male and female ACHE mutant zebrafish ($ache^{sb55/+}$). Animals were maintained and raised in standard conditions. The extracted brain tissue was homogenized in RIPA buffer and subjected to Western Blot analysis to determine differences in the relative protein expression levels.

Our preliminary data indicated that Smurf2 and GEP levels remain stable in the aging brain ($P = 0.301$, $P = 0.335$), and in the ACHE mutants GEP levels are increased compared to the wildtype controls ($P = 0.001$). Further analysis of the relationships between Smurf2 and $GABA_A$ levels and brain aging is ongoing.

We predicted that alterations in Smurf2 levels would parallel changes in key synaptic inhibitory proteins during the aging process, which was the case for the GEP levels. While Smurf2 may regulate inhibitory synaptic transmission, the exact roles of those synaptic proteins in the context of normal and delayed brain aging are not known well-understood and the subject of continuing study.

*With equal contribution.

P-09.03.3-006
Investigation of association between genetic variants of toll-like receptor 2 and 4 and ischemic heart disease in Kazakhstan population

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In recent years, express the hypothesis that aged individuals are vulnerable to infectious and other inflammatory agents and they become more prone to develop majority of severe age pathologies, including cardiovascular and oncology diseases, neurodegenerative diseases, type 2 diabetes mellitus and inflammatory diseases, etc. One of the central components of immune response is the family of toll like receptors (TLR). There are several opinions that single nucleotide polymorphisms (SNP) leading to a loss of function of the respective TLRs can be associated with age and increase the risk of age related diseases, especially cardiovascular diseases (CVD). However, many available studies focusing on TLR SNPs and CVD are with conflicting results. The aim of this study was to assess the potential interaction between genetic variants of TLR2 and TLR4 and Ischemic heart disease (IHD) in Kazakhstan population over 45 years old. We evaluated 148 patients with IHD and 144 healthy subjects aged 45 years and over (ethnical Kazakhs and Russians living in Republic of Kazakhstan). Polymorphic loci of the genes TLR2 rs5743708 and TLR4 rs4986790 were genotyped by PCR with subsequent restriction analysis. Our results indicated that the genotype and allele frequencies of TLR2 (Arg753Gln) and TLR4 (Asp299Gly) were not significantly different between the 2 groups ($P \geq 0.05$). Statistical analysis didn't elicit any association between studied gene polymorphisms and predisposition to IHD in individuals over 45 years old ($P \geq 0.05$). For these polymorphisms, age, fasting blood sugar and serum lipid levels were not also significantly different among different genotypes in the IHD and control groups. In conclusion, the data shows that there is no interaction between TLR2 and TLR4 and Ischemic heart disease (IHD) in Kazakhstan population over 45 years old. We plan to include other types of polymorphisms in TLR 2 and TLR 4 genes and Increase the volume of patient cohort in our future studies.

P-09.03.3-007
Evaluation of prognosis with total oxidant/antioxidant status and some oxidative stress parameters in patients with acute ischemic stroke

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Stroke is the third most common cause of death after coronary heart disease and cancer. Strokes are classified into two groups according to their pathology: ischemic stroke and hemorrhagic stroke. Ischemic strokes make up 87% and hemorrhagic strokes 13% of all strokes. During ischemic stroke, Oxidative stress has been shown to play a major role in the occurrence and progression, formed oxidants also affect cell membranes and genetic material such asDNA, RNA, and various enzymatic events, and they lead to cell damage. Some studies have shown oxidant-antioxidant status but have not shown the relationship with prognosis. This study has investigated the relationship between prognosis and total oxidant/antioxidant status and biochemical parameters in patients with acute ischemic stroke

58 patients, with acute ischemic stroke and 37 healthy controls we reenrolled in the study. Blood samples were taken within 1st and 7th days, and after 3rd months in the patient group for analysing serum total oxidant status (TOS), antioxidant status (TAS), catalase, arylesterase, and thiol. Prognosis was evaluated with National Institutes of Health Stroke Scale (NIHSS) andmodifiedRankinScale (mRS) scores.

There was no significantly difference between groups by means of serum TAS, TOS and catalase levels. But arylesterase (P: 0.07) and thiol (P: 0.031) levels were significantly higher in first 24 h blood samplingthancontrolgroup. Statistically significant negative correlation was observed between the 3rd month values of TOS and NIHSS score ($r = 0.410$, $P = 0.037$). But there was no correlation between mRS scores and serum TAS, TOS, catalase, thiol and arylesterase. Similarly, our findings suggested some serum oxidant levels were increased in acute ischemic stroke patients and total oxidant status might be used in evaluation of prognosis but larger studies are needed.

P-09.03.3-008
Amylin and preptin regulate glucose homeostasis in infertile women with polycystic ovary syndrome and poor responders undergoing IVF/ICSI

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Disrupted glucose homeostasis leads not only metabolic disturbance such as polycystic ovary syndrome (PCOS), but also influences oocyte growing. This study was designed to evaluate follicular fluid (FF) and serum levels of glucoregulatory hormones, amylin and preptin, in infertile women with PCOS and poor responders undergoing IVF/ICSI. Human follicular and serum were obtained from 20 infertile women with PCOS and 20 poor responder participants undergoing controlled ovarian stimulation (COS) with gonadotropin-releasing hormone antagonist protocol for IVF/ICSI treatment. FF and serum amylin and preptin levels were measured by ELISA. It was found that FF and serum amylin and preptin were lower in infertile women with PCOS when compared with poor responder participants. FF amylin and preptin concentrations were lower than that of the serum amylin and preptin concentrations. Decreased follicular fluid amylin and preptin levels suggest that amylin and preptin may have a physiological role in follicular maturation via controlling local glucose homeostasis. Despite high serum levels of amylin and preptin in PCOS their low concentration within the follicle may be main culprit of defective folliculogenesis seen in PCOS subjects. Similar to insulin resistance in PCOS subjects existence of amylin and preptin resistance support the critical role of both peptides in follicular maturation in PCOS.

Keywords: Follicular fluid; amylin; preptin; polycystic ovary syndrome; infertility.

P-09.03.3-009
The transcription initiation on P3 promoter of Xp10 bacteriophage in presence of p7 protein, a modulator of RNA-polymerase activity

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Many bacteriophages are able to manage the transcription system of their bacterial host for their own needs. For example, bacteriophage Xp10, in the early stages of infection of *Xanthomonas oryzae* inhibits transcriptional activity of bacterial RNA-polymerase on majority of promoters via p7 protein, except of bacteriophage P3 promoter responsible for expression of the bacteriophage 'middle' class genes.

The focus of this work is to study the mechanism of action of p7 protein in the transcription initiation and identification of the role of the individual elements of P3 promoter of Xp10 bacteriophage, enabling *X. oryzae* RNA-polymerase escapes inhibition by p7 protein.

We have designed a set of promoter probes representing the combination of sequences of p7-resistant P3 promoter and p7-sensitive T5N25 promoter. Using FRET-based assay it was shown that the truncated probes corresponding to promoter DNA downstream −26 position, relative to the transcription initiation start site, did not lead to dissociation of the sigma-factor. Longer probes, containing −35 promoter element, induce dissociation sigma-factor. The *in vitro* transcription experiments show that the deletion of region 4, a sigma-factor domain responsible for interaction with −35 promoter element during the transcription initiation, is not critical for inhibition of RNA-polymerase by p7 protein. Promoter probe with UP-element of P3 promoter had affinity to *X. oryzae* RNA-polymerase a several times higher than a probe containing the consensus UP-element for *E. coli* RNA-polymerase .

Summing up the results, it seems like the transcription initiation on P3 promoter of bacteriophage Xp10 can escape inhibition by p7 protein through a high affinity interaction between the UP-element and C-terminal domains of the alpha subunit of RNA-polymerase *X. oryzae*.

P-09.03.3-010
Distribution of soluble form of glial fibrillar acidic protein in the different areas of gerbils brain during development and aging

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Astrocytes are the most abundant cell type within the CNS and play an important role in CNS homeostasis and function. Glial fibrillary acidic protein (GFAP) forms the main astrocytic intermediate filament (IF). The overall level GFAP in different parts of the brain uneven and depends on the number of astrocyte cells. GFAP is very sensitive to any kind of neurodegenerative diseases and aging. During aging, a glial reaction is observed in the human brain, as well as in rat and mouse brains. The aim of our study was to investigate the quantitative astrocytes-specific protein GFAP in different areas of the gerbils brain at the first stages of postnatal development and aging. For the study 30 gerbils brains were used and divided into 5 groups ($n = 6$): 1: newborn animals (1 day), 2–4: 30, 90 and 180 days respectively, 5: animals aged 2 years. The animals were decapitated under mild anesthesia (thiopental), with isolated brain three divisions: the cerebellum, thalamus and hippocampus, which are then used to produce cytosolic protein fractions. The level of GFAP in the obtained fractions were determined according to the method of competitive ELISA. Newborn gerbils found no significant content of soluble form of glial fibrillar acidic protein in all investigated parts of the brain, and a sharp increase of amount within 30 days (in cerebellum – amounted to 1.12 ± 0.03 μg/100 mg tissue; to 90–180 days increased to 1.67 ± 0.11 μg/100 mg tissue, and began to grow again in older individuals aged 2 years). Unlike the cerebellum, the level of sGFAP in hippocampus and thalamus reached the maximum at 30 days p.d. (1.5–1.7 μg/100 mg tissue), and unchanged for 180 days. These results revealed that the most intensive development of astrocytes in the cerebellum to 90 p.d. of gerbils, and in the thalamus and hippocampus are formed within the first month of life.

Wednesday 7 September 12:30–14:30

Plant biochemistry and molecular biology

P-02.08.5-001 Whirly1 is involved in establishing an euchromatic status at HvS40 locus during drough stress induced leaf senescence in barley (*Hordeum vulgare* L.)

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The plastid-nucleus located protein Whiry1 acts as an upstream regulator of leaf senescence binding to the promoter of senescence associated genes (SAGs) like senescence marker gene *HvS40*.

In order to investigate the impact of Whirly1 on drought stress-induced senescence, transgenic barley plants with a knockdown of Whirly1 (*Hvwhy1kd*) were grown under untreated and drought stress conditions. The leaf senescence evolution was monitored by physiological parameters and gene expression studies of senescence and drought stress related genes. To reveal the epigenetic indexing at *HvS40* at onset of drought-induced senescence in wild type (WT) and *Hvwhy1kd* lines, stress-responsive loading with histone modifications at 6 gene regions of *HvS40* (2 regions in the promoter, one region around translation start site and 3 regions located in the gene body) was analysed by ChIP and quantified by RTq-PCR.

In barley, drought treatment caused acceleration of leaf senescence in wildtype (WT) plants, whereas *why1kd* lines showed a staygreen phenotype. Expression of senescence-associated and drought stress responsive genes expression was delayed in *Hvwhy1kd* indicating that Whirly1 protein acts as an upstream regulator of drought stress-induced senescence. The ChIP results showed that drought treatment is causing in WT a significant increase in the levels of H3K9ac all over the analyzed gene regions, correlating with a massive induction of *HvS40* expression, while drought stress caused no substantial increase of H3K9ac in *why1*kd plants.

The results suggest that drought induced expression of *HvS40* is under epigenetic control, and furthermore that WHY1 is involved in this epigenetic control level.

P-02.08.5-002 Anticancer activity of buckwheat burn virus

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Oncolytic viral therapy is based on the capabilities of selective lysis of tumor cells and is a prospective trend in cancer disease treatment. In vitro experiments showed that plant Rhabdoviruses does not have any direct cytotoxic effect upon Sarcoma 37 cells, causes induction of apoptosis in these cells and does not pose any threat to somatic cells of warm-blooded animals, which makes it possible to use this virus for therapy of malignant neoplasms. Buckwheat burn virus (BBV), the prototypic member of the family Rhabdoviridae, contains surface glycoprotein and which is lectin-active. Its carbohydrate branch can aid adhesion of lymphocytes to tumor cells. The present study has addressed the effect of BBV on cancer cell viability. All studies were carried out after 1 week of inoculated with Erlich Cancernome (2×10^6 cells/animal, i. p.) in 2 months male Balb/C mice treated at once with or without plant extract with BBV (15 mg/kg, i. p.). By fluorescent microscopy and using two due staining by acredine orange and propidium iodide it was found that in the 3rd day of administration of BBV lead to increasing of necrotic and apoptotic cells on 45% and 4% respectively versus to untreated group. At the same time the viability of investigated cells was impaired too and according to flow cytometry analysis using propidium iodide the amount of dead cells was elevated by fivefold (17.7% versus 3.5% in untreated group). Also as was shown in previously reports BBV decreased activity of macrophages in the early stages after injection and it may have a positive effect when using this drug in tumor therapy. When using this drug appears to slow down the possibility of a sharp activation of macrophages, and as a consequence of the development of cytotoxic effect will be prolonged.

Key words: Rhabdoviruses, buckwheat burn virus, cancer, cell viability.

P-02.08.5-003 Antimicrobial activity of some plant materials used in Armenian folk medicine

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Plants are considered as one of most promising sources for new antimicrobials, based on the evidence of their use in folk medicine to treat various infectious diseases since ancient times. Despite relatively small area size, Armenia has large diversity of flora with many endemic species. The main goal of this study was the screening of various parts of 28 herbs (widely being used in Armenian folk medicine) for their antimicrobial activities in order to select most prospective plants for further comprehensive studies.

Plant crude extracts were obtained with maceration technique using five solvents: water, methanol, chloroform, acetone and hexane. Agar well diffusion assay was used to evaluate antimicrobial properties of plant crude extracts at 500 μg/ml concentration against *Escherichia coli* VKPM-M17, *Pseudomonas aeruginosa* GRP3, *Bacillus subtilis* WT-A1, *Salmonella typhimurium* MDC 1754 and *Staphylococcus aureus* MDC 5233, *Candida albicans* WT-174 and *Candida guilliermondii* HP-17. Statistical analysis was done using GraphPad Prism 5.03.

Crude extracts of all tested plant materials expressed antimicrobial activity against at least one test strain. Most of the tested extracts inhibited growth of both Gram-negative and Gram-positive bacteria. In contrast, only some plant materials exhibited inhibitory activity against yeast strains. According to obtained data *Sanguisorba officinalis*, *Rumex confertus*, *Hypericum alpestre*, *Lilium armenum* and *Agrimonia eupatoria* possessed the highest and broadest antimicrobial activity. Moreover, the results showed that acetone was the most effective solvent for solubilizing antimicrobial compounds from plant materials followed by methanol, chloroform, hexane and water.

The results demonstrated high antimicrobial activity of medicinal plants used in Armenian traditional medicine. Five plant species were selected for further comprehensive studies. Besides, acetone was proposed as efficient solvent in antimicrobial screening protocols.

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P-02.08.5-004
Effects of aluminum stress on photosystem-I apoprotein A2 gene (psaB) transcription level in lichen *Xanthoria parietina* (L.) Th. Fr.

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In this study the effects of shortrerm aluminium (Al) toxicity on the lichen *Xanthoria parietina* (L.) Th.Fr. were investigated at physiological and transcriptional level.

Lichen thalli were treated with $AlCl_2$ in different doses (0.25, 0.5, 1 and 5 mM). Lipid peroxidation and chlorophyll integrity were determined by spectrophotometer. Expression level of psaB gene was also investigated.

Chlorophyll a content was significantly ($p < 0.05$) decreased after 48 hours treatment with 1 mM and 5 mM of Al, while chlorophyll b content was increased significantly due to treatment with increased concentration of aluminum. Also treatment with 0.25 and 0.5 mM Al for 24 hours increased the gene expression level of *psaB* by 35.6% and 21.3% respectively.

Our results indicated that aluminum treatment has decreased the chlorophyll biosynthesis and increased the lipid peroxidation depending on time and concentration. This study also demonstrates that the PSI can be readily photo-inhibited by aluminum stress.

In conclusion, 5 mM Al exposure for 48 hours could damage the electron transport in Photosystem I.

P-02.08.5-005
Nigella sativa reduces paracetamol-induced nephrotoxicity and oxidative stress in rats: biochemical evaluation

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Background: *Nigella sativa* L. (Ranunculaceae) (NS) is traditionally used to treat many conditions such as inflammation. This study evaluates the effects of NS seeds ethanol extract in paracetamol-induced acute nephrotoxicity in rats.
Material Method: Forty-eight female Wistar Albino rats were divided into eight groups: I = sham; II = sham + 1000 mg/kg NS; III = sham + 140 mg/kg (N-acetyl cysteine) NAC; IV = 2 g/kg paracetamol; V = 2 g/kg paracetamol + 140 mg/kg NAC; VI, VII and VIII = 2 g/kg paracetamol + 250, 500 and 1000 mg/kg NS, respectively. Paracetamol administration (oral) was carried out 1 h after NS and NAC administrations (oral), and all animals were sacrificed 24 h later.
Result: Urea and creatinine levels were determined in serum, while glutathione, malondialdehyde levels and superoxide dismutase activity were determined in the kidney tissues. There were significant increases in the serum levels of urea and creatinine in the paracetamol-administered group. Serum levels of urea and creatinine were decreased in all groups administered NS with paracetamol. NS administration dramatically restored SOD, GSH, and MDA levels in the kidneys.
Conclusion: The results suggest NS has a significant nephroprotective activity on paracetamol-induced nephrotoxicity. It may be suggested that the antiinflammatory and antioxidant effects of NS ethanolic extract originated from different compounds of its black seeds.

P-02.08.5-006
The study of problems of preservation of the birches

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Nature of deciduous trees have a whole range of various medicinal properties. Instead of synthetic hormone substitutes, you can use medicinal infusions and decoctions of natural phytohormones are widely used in both folk and professional medicine. One of these plants is birch, its young leaves and buds. However, they also must be used with caution because overdose of these compounds is very dangerous, not only can you not get the desired effect, but also face the opposite of his action. In our research to mass replication of plants (different types of birches (Betula ajanensis, Yarmolenko, Jacguemontii, Maximowiczii, ulmifolia, Middendorffii, Kelleriana, tianschanica)) we use nutritional medium excluding the application of phyto promoters in order to prevent mutation. The object of research serve as the old, the sick, being on the verge of extinction, mature trees as explant meristema. Since from the moment of calling experience and most cultivation occurs at nutritional medium without hormones. As a result of molecular analysis we get without virus, genetically identical plants.

Molecular certification of different types of birches of interest, both in terms of organizing, and in terms of selection and genetic improvement of valuable forms, identification of lines selected from natural populations and clones obtained in vitro. Relationship between clones and installed parent form by comparing profiles amplific PCR products using ISSR-marking. According to the results of carried out works really recovered clones obtained from one source tree, indicating the potential for certification of clones studied forms of birches PCR. A study performed in the framework of the State grant project "Conservation of breeding valuable species of birches".

P-02.08.5-007
Fractionated triterpenoid glycosides from sea cucumber inhibit invasion and metastasis in human cancer cells

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Sea cucumbers are slow-motioned invertebrates. Holothuria polii Delle Chiaje, 1824 is widely distributed sea cucumber in İzmir coastline (Turkey). It secretes saponins i.e. triterpenoid glycosides (TTG) as secondary metabolites. The aim of this study is to evaluate anti-invasive and anti-migrative effects of fractionated TTGs obtained from H. polii on HT-29, T84 and UPCI-SCC-131 cancer cell lines. The semi-purified TTGs was extracted from H. polii collected from coast of İzmir-Dikili. The four different fractions (fraction A-D) were collected by using HPLC (High-performance liquid chromatography) and characterizated with MALDI-MS/MS. The fractions obtained from H. polii extract include Holothurin A (1243.50 m/z) and 24–dehydroechinoside A, Scabraside A or Fuscocinerosides B/C isomer (1227.50 m/z). Anti-

invasive and anti-migrative effects of the fractions on the cancer cell lines were detected with xCELLigence RTCA DP system. The results showed that Fraction A-D inhibited migration and invasion of human cancer cell lines at 6th and 12th hours compared to control group. This study shows that Holothurin A, 24–dehydroechinoside A, Scabraside A or Fuscocinerosides B/C isomer could be evaluated as promising anti-cancer agents for human cancers.

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P-02.08.5-008
Alternative splicing regulation of SR proteins in response to environmental stress in Chinese cabbage

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Serine/arginine-rich protein (SR protein) family, which acts as RNA-binding protein, plays a major role in post-transcriptional regulation of pre-mRNA, such as alternative splicing (AS). These proteins cause pleiotropic effect by regulating AS of pre-mRNA in a tissue and developmental stage-specific and stress-responsive manner in *Arabidopsis*. Here, we identified 31 genes encoding SR proteins in Chinese cabbage (*Brassica rapa* Chiifu-401) from *Brassica* database and analyzed their phylogenetic relationship. *B. rapa* has 31 types of SR protein that are classified into common (SR, RSZ and SC) and plant specific (SCL, RS2Z, RS and SR-like) subfamily similar with *Arabidopsis*. Interestingly, the AS pattern of most *SR* genes changed at the late stage (14 and 21 days after germination). To verify the correlation between *SR* genes and environmental stress, we screened the AS pattern of *SR* genes to various abiotic stress using RT-PCR and a microarray analysis. In particular, the expression level and the AS pattern of Bra015576 and Bra018581 were affected significantly by heat stress. These results suggest that the AS regulation by SR protein correlates with adaptation mechanism to the environmental stress in Chinese cabbage.

P-02.08.5-010
Characterization of recombinant Prolyl oligopeptidase from *Myxococcus Xanthus* and potential use in gluten hydrolysis

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A recombinant Prolyl oligopeptidase from *Myxococcus xanthus* was purified with a specific activity of 224 U mg(-1) by using Nickel-Metal-Chelate affinity chromatography and gel permeation chromatography. The recombinant enzyme had a monomeric molecular weight of 70 kDa. Its isoelectric point, determined by two dimension polyacryl-amide gel electrophoresis, was close to 6.3. The optimum pH and temperature was estimated as 7.5 and 37 °C, respectively. The purified enzyme was stable from pH 6.0–8.5 and able to thermal stability up to 37 °C. The K(m) and V(max) values were 0.2 mM and 3.42 micromol/min per mg. The enzyme exhibited hydrolytic activity for Suc-Gly-Ala-pNA, Suc-Gly-Pro-pNA, Z-Gly-Pro-pNA, IGF-1, Substance P, whereas no activity for H-Gly-Pro-pNA, H-Val-Ala-pNA, H-Arg-Pro-pNA, H-Ala-Pro-pNA, Glu-Ala-pNA, Pro-pNA, Leu-pNA. Its proteolytic activity was inhibited by active-site inhibitors of serine protease, Z-Pro-Prolinal PMSF, and metal ions, Cd^{2+} and Hg^{2+}.

The potential use of the enzyme was tested by the hydrolysis of the wheat gluten. The resulting gluten hydrolysate were characterized by means of their antioxidant, antibacterial, trypsin inhibition and Prolyl oligopeptidase inhibition activities.

Keywords: Serine protease, Prolyl oligopeptidase, Bioactive peptides, 2,4,6-trinitrobenzene sulfonic acid.

P-02.08.5-011
The development of barley seed protein library for targeted analyses

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Proteomic analysis is probably the best approach to analyze seed germination. However, it is difficult to analyze complex samples and there are many obstacles that must be faced in order to achieve a reasonable proteome coverage. For example, the barley (*Hordeum vulgare*) genome was fully sequenced in 2012, but the UniProt database contains less than 1000 reviewed sequences, which is approximately 16-fold less than for *Arabidopsis thaliana*. Here, to improve the barley proteome coverage, we employed several fractionation methods including polyethylene glycol precipitation, strong cation exchange chromatography, Off-Gel separation, SDS-PAGE and acetonitrile elution gradient. Proteomic analyses were performed using an LC-MS-based analyses and an UHR q-TOF mass spectrometer. The candidate peptides were targeted via Selected Reaction Monitoring (SRM) and Triple-stage quadrupole (TSQ) mass spectrometer. In total, 4092 proteins were identified, which represents a three- to four-fold increase compared with the standard shotgun analysis of the same sample. Out of these, 2240 were only accessible by one of the techniques and, besides, the detection limits were not similar. We hypothesized that an SRM-based targeted analysis will allow detection and quantitation of most of these proteins, even without the application of proteome fractionation. We can conclude that all peptides from the library with MS/MS spectra of the total intensity above 10,000 are easily detectable in the total protein extracts.

P-02.08.5-012
Transcriptome sequencing based identification of alternative oxidase genes in white water-lily, *Nymphaea alba*

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Alternative oxidases (AOXs) are the terminal oxidases in the respiratory electron transport chain of plants. They reduce molecular oxygen to water with low proton translocation across the inner mitochondrial membrane. In plants, AOXs increase local tissue temperature to release volatile compounds thereby attracting pollinator insects and regulation of mitochondrial retrograde signaling pathway. Regulation of retrograde signaling pathway is currently under investigation to improve cultivation studies in many plants. Water lilies are aquatic ornamental and economically valuable plants classified under *Nymphaea* family. *Nymphaea alba*, white water-lily, has a special focus since its applications in landscaping of parks and gardens, farming as vegetable and medical applications. However, cultivation of *N. alba* is a challenging process. We hypothesized that by controlling alternative oxidases, success rate can be increased for *N. alba*

cultivation. To identify alternative oxidase encoding genes in *N. alba,* we performed transcriptome analysis. By using transcriptome analysis data, *AOX* gene sequences, subcellular localization of AOX proteins and structural modelling of AOX proteins were predicted. In 272934 transcripts, database search with Trinotate tool revealed 77 transcripts with AOX domains characterized in known alternative oxidases. Blast analysis of these 77 sequences with known AOX proteins revealed three distinct *AOX* genes (*Nalba-AOX1, Nalba-AOX2* and *Nalba-AOX4*). After subcellular localization analysis of three identified AOX proteins by using TargetP server tool, *Nalba-AOX1, Nalba-AOX2 are predicted as mitochondrial while Nalba-AOX4 is localized in chloroplasts.* Template based structural modelling results showed that all identifed proteins are statistically similar to known structure models of corresponding AOXs.

P-02.08.5-013
Antioxidant and antimutagenic activity of *Limonium gmelinii* (fam. Plumbaginaceae) and *Inula britannica* (fam. Compositae)

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Most environmental contaminants have toxic and mutagenic effects on living organisms as a result of the activation of free radical formation and inhibition of reparation activity. It is becoming relevant to search for protectors of natural origin from the effects of xenobiotics. Many biologically active substances (BAS) of inartificial origin are found to be antioxidants and can increase the body's resistance to the toxic and mutagenic effects of a wide range of pollutants.

The aim of the study was to investigate the antioxidant and antimutagenic properties of BAS from medicinal plants *Limonium gmelinii (Plumbaginaceae)* and *Inula britannica (Compositae)*.

The antioxidant potential of plant extracts was determined by the activity of superoxide dismutase (SOD), catalase, and the content of malonic dialdehyde. Mutagenic and anti-mutagenic properties of the extracts were determined in the test by counting chromosomal aberrations in root meristem of barley seeds. Barley seeds were treated with an aqueous solution of unsymmetrical dimethyl hydrazine (UDMH), which is highly toxic I class hazardous material, well known pro-oxidant.

The results showed that UDMH enhanced the process of lipid peroxidation and decreased the mitotic activity. Treatment of barley seeds with extracts from *I. britannica* and *L. gmelinii* and their germination in the presence of stress factors stimulated antioxidant defenses in the primary roots of barley seeds. Increase of the activity of SOD and catalase, and reduction of peroxidation level of lipids were observed. Cytogenetic study showed no mutagenic activity in plant extracts. When effects of plant extracts and UDMH were combined there was a significant reduction in the frequency of structural mutations, induced by the toxicant.

Conclusion about the presence of the antioxidant and antimutagenic activity in the studied plant extracts is made.

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P-02.08.5-014
Comparative analysis of cytokinin dehydrogenase inhibition and trans-zeatin treatment in Arabidopsis seedlings

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Cytokinins are plant hormones regulating many processes during plant life ranging from germination to senescence. Manipulation of cytokinin levels and their impact on plant vitality, production and ability to defend against stresses is in great interest of agriculture. In this work we focused on comparison of inhibitor of the cytokinin degradation INCYDE (2-chloro-6-(3-methoxyphenyl)aminopurine) and exogenous application of *trans*-zeatin on *Arabidopsis thaliana* seedlings. Transcripts of genes regulating cytokinin metabolism were analysed by RT-qPCR analysis. Classical cytokinin root essay revealed that INCYDE effect is comparable to that of *trans*-zeatin in a similar concentration-dependent manner. Besides a negative effect on the primary root length, both substances induce flavonoid accumulation and an increase in the root hairs formation. Histochemical staining of transgenic plants expressing glucuronidase (GUS) under cytokinin-responsive promoter of *ARR5* gene revealed increased GUS activity in cotyledons following INCYDE treatment suggesting diverse localization of cytokinin modulation upon *trans*-zeatin and INCYDE treatment, respectively. Possible molecular differences originating in different cytokinin population and distribution following *trans*-zeatin or INCYDE treatments were monitored on the level of gene expression and via an LC-MS proteome analysis in roots and shoots of 14-day-old plantlets. RT-qPCR analysis revealed an alteration in cytokinin metabolism that could explain observed differences on the proteome level between INCYDE and *trans*-zeatin treated seedlings. Pharmacologically inhibited cytokinin degradation could be very efficient tool for modulation of cytokinin levels. Interestingly, the application of INCYDE and *trans*-zeatin shows a contrasting spatial and temporal pattern on molecular levels. INCYDE represents potent growth regulator with interesting properties useful for agriculture.

P-02.08.5-015
The expression yield of prokaryotic alpha-amylase is significantly magnified by molecular cloning techniques

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Randomly hydrolyzing glycosidic bond alpha-amylase has been traditionally employed in bread and similar industries. In that regard, increasing the overall expression level of the enzyme is a crucial concern in biotechnology.

To reach the goal, appropriate alpha-amylase producing species and expression vector were carefully selected. Therefore, genome of *Bacillus subtilis* was extracted and amplified by polymerase chain reaction (PCR) using specifically designed primers. Subsequently, the extracted gene was inserted in expression vector pHT43 and transferred to *E. coli* as intermediate host followed by *Bacillus subtilis* host replacement. The recombinant vector was expressed in *Bacillus subtilis* and the expression was evaluated by agarose gel electrophoresis. Relative purification of the recombinant enzyme was performed by 50 kDa filtration to remove impurities. To identify the biochemical characteristics, starch was used as specific substrate to measure enzyme activity

and the enzyme was exposed to various pH and temperatures. The extra-cellular expression of alpha-amylase enzyme was successfully elevated by 5 folds in comparison to the native enzyme. The optimum temperature and pH for the enzyme was carefully determined as 70 °C and 6, respectively. The enzyme was stable at 50 °C, but thermal stability was dramatically decreased at higher temperatures up to 70 °C. Kinetic parameters were also measured; Vmax was 1.998 U/ml min and Km was 3.998 mg/ml.

It is concluded that the elevated expression extent of recombinant alpha-amylase together with appropriate qualifications could make the clone a good choice for various industrial applications.

P-02.08.5-016
Expression of aluminum responsive microRNAs in resistant and susceptible flax (*Linum usitatissimum* L.) genotypes

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Toxicity of aluminum (Al) is a major reason of crop losses in acidic soil. Gene expression regulation *via* microRNA (miRNA) is important mechanism of plant response to Al stress. In the present work, alterations of microRNA levels in flax plants upon exposure to Al were investigated.

Flax seedlings of cultivars TMP1919, Lira and lines G-1071/4_o, G-1071/4_k were treated for 4 and 24 hours with 500 μM $AlCl_3$ solution or distilled water (control). Twelve small RNA libraries were constructed and sequenced using Illumina GAIIx. To identify known miRNAs, obtained sequences were aligned with miRNAs from miRBase (http://www.mirbase.org/). Fold change value was calculated to identify up- and down-regulated miRNAs under Al stress.

In total, about 40 million raw reads were obtained and 109 conserved miRNAs from 26 families were identified. Significant expression alterations in flax plants under Al treatment were shown for miR319 and miR390. Expression level of miR319 was varied in similar way in resistant and susceptible to Al genotypes: miR319 was up-regulated after 4 hours of $AlCl_3$ exposure and down-regulated after 24 hours. MiR390 expression was increased after 4 hours of $AlCl_3$ exposure and decreased after 24 hours in susceptible to Al flax genotypes (Lira, G-1071/4_o), while in resistant genotypes (TMP1919, G-1071/4_k) miR390 level was decreased after both 4 and 24 hours of Al treatment.

In other plant species, miR319 and miR390 were identified as Al-responsive. MiR319 targets mRNA of TCP (Teosinte Branched/Cycloidea/PCF) transcription factors, which control plant growth. MiR390 targets mRNA of TAS3 protein, which regulates lateral root growth *via* degradation of ARFs (auxin response factors). In flax, the involvement of miR319 and miR390 in response to Al stress was shown for the first time. Moreover, we revealed diverse expression alterations of miR390 in susceptible and resistant to Al genotypes. This work was financially supported by grant 16-16-00114 from the Russian Science Foundation.

P-02.08.5-017
Association genetics of phenylalanine ammonia lyase (PAL) and cinnamyl alcohol dehydrogenase (CAD) enzymes involved in lignin biosynthesis of european black poplar (*Populus nigra*)

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Populus nigra L. are considered as one of the most economically significant forest trees with respect to production of wood, biomass, and other wood-based products. While wood quality and biomass are directly associated with high cellulose content, lignin emerges as an undesirable polymer for both pulp and biofuel manufacturing industries. The aim of the study is by choosing the superior and eliminating the inferior clones to make a contribution to woody feedstock development and to improve wood quality of *Populus nigra*.

To estimate association genetics of PAL and CAD enzymes which have important functions in lignin biosynthesis, the important germplasms of *Populus nigra* has been sampled from 3 year old poplar trees (285 clones x 2 replicates x 2 ramets) which were grown in Behiçbey Plantation clone bank in Ankara. Additionally, five commercially registered clones and six foreign clones were included to the study to make comparison.

The average mean values of cellulose, lignin and glucose content were calculated as 21.8 ± 16.29 μg/ml, 23 ± 4.64 μg/ml, and 35 ± 9.71 μg/ml, respectively. Even though for PAL and CAD enzymes, data gathering process have been still resuming, particular clones have been separated from all in terms of PAL and CAD activities as expected.

Key words: *Populus*, poplar, lignin, PAL, CAD, genetic variation, feedstock

P-02.08.5-018
Proteomic analysis of the molecular mechanisms of the response of plant seeds to pre-sowing treatment by stressors

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Seed treatment with non-ionizing low-level radiation (NR), such as cold plasma (CP) or electromagnetic field (EF), is a modern eco-agricultural technology for stimulation of plant germination and performance. The molecular determinants of seed response to these treatments are not established and no genomic studies of plant seed response to NR have been reported. We studied the effects of pre-sowing seed treatment, using vacuum (7 min), radio-frequency EF (5–15 min) and CP (2–7 min), on germination and growth of non-oilseed *Helianthus annuus*. To gain an insight into the molecular mechanisms underlying effect of NR on sunflower seed germination and dormancy, we estimated changes induced in the balance of plant hormones and differential protein expression. The results of the germination tests and estimation of seedling morphology showed that response develops in time and is stronger when sowing is performed in 7 days

in comparison to 3 days after seed treatment. The 2D DIGE analysis revealed 38 differentially expressed proteoforms in kernels of seeds treated with CP or EF. Proteins involved in biological processes of seed maturation, response to stress, response to abscisic acid stimulus, processes of organonitrogen compound metabolism and glucose catabolism were identified. While expression patterns for majority of the proteins were highly specific to CP and EF treated seed kernels, accumulation of several proteoforms of seed storage proteins (SSP), including vincilin-like, miraculin-like protein and albumin-8 were common for both experimental groups. This suggested that response to NR treatment could be at least partially associated to function of SSPs in response to oxidative stress that protects proteins required for seed germination and seedling formation. Variation of abundance of distinct proteoforms of helianthinin, vicilin-like and 11s globulin-like SSPs suggested that post-translational modifications are involved in regulation of the function of SSPs.

P-02.08.5-019
Suppression of lipopolysaccharide-induced inflammatory responses in RAW 264.7 macrophages by tuber extract of *Cyclamen* L.

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Turkey is a prominent centre of plant diversity, being the meeting point of three main floristic zones. Geophytes which have underground storage organs such as, tubers, bulbs and rhizomes. *Cyclamen* L. is a tuberous geophyte traditionally used by some people for treating whooping cough, headaches or sinusitis, and confirmed to have antioxidant, analgesic and anti-inflammatory properties by several reports. A prolonged inflammatory response is often associated with chronic diseases such as cancer, arthritis and autoimmune disorders. Recently, plant based products are used as an alternative and complementary treatment of these diseases. In this respect, the present study was aimed to determine the effects of three *Cyclamen* tuber extracts on LPS-induced inflammatory responses of murine RAW 264.7 macrophages.

Firstly, *C. cilicium* (endemic), *C. pseudibericum* (endemic) and *C. graecum* subsp. *anatolicum* were collected from different localities of Turkey. The tubers of plants were air-dried and grounded to fine powder and then extracted with ethanol. Cell viability assay was performed to evaluate the nontoxic concentration in cell line by MTT assay. Several measurements were performed including TNF-α, NO and IL-8 concentration assay by Elisa after treatment compared to non treated cells to determine the anti-inflammatory activity. Also, TNF-α and iNOS mRNA levels were evaluated by quantitative RT-PCR.

The cytotoxic activity which is considered safe on RAW.264.7 cell were found as 0.5–5 μg/ml. Studied *Cyclamen* taxa inhibited TNF-α and IL-8 release on LPS stimulated-RAW.264.7 in a concentration-dependent manner. Among the three *Cyclamen* tuber extracts evaluated, the highest nitrite-associated NO inhibitory activity was obtained from *C. pseudibericum* compared to other two *Cyclamen* L. taxa. Collectively, these results suggest that *Cyclamen* tuber extracts possess anti-inflammatory properties.

P-02.08.5-020
In vitro hypoglycemic activity of *Ziziphus Jujuba*

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Recent reports have indicated that continuous treatment with nutritional jujuba (*Ziziphus jujuba Miller*) fruit extracts in diabetic rats improved glucose utilization and produced a significant decrease in the blood glucose. In the present study, hypoglycemic activity of *Z. jujuba* was investigated using various in vitro techniques.

The hypoglycemic effect of *Z. jujuba* in phosphate buffered saline which grown in Balıkesir was studied by measuring glucose adsorption, glucose diffusion and glucose uptake by yeast cells. The glucose content in the solution measured by spectrophotometrically with commercially kits.

The adsorption capacity of the *Z. jujuba* was found to be directly proportional to the molar concentration of glucose. The glucose binding capacity of extract increased in higher glucose concentratrations. There was significant differences were observed between the adsorption capacities of *Z. jujuba* and control samples ($p < 0.05$). The rate of glucose diffusion was directly proportional to the time. Diffusion rate was significantly lower in the solution containing *Z. jujuba* compared to control ($p < 0.05$). The extract demonstrated significant inhibitory effects on movement of glucose into external solution across dialysis membrane compared to control. The rate of glucose transport across cell membrane in yeast cells was observed to be inversely proportional to the molar glucose concentration. *Z. jujuba* inhibited glucose transport across the yeast cells.

The results showed that *Z. jujuba* reduced glucose levels at least by three mechanisms. First by increasing glucose adsorption capacity during postprandial hyperglycemia; second by retarding glucose diffusion rate and third, at the cellular level by inhibiting glucose transport across the cell membrane. All of these decreased the absorption of glucose in the intestinal cells and the concentration of postprandial serum glucose.

P-02.08.5-021
Cucurbitacin B increased the anticancer effect of imatinib mesylate through inhibiton of matrix metalloproteinase-2 expression in colorectal cancer cells

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Several natural products have been investigated for their anticancer effects. Among these, Cucurbitacin B (CuB) has been reported as its inhibitory effects on cancer cell proliferation. Matrix metalloproteinases (MMPs) belong to endopeptidase family and they are received as potential biomarkers for several types of cancer. The aim of this study is to investigate the effect of CuB in combination with imatinib mesylate (IM) on MMP-2 mRNA expression of human SW480 colorectal carcinoma cells.

The cytotoxicity analysis was performed via MTT assay. Muse cytofluorimetric analysis system was performed to evaluate apoptotic cell population. The MMP-2 mRNA expression was determined by quantitative real-time PCR. This study was supported by Scientific and Technological Research Council of Turkey

Grant, SBAG-114S871. Data obtained from the cell culture experiments were expressed as mean ± SD and One-way ANOVA test was applied for multiple comparisons.

CuB alone significantly inhibited cell growth at 10 μM and higher concentrations. The most potent effect was observed in CuB-IM combination treatment with 3.51 μM IC_{50} value. In CuB-IM treated group, the apoptotic effect was higher than CuB and IM treated groups. CuB-IM induced apoptosis significantly at 10 μM concentration when compared to control and 1 μM ($p < 0.05$). CuB alone showed inhibitory effects on MMP-2 mRNA expression at 1 μM and higher doses significantly ($p < 0.05$).

The results showed that the combination treatment of CuB with imatinib synergistically inhibited human SW480 cell growth and induced apoptosis by increasing the anti-histone antibody-bound nucleosom levels and Annexin V binding. Although CuB could inhibit MMP-2 expression alone at higher treatment doses, it enhanced the inhibitory effect of IM on MMP-2 synergistically in a dose dependent manner. In conclusion, this study suggests that CuB combined with imatinib mesylate may enhance the effects of chemotherapy in patients with colorectal cancer.

P-02.08.5-022
Investigation of antibacterial effects of *Zizyphus jujuba*

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Plants are most important parts of natural resources that alternatively referred to synthetic drugs for reasons such as being less side effects and lower costs. *Ziziphus jujuba Miller* (*Z. jujuba*), a plant used in traditional medicine, is one of the most important Ziziphus species belonging to Rhamnacea family. The fruit and seeds of this plant are used different purposes such as anti-inflammatory, antioxidant, immune-stimulant and wound healer. In this study, we investigated the antibacterial effects of *Z. jujuba*.

The aims of this study were to screen the antibacterial activity of *Z. jujuba*. The extract was obtained from *Z. jujuba* fruits pulverized with the aid of ball mill using 50% aqueous-ethanol solution. Extracts were screened for antimicrobial activity against six different standard strains of bacteria by determining minimum inhibitory concentration (MIC) according to CLSI criteria. Serial dilutions are made between 64 mg/ml and 0.031 mg/ml concentration range. The lowest concentration of wells that no visible growth has been accepted as MIC value. Materials in the MIC and lower concentrated wells were transferred to 5% Sheep Blood Agar petri dishes for calculation of minimal bactericidal concentration (MBC). The lowest concentration that no colony formation has been accepted as MBC value.

jujuba showed the most potent effect on strain of *S. aureus* ATCC 29213 is Gram-positive cocci (MIC: 2 mg/ml). The MIC values of other Gram-positive bacteria *S. aureus* ATCC 43300, *E. faecalis* ATCC 51219, *L. monocytogenes* F 1483 and *M. smegmatis* CMM 2067 were detected as 8, 16, 8 and 8 mg/ml respectively. MIC values of Gram-negative bacilli were detected as >64 mg/ml.

Consequently, *Z. jujuba* was found to be effective on Gram-positive cocci bacteria (*S. aureus* ATCC 29213, *S. aureus* ATCC 43300 and *E. faecalis* ATCC 51219). The strongest effect was observed on *S. aureus* ATCC 29213 strain. In contrast, extract showed less effect on Gram-negative bacilli.

P-02.08.5-023
Selective cytotoxic effect of *Morus rubra* extract in human lung cancer cells through enhancing apoptosis and cell cycle arrest

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Cancer is a disease that develops as a result of unlimited proliferation of abnormal cells that occurs due to loss of control over the mechanisms of normal growth and differentiation of cells. *Morus rubra*, known as "red mulberry" belongs to family of *Moraceae*. For many years, the fruits of *Morus* species have been used to treat many diseases in traditional medicine. Biological effects of *Morus* species is predominantly attributed to its content of polyphenolic compounds. Many studies have evaluated the cytotoxic effects of different *Morus* species, but there is no study about cytotoxic effect of *M. rubra*. In this study, we aimed to evaluate the cytotoxic effect of *M. rubra* extract in human lung cancer cells (A549) with regard to apoptosis, cell cycle and mitochondrial membrane potential.

Cytotoxic effect of *M. rubra* extract on human lung cancer cells was determined using MTT assay. Then, mechanisms of cytotoxic activity of *M. rubra* extract on A549 cells were examined in terms of cell cycle, apoptosis and mitochondrial membrane potential using flow cytometric methods.

M. rubra extract exhibited selective toxicity against A549 cells compared to normal foreskin fibroblast cells. We determined that *M. rubra* extract increased cell cycle arrest at G_1 phase, the level of apoptotic cells and decreased mitochondrial membrane potential in A549 cells.

Our results showed that *M. rubra* extract has pro-apoptotic and antiproliferative effect in A549 cells. Further studies are also needed to fully mechanisms underlying this effect of *M. rubra* extract.

P-02.08.5-024
Dipeptidyl peptidase IV inhibitory activity of *Arctium tomentosum* L.

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Type 2 *Diabetes mellitus* (T2DM) is rapidly growing metabolic syndrome of multiple aetiologies causing hyperglycaemia with insulin resistance at cellular level. A novel approach in the treatment of T2DM is based on preventing of rapid inactivation of the incretin hormone glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) by dipeptidyl peptidase-IV enzyme.

In this study; Dipeptidyl peptidase IV (DPP-IV; EC 3.4.14.5) inhibitory activity of the aqueous and methanolic extracts of *Arctium tomentosum* leaves were successfully tested in vitro

conditions. Our study revealed that both aqueous and methanolic extracts obtained from test material had a significant DPPIV enzyme inhibitory activity in *changing ratio*. The IC_{50} values were also determined by nonlinear regression curve fit using Graph pad Prism 5.0 with appropriately diluted of lyophilized *Arctium tomentosum*. Diprotein-A (Ile-Pro-Ile) was used as reference inhibitor. *A. tomentosum* aqueous extracts showed IC_{50} 4.6 μg/ml while the standard (positive control) Diprotin A displayed the IC_{50} value of 10.1 μg/ml.

This study demonstrates that *A. tomentosum* aqueous extracts could be a good lead for further development as a new antidiabetic agent.

P-02.08.5-026
DNA recognition determinants of *Arabidopsis thaliana* B3 transcription factors

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Transcription, one of the most important cellular processes, is regulated by transcription factors (TF), proteins that often directly interact with gene promoter sequences. TF binding to DNA is mediated by various DNA binding domains. The B3 TFs constitute a large, plant-specific protein family (approx. 10% of all TF proteins in the flowering plants), which is characterized by the presence of one or several small (approx. 110 amino acids) B3 DNA binding domains. Currently the B3 TFs are divided into four groups (LEC2-ABI3/VAL, ARF, RAV and REM). The preferred recognition sites were identified for representatives of all groups except the REM family. Currently, only a single structure of a DNA-bound B3 domain (ARF1, ARF family) is available, thus the mechanism of site-specific DNA recognition by the LEC2-ABI3/VAL and RAV B3 domains remains poorly understood.

Based on the ARF1-DNA structure (PDB 4ldx) we have built homology models of DNA-bound B3 domains from A. thaliana ABI3 (LEC2-ABI3/VAL family) and NGA1 (RAV family) transcription factors, mutated putative DNA-interacting amino acid residues and characterized the DNA binding ability of the purified mutants using electrophoretic mobility shift assay and a set of radiolabelled DNA substrates carrying various variants of the optimal recognition site.

We confirm the importance of several positively charged amino acid residues, which are conserved between the ABI3/NGA1 B3 domains and structurally related DNA-binding domains of bacterial restriction endonucleases EcoRII, BfiI and NgoAVII; furthermore, we identify residues in the 'N-arm' and 'C-arm' loops that may be involved in specific interactions with the DNA bases. Our results therefore help us refine the homology models of the DNA-bound B3 domains and in the future may help us predict the DNA binding properties of currently uncharacterized B3 domains.

P-02.08.5-027
Immunohistochemical analysis of inhibitory effects of *Origanum hypericifolium* oil on Dipeptidyl peptidase IV in streptozotocin-induced diabetic rats

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Diabetes mellitus (DM) is a serious metabolic disorder with micro- and macro-vascular complications that result in a significant morbidity and mortality. GLP-1 and GIP have significant role in pancreatic beta cells and prevention of inactivation of them by dipeptidyl peptidase IV (DPP IV) inhibition is a novel approach to treatment of DM. *Origanum hypericifolium* (Lamiaceae) is an endemic Turkish plant and its essential oil is mainly composed of monoterpenes including carvacrol and thymol. Streptozotocin (STZ) is used to induce diabetes in rats. The aim of this study is to investigate the inhibitory effects of *O. hypericifolium* essential oil on the DPP IV in STZ-induced diabetic rats. The animals (female Spraque-Dawley rats) were assigned to four groups (Group 1: Control, Group 2: STZ-induced diabetic, Group 3: *O. hypericifolium* injected, Group 4: STZ-induced diabetic and *O. hypericifolium* injected). DM was experimentally induced in Groups 2 and 4 by a single intraperitoneal injection of STZ at a dose of 45 mg/kg body weight. In Groups 3 and 4, rats were intraperitoneally injected with *O. hypericifolium* oil at a daily dose of 1 ml/kg body weight for 42 consecutive days. At the end of the experimental period, all animals were sacrificed by cervical dislocation under ether anesthesia and liver and kidney tissues of each animal were rapidly excised. Tissues were fixed in Sainte-Marie fixative. After routine histological processes, samples were embedded in paraffin, immunohistochemical staining for DPP IV was performed on sections and then they were photographed. The immunohistochemical reaction intensity differences were observed between the groups. In conclusion, the immunohistochemical distribution of DPP IV in the tissues that the test oil was applied in the diabetic rats may be important for the investigation of the inhibitory effects of oil on the enzyme. Moreover, our findings suggest that *O. hypericifolium* oil may be used for prevention of diabetic diseases.

P-02.08.5-028
The Effects Of MEC17 gene mutation in *Chlamydomonas reinhardtii* E

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Introduction: All eukaryotic cells need microtubules for purposes of nuclear and cell division, organization of intracellular structure, and intracellular transportation, as well as ciliary and flagellar motility. Microtubules are made of polymerized α/β-tubulin subunits. MEC17 is important for microtubules, because it encodes the enzyme that adds acetyl groups to Lysine 40 (K40) of tubulin. K40 is largely conserved in α-tubulins of many eukaryotes, and acetylation is thought to stabilize microtubule structure. In algae, the effect of acetylation by MEC17 on flagellar motility and phototaxis has not been tested previously.

Materials and methods: In this study, *mec17* mutant *Chlamydomonas reinhardtii* cells were compared to wild-type cells to see the effect on flagellar motility and phototaxis. We tested phototaxis, eyespot size and quantity under the microscopy. In addition to this, we fixed cells and examined them by immunofluorescence microscopy using antibodies to tubulin, acetylated tubulin, and photoreceptor.

Results: We observed that some *mec17* mutant cells contain more than one eyespot.

We detected no acetylated-tubulin (Ac-tub) by immunofluorescence. The cells still phototax and have normal motility

Discussion and Conclusion: Interestingly, *mec17* cells still have the ability to phototax and they have normal flagellar motility, even though they contain occasional additional eyespots and no Ac-tub.

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P-02.08.5-029
Chlorella vulgaris as a model system for screening of plant growth modulators

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The discovery of new plant growth modulators became extremely important task as an alternative approach to overcome plants resistance to herbicides and pesticides, which leads to harmful action on plants and land rising, environmental and ecological problems. Small molecules provide agricultural biotechnology with valuable tools, which help to circumvent the need for genetic engineering and offer unique benefits to modulate plant growth and development.

We developed a system to explore molecular modes of action of plant growth modulators using *Chlorella Vulgaris* model. Our model allows applying High Content Screening approach in 96-well plate format for fast and robust effect assessment of large number of tested modulators. *Chlorella V*. was grown in climate chamber under optimized constant temperature (20 °C) and light conditions (16:8 hours/light:dark). Modulating effect of tested compounds was estimated by spectrophotometric measurement of microalgae density at the beginning of the experiment (start point- 0.1OD) and 48 hours later.

To validate our system we used known cytokinins and auxins (10 mM) as positive controls of growth stimulation. We showed that in presence of each compound the density of *Chlorella V*. was increased in 7–11 times range, compared with only 2 times increase in control group. Eight new chemicals (10 μM), which demonstrated modulation effect on *Nicotianatabacum L*. pollen and *Arabidopsis thaliana* models, were tested on developed *Chlorella V*. model. Positive controls showed no stimulating effect at this concentration, while tested compounds were confirmed as hits and increased the density up to 400%.

We demonstrated that developed model system, based on *Chlorella V*., is an effective system for primary screening of plant growth modulators. The main advantages of this system are short time of assay, simplicity of performance, possibility of automation and low cost. Selected hits can be recommended as perspective candidates for future test on crop field.

P-02.08.5-030
Development of SNP markers linked to the downy mildew resistance in sunflower (Helianthus annus L.) by competitive allele specific PCR

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Sunflower is under a big threat of downy mildew which is a fungal disease caused by *Plasmopara halstedii*. The disease can cause up to an 80% yield loss in sunflower production. Downy mildew Resistance genes (*R*) denoted as *Pl* has been discovered to date in sunflower. In recent years, Single Nucleotide Polymorphism (SNP) markers have become widely used in plant breeding programs. In this study SNP markers have been currently developing for Pl_6, Pl_8, Pl_{13}, Pl_{arg} genes by Competitive Allele Specific PCR (KASP) assay which enables bi-allelic scoring of SNPs and insertions/deletions (Indels) at specific loci. In total 66 Sequence Tagged Site (STS) sequences from NCBI were aligned for Pl_6, Pl_8, Pl_{13}, Pl_{arg} genes to identify conserved regions for each gene. Based on the conserved regions, specific PCR primers were designed in order to make sequencing of these genes in five crosses and their F_2 progenies. Sequence data will be used to design an allele specific primer maches the target SNP and amplifies the target region with the common reverse primer provided by KASP Genotyping Assay. SNP markers linked to *Pl* genes which are being developed in this study, have the potential to be used in Marker Assisted Selection (MAS) for sunflower breeding programs.

P-02.08.5-031
Investigation of the antidiabetic effects of *Hibiscus sabdariffa, Teucrium polium* and *Myrtus communis* in HEPG2 cells line

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Some antidiabetic plants currently are used in alternative treatment of Type II diabetes. *Hibiscus sabdariffa, Myrtus communis and Teucirum Polium* plants are also known for their antidiabetic properties. *Hibiscus sabdariffa, Myrtus communis and Teucrium Polium*; depending on the impact on Hepg -2 cells to investigate the possible mechanisms of Type II Diabetes with researches on glucolysis and glucogenesis pathways gene expressions (PK_M, GLUT-2,PEPCK). Plants were obtained in dried state from reliable herbalists in Denizli and Mersin. Plants treated with the extractor device. Plants obtained aqueous extract was subjected to lyophilization process. Human cancer cells have been used throughout the study. The cytotoxicity of the cells was measured by ELISA plate reader . Total RNA was isolated using trızol® solution was carried out according to the instructions of the manufacturer's (Thermo Scientific) recommended procedures were performed, but we have to optimize our own laboratory conditions. PK_M, GLUT-2,PEPCK genes were synthesized by BİONEER. During our study the activation of certain genes (PK_M, GLUT-2,PEPCK) were examined by Real time PCR. In our study Hibiscus sabdariffa, Mrytus communis and *Teucrium Polium* plant to extract applied Hepg -2 cell line in the gluconeogenesis and glycolysis pathways in involved in some important genes (PEPCK, PK_M, GLUT- 2) analyzed the expression levels . *Teucrum Polium* plant extract is applied in hepg - 2 cells the glycolysis pathway genes (PK_M GLUT- 2) an increased expression also genes of gluconeogenesis pathway (PEPCK) were not decreased. However *Hibiscus sabdariffa* and the expression of genes involved in glycolysis and gluconeogenesis pathway *Mrytus communis* plants were observed to have a full effect as diabetic or hypoglycemic . In this context, it is considered that the plant *Teucrum Polium* on the line hepg -2 cells showed antidiabetic effect.

P-02.08.5-032
Purification of β-glucosidase from Malatya apricot (*Prunus armeniaca* L.) seeds and some of its biochemical properties

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Introduction: β-Glucosidases are one of the key enzymes in carbohydrate metabolism and located in glycosyl hydrolases (EC 3.2.1) family. Plant β-glucosidases have biotechnological significance as they are effective on glycosidic bonds of flavor and aroma precursors in plants. β-Glucosidases that located in fruit seeds are important because they affect the amygdalin. Aim of this study is purification and partially characterization of β-glucosidase from Malatya apricot seeds.
Materials and methods: Apricot seeds were homogenized with extraction buffer to prepare of crude extract. The enzyme protein was precipitated with 60% ammonium sulfate then purified by hydrophobic interaction chromatography using Sepharose 4B-L-tyrosine-1-naptylamine gel. *para*-Nitrophenyl β-D-glucopyranoside (*p*-NPGlc) was used as substrate to determine biochemical properties of the enzyme. The optimum pH was determined using buffers between pH 2–12 and thermal optima was determined using 25–85 °C temperature range. Inhibitory effects was determined with 1 mM substances.
Results: The enzyme was 11.1-fold purified with yield of 14%. Purified β-glucosidase from apricot seed was visualized about 60 kDa molecular weight on SDS-PAGE. The kinetic parameters were determined against *p*-NPGlc substrate as Km and Vmax values of 2.5 mM and 58.1 EU, respectively. The optimum pH and temperature were determined 5.0 and 55 °C respectively. Effects of $CaCl_2$, KCl, NaCl, $MgCl_2$, K_2SO_4, Na_2SO_4, $CuSO_4$, $FeCl_3$, Pb(II) acetate, $AgNO_3$, $ZnCl_2$ and glucose on purified enzyme activity were investigated. KCl, Na_2SO_4, Pb(II) acetat and $CuSO_4$ reduced the enzyme activity.
Discussion and conclusion: In this study, β-glucosidase was purified from Malatya apricot seed and some of its biochemical properties were determined. Because this enzyme has pharmaceutical importance hydrolyzing amygdalin. The results showed that immobilized almond β-glucosidase was used to break amygdalin and release -CN compound that effective to shrink cancer mass.

P-02.08.5-033
A new affinity gel for purifing polyphenol oxidase enzyme

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Polyphenol oxidase (PPO) enzyme, sometimes called as phenol oxidase, catecholase, phenolase, catechol oxidase or tyrosinase, is considered to be an o-dipenol. PPO (EC 1.14.18.1), a multifunctional copper containing metalloenzyme, is widely distributed in nature. PPO exists in many kinds of plants and fungi, such as banana, mushroom, butter lettuce, Napoleon grape, potato, coffee, marula fruit, artichoke heads, longan fruit, tobacco, wheat flour.

In this study, a novel affinity chromatography gel was synthesized for purifing PPO enzyme. The affinity chromatography gel was synthesized by coupling aniline as a specer arm to CNBr activeted Sepharose-4B. Then, p-amino benzoic acid was coupled to aniline as a ligand. PPO was purified from *Musa sapientum var. Cavendishii* (banana) by using Sepharose-4B-aniline p-amino benzoic acid affinity chromatography gel. % 4.49 yield and 33.4 fold purification were achieved. SDS-polyacrylamide gel electrophoresis of the enzyme indicates a single band with an apparent MW of 35 kDa. The V_{max} and K_m of the purified enzyme were determined 42,628 U/ml min and 9.27 mM, respectively.

P-02.08.5-036
Phenolic content and antioxidant capacity of gamma irradiated olive leaves

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In this study, dried in diffirent ways (such as microwave, infrared, convection heaters and under normal atmospheric conditions) olive leaves has been used as experimental material. Radiation has been applied to dried olive leaves in three diffirent dosages in room temparature. The amount of radiation to be implemented to the samples have 3, 5, 10 KGy/min. In this study, how gamma rays (radiation) effects phenolic components, total phenolic content and antioxidant capacity of dried olive leaves has been determined. The phenolic components, total phenolic content and antioxidant capacity were analysed with HPLC, folin ciocalteu and DPPH radical scavenging method, respectivelty.

Gamma rays is well known as a decontamination method for many foodstuffs and plant materials, being an environment friendly and effective technology to resolve technical problems in trade and commercialization. For this reason, nowadays it is utilized as an alternative method of sterilization. Gamma rays are of ionizing radiation. When ionizing radiation interacts with matter, generally it causes a break in the molecular bonds and/or breaks the bonds between the molecules. These intermediates have unpaired electron and called free radical. Gamma radiation or the radiation-induced free radicals would break or cause damage to the DNA molecules of living organisms. Gamma irradiation is predict to change phenolic content and antioxidant capacity in living tissues. Phenolic compounds are secondary plant metabolites naturally present in fruits and vegetables. In recent years there has been a growing interest in food phenolics because of their potential health benefits mainly due to their antioxidant and free radical scavenging activity.

P-02.08.5-037
Investigation of horizontal gene transfer (HGT) from genetically modified plants to bacteria

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Despite the controversy about potential risks posed by genetically modified plants on human health, environment and microorganisms, cultivation area of these crops increases day by day. This increment has revealed concerns especially related to HGT. HGT studies indicated that antibiotic resistance genes in GM plants have a potential to transfer to soil microorganisms. In this study, HGT of widely used genetic elements such as regulatory sequences, from transgenic plants to bacteria was investigated.

Three soybean feed and four seed examples from Turkish Feed Manufacturers' Association were used for genetic analysis

based on foreign gene determination. GMO analysis were conducted by CaMV 35S promoter-specific PCR in genomic DNAs. In GM positive samples, genomic DNAs sheared into appropriate fragment size by ultrasonication for the purpose of bacterial transformation. Presence of 35S promotor region in fragmented DNAs was proved by PCR. *Escherichia coli* DH5α strain was transformed by fragmented DNA samples according to $CaCl_2$ method. 35S promotor sequence screened by using PCR in bacterial genomic DNAs.

As a result of GM screening, all feed and three of the seed samples were found to be transgenic. Ultrasonication conditions were optimized for shearing DNA's to 450–500 bp for bacterial transformation. Fragmented DNAs confirmed for carrying intact 35S promotor sequence. None of bacterial genomic DNAs were found to be 35S-positive.

According to the transformation results, absence of 35S promotor sequence in all tested bacterial genomic DNAs, proved the DNA samples belonging to GM plants can not transfer into compotent *E. coli DH5* under laboratory conditions. For verification of this finding, transformation studies will continue with *Acinetobacter baylyi* BD413 strain which is naturally competent soil bacterium for natural transformation. We believe that all of our findings will contribute to constitute transformation system which can be used as model in HGT studies.

P-02.08.5-038
Preliminary studies on differential methylation in A and D sub-genomes of Upland cotton (*Gossypium hirsutum* L.)

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Four species of cotton (*Gossypium* L.) provide raw materials for the textile industry. Among the four species, two have diploid genome and another two have tetraploid genome. Tetraploid genome consists of A and D sub-genomes. A sub-genome belongs to Asian cotton while D-sub genome belongs to American cotton. Previous studies revealed that D sub-genome of *Gossypium* species contributes to the superior yield and quality of tetraploid *Gossypium* L. species (AtDt).

DNA cytosine methylation of four regions of DNA sequences located on A and D sub-genomes of *Gossypium hirsutum* L. Texas Marker 1 (TM-1) was investigated using bisulfite sequencing technique.

Among the regions studied two could not be located on sub-genomes due to sequence identity match between A and D sub-genomes. On the other hand two DNA regions could be located on A and D sub-genomes using the BLAST searches. Some of the DNA sequences located on different sub-genomes showed polymorphic nucleotides including C/T and G/A polymorphisms. In silico analysis indicated that some alleles located on different sub-genomes of cotton have C/T and G/A polymorphisms. C/T polymorphisms between the sub-genomes could be thought as unmethylated cytosine using the bisulfite sequencing technique. This indicated that an extra attention needs to be paid in DNA total cytosine methylation studies in polyploid species such as cotton using bisulfite sequencing, methylation sensitive amplification polymorphism (MSAP), whole-genome bisulfite sequencing (WGBS). Otherwise, T in C/T polymorphism between the sub-genomes could be thought as unmethylated cytosine. Based on the two genomic regions we could conclude that A sub-genome may be more methylated than D sub-genome. Differential methylation of genes located on different sub-genomes may provide another mechanism responsible for differential gene expression of genes located on different sub-genomes.

P-02.08.5-039
Cleaved minisatellite locus (CML) markers for fingerprinting of cotton cultivars grown in Turkey

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After their discovery by Alec Jeffreys in 1984, minisatellites have been used in genetic studies of many organisms. Minisatellites, also called variable number tandem repeats (VNTRs), are composed of arrays of longer repeats mostly dispersed throughout heterochromatin (centromeres and telomeres). Direct amplification of minisatellite regions of DNA using a single core primer is a powerful method to amplify minisatellites (DAMD-PCR). Although the DAMD-PCR technique has been applied to many plant species, the level of polymorphisms in cotton (*Gossypium* L.) is very low due to very narrow Turkish cotton genetic base. The objective of this study was to improve the level of polymorphisms by cleaving minisatellite loci by restriction enzyme digestion.

Genomic DNA samples of twenty-one Turkish cultivars, Pima 3-79, TM-1 and PS-7 were extracted. Twenty-one minisatellite primers were screened using the DAMD-PCR technique. Monomorphic amplicons were digested using several restriction enzymes. Three to five micro liters of amplified products were digested with various restriction enzymes. Digested products of minisatellite loci were separated in 3% high resolution agarose gels.

Comparison studies of digested and undigested markers revealed that cleaved minisatellite markers showed polymorphisms in those cotton lines that could not be differentiated by microsatellite and minisatellite markers. This approach was called cleaved minisatellite locus markers (CML). The amplification reactions of minisatellites used touch-down (TD) cycling conditions. The use of TD offered a simple and rapid means of optimizing polymerase chain reaction (PCR), increased specificity, sensitivity, and efficiency without the need for lengthy optimizations of minisatellite primers. The CML markers were obtained at a 55 °C annealing temperature, which is a temperature higher than those used in random amplified polymorphic DNA (RAPD) inter-simple sequence repeat (ISSR) markers.

P-02.08.5-040
Association between cytosine methylation and tissue specific expression of microsatellites

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Heritable covalent modification of DNA, RNA or protein without altering their primary sequences is defined epigenetics. Because all biological events are influenced by epigenetics, it is one of the most important fields in science. DNA methylation is one of the most important epigenetic mechanisms. DNA cytosine methylation is a process by which methyl groups are added to cytosine bases of DNA. Microsatellites, also knows simple sequence repeats are DNA sequences consisting of 1–6 nucleotide repeats. There is a large body of information regarding the relationship between microsatellite instability and abnormal gene expression, and between DNA methylation and altered gene expression. However, there is limited information on cytosine methylation of microsatellites. In the present study, we investigated whether there is any association between cytosine methylation and tissue specific expression of microsatellites.

Genomic DNA samples of various tissues and developmental stages of pepper line Demre Sivrisi (*Capsicum annuum* L.) and cotton line TM-1 (*Gossypium hirsutum* L.) were extracted. cDNA samples were synthesized using mRNA expressed in pepper

tissues. Cytosine methylation levels were investigated using bisulfite sequencing methods.

Screening studies of microsatellites revealed that some genes containing microsatellites were differentially expressed in tissues and developmental stages of pepper. Microsatellite containing genes that expressed differently among tissues had also showed different methylation levels in CG, CHG and CHH (where H refers to A, C or T) contexts. Methylation level differences between microsatellites were also observed. As far as our knowledge, it is the first report on differential expression of genes containing methylated microsatellites.

P-02.08.5-041
PCR-LG: An alternative way to assign the chromosome location of genes/markers in cotton

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Assignment of genes and DNA markers on chromosomes is very important in life sciences, especially for plant breeding and medicine. There are several methods for the assignment of a gene or DNA sequence to a specific location on a chromosome. For example, the most widely used technique is the assignment of fluorescently-labeled gene or DNA sequences (markers) on chromosomes using the fluorescently-labeled gene (for instance, FISH technique). Another example is the construction of a genetic map (linkage map) which orders the targeted genes along the DNA strand based on recombination frequency. Sequencing is the most precise technique in which coding (gene-containing) and noncoding DNA region of genes could be located on a chromosome molecule. Aneuploid lines could also be used to locate genes in a specific chromosome, but maintenance of these lines is difficult. Here we report the use of chromosome substitution lines to indirectly locate genes/markers on chromosomes.

We used chromosome substitution lines (CSLs) that carry a chromosome pair or chromosome arms from *Gossypium barbadense* L. while the rest of chromosomes belong to *G. hirsutum* L. A total of 10 CHLs, a homozygous cotton line TM-1 and a double haploid line Pima 3–79 were used as plant materials. Twenty microsatellite primer pairs were utilized in touch-down polymerase chain reactions.

We developed a method, called Polymerase Chain Reaction to Locate Gene (PCR-LG), to assign genes/markers on chromosome or chromosome arm. With the use of PCR-LG approach any polymorphic genes/markers between TM-1 and Pima 3–79 (*G. hirsutum* and *G. barbadense*) could be assigned to a chromosome or chromosome arm. Results indicated that if 26 CSLs were used any polymorphic markers (genes) with known primer pairs could be assigned to cotton chromosomes. Although we used cotton chromosome substitution lines to validate the proposed technique, it could be applicable all the species that have chromosome substitution lines.

P-02.08.5-042
Pecularities of genome variability of antarctic hairgrass *Deschampsia antarctica* Desv. from the Maritime Antarctic

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Deschampsia antarctica Desv. (*Poaceae*) is the only grass species native to the Antarctic region, adapted to harsh environmental conditions. However, reasons for its unique success remain unexplored. Stressful environmental factors can influence a plant genome and cause changes in the chromosome number and morphology and increase genetic variation. Therefore, the purpose of our research was to explore alterations in the *D. antarctica* genome both at the chromosomal and molecular levels and to investigate species genome stability using in vitro tissue cultures.

Plants used for the study were grown in vitro from seeds collected in the Argentine Islands region during 2008–2014. Chromosome number was determined in plant root apical meristems and specimens prepared from tissue cultures. rRNA genes localization were determined using the FISH technique. Molecular-genetic analysis was performed using PCR with polymorphic ISSR-primers.

New forms of chromosome polymorphism, associated with aneuploidy (2n = 13-27), polyploidy (2n = 13-39) and the occurrence of additional B-chromosomes (2n = 26 + 1-3B), were observed. FISH analysis also confirmed that genotypes with a different chromosome numbers varied in the number of 5S rDNA and 25S rDNA sites. Assessment of genetic variation demonstrated a low level of diversity: differences between the plants with different chromosome numbers do not exceed the level of within-population variation.

Cytology analysis of *D. antarctica* cultured tissues revealed aneuploidy (up to 60.6 %) with predominance cells with diploid and near-diploid chromosome number irrespective of plant's initial karyotype (diploid, mixoploid or polyploid).

We assume that discovered intraspecies chromosomal polymorphism is a manifestation of quick genome reaction to harsh Antarctic conditions. Whereas the results of molecular-genetic analysis and study of cell cultures of this species suggests on the relative stability of *D. antarctica* genome.

P-02.08.5-043
Angiotensin converting enzyme inhibitory activity of *Morchella esculenta* (L.) Pers

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Hypertension is a multi-aetiological, chronic pathophysiology that leads to multi-organ dysfunctions like cardiovascular diseases, strokes, and renal complications. Natural extracts play an important role in traditional medicines for the treatment of hypertension and are also an essential resource for new drug discovery. Mushrooms are use as therapeutics in alternative and complementary medicine as functional food because of contain a large number of biologically active components that offer health benefits and protection against many degenerative diseases.

Morchella esculenta is one of the most highly priced edible mushrooms worldwide. It contains a wide range of active constituents which include tocopherols, carotenoids, organic acids, polysaccarides and phenolic compounds which exhibit a wide range of medicinal and pharmacological properties including anti-microbial, anti-inflammatory, immunustimulatory, antitumor and antioxidant.

In this study; the in vitro angiotensin converting enzyme-I (ACE-I) inhibitory activity of *M. esculenta* peptides were generated by alcalase hydrolysis were studied. The 5 kDa < peptides < 10 kDa in the ultrafiltration fractions displayed highest ACE inhibition (85.9 ± 5.09% at 140 μg/ml).

The results indicate that *M. esculenta* derived peptides may have potential as functional food ingredients in the prevention and management of hypertension.

P-02.08.5-046
Modulations of antioxidant enzymes, GSTs and catalase, by salvia absconditiflora in HEPG2 cell line

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Oxidative stress is considered to play a important role in the pathogenesis of aging and several degenerative diseases, such as cancer. In order to cope with an excess of free radicals produced upon oxidative stress, humans have developed several mechanisms for maintain redox homeostasis. These protective mechanisms either scavenge or detoxify ROS, block their production, and include enzymatic and nonenzymatic antioxidant defenses. In enzymatic defenses include Glutathione S-Transferases and Catalase enzymes.

Many epidemiological studies have revealed that there is a strong correlation between consumption of polyphenol-rich foods and the prevention of certain diseases like cancer, cardiovascular diseases and aging. Phenolic compounds are abundant in all plants. So, they form an integral part of the human diet. Salvia species, commonly known as sage, have been used since ancient times for more than 60 different ailments ranging from aches to epilepsy. There are around 900 species of Salvia, 95 of which are represented in Turkey including *Salvia absconditiflora*.

In this study, *S. absconditiflora* collected from METU Campus (Ankara, Turkey) is extracted with methanol and water. Effects of the water and methanol extracts on the mRNA expressions of antioxidant enzymes GSTM1 and Catalase in HepG2 cells were investigated by q-RT-PCR technique. It was also monitored the effects of the extracts on the enzyme activities of GSTs and Catalase by spectrophotometrically.

Water and methanol extracts decreased GSTs mRNA expression in HepG2 cells for 48 hours and 72 hours incubation and methanol extract decrease catalase mRNA expression for only 72 hours incubation. On the other hand, extracts highly increased the GSTs and Catalase activities in both hour incubation.

Overall, these results indicate that *S. absconditiflora a*nd/or its components have regulatory activities on antioxidant enzymes and they may have a potential as a therapeutic agent in the treatment of cancer.

P-02.08.5-047
Transcriptomics and proteomics approach to drought stress mechanism in wheat

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Identification of novel stress-responsive genes and their role in drought response is an important area for the improvement of the crops. Drought-related genes were investigated in leaves and roots of three wheat genotypes after different drought stress treatments by RNA sequencing (RNA Seq) technology and de novo assembly was performed before comparative transcriptome analysis. Analyzing 311 gigabases of 100 bp paired end Illumina reads from a hexaploid wheat poly(A) RNA library, we identified common and new differentially expressed transcripts. Selected differentially expressed genes were confirmed by qRT-PCR. We also performed root proteome analysis with nano electrospray ionization source coupled to a high-performance liquid chromatography system (nanoUPLC−ESI−qTOF−MS) to identify drought-related proteins. Totally 191 proteins were differentially expressed in root tissues of tolerant and non-tolerant wheat genotypes. Responses of antioxidative defense system to drought stress were comparatively studied in the same wheat cultivars. Similarities between protein and RNA levels help increase our confidence in novel biomarkers, differences may also reveal other post-transcriptional regulatory junctures. All these analyses will allow us to get a better idea about the possible role of these genes in the drought-response mechanism. The drought-related genes that are functionally characterized could be introduced into agronomically important wheat cultivars. This work offers a resource for accelerating drought-related gene discovery and improving this important crop.

P-02.08.5-048
Isolation and characterization of a hexose converter from olive

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Introduction: Hexose sugars are key components of glycolysis and photosynthesis. The genes regulating their conversion into one another, is therefore, of great importance for the control of carbon metabolism. In this study, we report isolation and characterization of a cDNA associated with conversion of hexoses in olive. The cDNA putatively named aldolase based on bioinformatic and experimental analyses.

Material-Methods: Characterization based on nucleotide and amino acids were conducted using bioinformatic tools such as nucleotide and protein BLAST, BioEdit, Primer3, FinchTV, CLC Genomic Workbench, ExPASY, TargetP, SOSUI and Web Promoter Scan. Comparison of the genomic and cDNA sequence of the gene and detailed bioinformatic analyses including cellular location, hydropathy analysis, amino acid-nucleotide composition and predicted 3D structure were also conducted using the bioinformatics tools mentioned above. Temporal expression pattern of the putative aldolase were conducted using real-time PCR experiments. SDS and Western blot analyses were completed while biochemical analyses are ongoing.

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Results: The cDNA clone identified from a cDNA library we constructed was analyzed and labeled as a putative aldolase based. This similarity was confirmed with detailed BLAST analyses. Real-time PCR analyses revealed the putative gene expressed 3–5 fold more than the housekeeping gene (GAPDH) in leaves. Polymorphism analysis revealed olive aldolase had multiple SNPs among about 30 cultivars while Ayvalık and Cormona cultivars were the closest to each other based on this sequence. The putative aldoase cDNA was transferred into bacteria to express the protein it encodes. The protein was displayed on SDS-PAGE and Western blotting analyses which will be followed by biochemical characterization assays.

Acknowledgements: This work was supported by TÜBİTAK with grant number 110O108.

Keywords: *Olea europaea* L., Bioinformatic analyzes, hexose aldolase, Real-time PCR, SDS-PAGE.

P-02.08.5-049
Genetic mechanism of oleocanthal biosynthesis in olive

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Oleocanthal is an important secondary metabolite that has been reported to be useful against important human diseases including cancer. The aim of this study was to identify and characterize the key biochemical and genetic components of oleocanthal biosynthesis. To determine the biochemical components of the pathway, multiple olive cultivars along with their spatial and temporal points were determined. The expression levels of multiple candidate genes were also aimed via real-time PCR. Nucleotide BLAST and protein BLAST (for comparison of the similarity of the candidate genes with that of other organisms) were conducted on NCBI web page. Phylogenetic tree construction, amino acid composition analysis, nucleotide composition analysis, hydropathy analysis and translations through ExPASY were conducted. Primer3 was used to design forward and reverse primers to amplify the target genes from different olive tissues at different times. Analysis of the first candiate gene with BioEdit program revealed that A+T ratio was more than G+C according to the nucleotide composition analysis. According to amino acid composition analysis isoleucine, lysine and leucine were more than other amino acids while Kyte&Doolittle hyddropathy analysis revealed that the protein was hydrophilic. Abundance of hydrophobic amino acids (leucine and isolecine) along with an abundant hydrophilic amino acid (lysine) suggest the existance of hydrophobic pockets in the protein which may mean a membrane bound protein or a sitoplasmic protein with a strong hydrophobic core. The molecular weight of the protein was 56 kDa with a pI of 9.21. The protein was found to have a signal peptide. According to the $SOSUI_{GramN}$, intracellular localization was found to be in the inner membrane. Analysis of other candidate genes contiunes.

Acknowledgements: This study was supported by TUBITAK with grant number 110O108.

Key words: Olive, *Olea europaea* L., Secologanin, Polymorphism, Allele diversity

P-02.08.5-050
Antioxidant potentials of propolis and its bioactive components, and their effects on CYP2E1 gene expression in HT-29 adenocarcinoma cell line

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Propolis is a resinous mixture that is collected by honeybees from plants, and is combined with beeswax and secretions from the bee's salivary glands plus some pollen. It is a rich mixture of polyphenols, flavonoid aglycones, phenolic acids and their esters. It has been used in traditional medicine for thousands of years because of these ingredients. Propolis, just like honey, has been the subject of many studies due to its antimicrobial, antifungal, antiviral and hepatoprotective activities. The cytochrome P450 enzymes are involved in phase I xenobiotic and drug metabolism. CYP2E1 is present in high levels in human tumors demonstrated by qRT-PCR and immunohistochemical studies.

In this study, Propolis collected from Datça (Mugla, Turkey) is extracted with 70% ethanol. Phenolic contents of the propolis were measured by LC-MS/MS. Cytotoxic effects of the propolis on HT-29 human colon adenocarcinoma cell lines were examined via XTT colorimetric cell proliferation assay. Effects of propolis extract and its main bioactive component caffeic acid on the expression of phase I detoxification enzyme CYP2E1 in HT-29 cells were investigated bu using q-RT-PCR technique.

IC_{50} values for DPPH radicals scavenging activities of the extract was calculated as 0.042 mg/ml. TPC and TFC were determined as 168.6 mg GAE/g extract and 141.8 mg QE/g extract, respectively. Caffeic acid content of the extract was measured as 10.6 μg/g extract. IC_{50} values for XTT assay in 48 hours incubation with the extract and caffeic acid were evaluated as 1.16 mg/ml and 0.149 mg/ml, respectively. Propolis extract and its main phenolic content caffeic acid significantly dicreased CYP2E1 mRNA expression with 2.4 and 5.3 fold, respectively in HT-29 human colorectal adenocarcinoma cells for 48 hours incubation.

Overall, these results indicate that propolis and/or its components have regulatory activities on CYP2E1 expression and they may have a potential as a therapeutic agent in the treatment of cancer.

P-02.08.5-051
Effects of histone H3 lysine 9 inhibition on gene expression profile in tobacco (*Nicotiana tabacum* L.)

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Differentiation is the characteristic of multicellular organism. Cellular dedifferentiation underlies topical issues in biology such as reprogramming in stem cell research, regeneration and nuclear cloning, and has common features in plants and animals. The state of dedifferentiation is evidenced by changes in cell morphology, genome organization and the pattern of gene expression as well as by the capability of plant tissues to differentiate into multiple types of cells depending on the type of stimulus applied.

Chromatin reorganization appears to be a fundamental theme in cellular dedifferentiation and reentry into the cell cycle both in plants and animals. The chemical modifications of histone proteins which are structural units of the nucleosome, generate

'codes' for the recruitment of proteins or protein complexes that affect chromatin structure and gene expression according to 'histone code' hypothesis. Methylation of histone H3 at lysine 9 residue by specific methyltransferases SUV39H1 in humans and KYP/SUVH4 in *Arabidopsis* generates a'code' for the recruitment of HP1 proteins.

In this study, to enhance the dedifferentiation efficiency, chaetocin which inhibits SUV39H1 has been used at the germination stage of tobacco seeds in in vitro conditions. Our results showed that chaetocin induced callus formation from leaf discs of tobacco in the early stage of the inhibitor application. Chaetocin can enhance reprogramming of plant cells in seed development treatment as callus induction.

It is known that the formation of callus which is the non-differentiated cell community in plants, is a consequence of the changes in the gene expression profile. It has been found that epigenetic modifications play a crucial role in some of these changes. The definition of the genes related to callus formation by the inhibitation of an epigenetic modification -H3K9 methylation- in tobacco might be used to bear on various dedifferentiation-driven cellular processes.

P-02.08.5-052
Apoptotic effects of *Rheum ribes* plant extract on MCF-7 cancer cell lines

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Induction of tumor cell death by the use of some phytochemicals that consumed through diet, and derived from medicinal plants opens up new horizons for cancer treatment researches. *Rheum ribes* species, which is studied in this research, is one of the commonly used herbs in pharmacological researches. The high content of phenolic compounds in *R. ribes* extracts were known to be responsible for the high antioxidant and antibacterial activities. Our aim in this study is to assess cytotoxic and apoptotic changes by way of implementing methanol extract of the *Rheum ribes* (root) to the MCF-7 breast cancer cell line.

Cytotoxic effect of *Rheum ribes* extract was evaluated by using the XTT (2,3-Bis(2-metoksi- 4-nitro-5-sulfofenil)-2H-tetrazolyum) test. In order to determine the dose of IC50, plant extracts were applied as time and dose dependent in the range of 10–500ug. In 72nd hour, the IC50 value is determined as 400ug. To examine the apoptotic effects of the extract, total RNAs were isolated from dose group and the control cells firstly, then cDNAs were synthesized. Expression profile of the target genes(Caspase-3, Caspase-7, Caspase-8, Caspase-9, Bax, Bcl-2, Fas) are determined by qPCR.

According to the results, when the control group compared with the cells, it was determined that, while 17.33 and 3.05-fold respectively increase in the gene expressions of Caspase-3 and Caspase-7 of dose group cells, 15.45-fold decrease in the gene expressions of Bcl-2. No significant difference was observed in the other genes examined. Based on the obtained data, we believe that methanol extract of the *Rheum ribes* induces apoptosis by activating intrinsic pathway.

As a result, this plant species can be a new and effective therapeutic candidate for the medical world in search of alternative anti-cancer approaches, and could shed light on the work to be done in this area.

P-02.08.5-053
The effect of ferulic acid and 5-fluorouracil combination on apoptosis in PC-3 human prostate cancer cells

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Prostate cancer is quite often seen in industrialized countries and has the second most common death rate due to cancer after lung cancer in men. 5-Fluorouracil (5-FU) is a pyrimidine analog and cell cycle-targeting drug by inhibiting DNA synthesis. It has been widely used for treatment of several cancers such as gastric, colorectal, and breast cancers. Phenolic compounds found in foods are potential antioxidants of harmful oxidative processes related to cancer and also important due to induction of different mechanism such as apoptosis. Ferulic acid (FA; 4-hydroxy-3-methoxycinnamic acid), a phenolic compound, is abundant in fruits and vegetables. The purpose of this study was to investigate combination effect of FA and 5-FU on apoptosis in PC-3 human prostate cancer cell line.

The effects of 5-FU, FA, and combination of both of them on cell viability were determined by XTT method. Total RNA isolation was conducted using TRIzol Reagent. Expressions of important genes in apoptosis including *CASP3*, *CASP7*, *CASP8*, *CASP9*, *BCL2*, *BAX*, *FAS* and *CYCS* were investigated in four groups by qPCR.

The IC_{50} doses of FA and 5-FU were found to be 300 μM and 60 μM for 48 hours in PC-3 cells, respectively. In order to determine combination effect, PC-3 cells were treated with $<IC_{50}$ doses (200 μM FA and 40 μM 5-FU). According to qPCR results, a significant increase in the expressions of *BAX* and *CYCS* genes and a decrease in the expression of *BCL2* were observed in the FA group, compared with the control group cells. After the treatment with 5-FU, the expression of *CASP8* was significantly increased compared with the control group. Furthermore, combination of FA and 5-FU significantly increased expression of *CASP7*, *CASP8*, *FAS* and *CYCS* genes.

In conclusion, comparing with the single treatments, it was observed that combination of FA and 5-FU affected expression of different genes in apoptosis in PC-3 cell line.

P-02.08.5-054
Combination of ferulic acid and gemcitabine affects expression of some apoptotic genes in PC-3 human prostate cancer cells

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Prostate cancer, the second causing of cancer-related death in men, is an important cancer type due to the late age of onset, slow the progression, high incidence. Gemcitabine is an effective agent in several cancer types including non-small cell lung cancer, pancreatic, bladder and breast cancer. It is known that gemcitabine is used single agent or as a part of combination treatment in prostate cancer therapy. Nowadays, new treatment methods have been tested for cancer therapy including natural compounds with low toxicity. Ferulic acid (FA) one of these agents, an

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abundant phenolic compound found in various fruit and vegetables. In this study, objective was to investigate combination effect of ferulic acid and gemcitabine on apoptosis in PC-3 human prostate cancer cell line.

Cell viability was determined by using XTT method after the treatment with gemcitabine, FA and combination of both of them. Total RNA was isolated with TRIzol Reagent. Expressions of *CASP3*, *CASP7*, *CASP8*, *CASP9*, *BCL2*, *BAX*, *FAS* and *CYCS* genes are important in apoptosis, were evaluated in four groups by qPCR.

The IC_{50} doses of FA and gemcitabine were found to be 300 μM and 50 μM for 48 hours in PC-3 cells, respectively. For determination of combination effect, PC-3 cells were treated with $<IC_{50}$ doses (200 μM FA and 35 μM gemcitabine). When compared with the control group, qPCR results illustrated, a significant increase in the expressions of *BAX* and *CYCS* genes whereas a decrease in the expression of *BCL2* gene in the FA treatment group. After the treatment with gemcitabine, the expression of *CASP3* and *FAS* genes were significantly increased compared with the control group. Furthermore, combination of FA and gemcitabine significantly increased expression of *CASP3, CASP7*, *CASP8*, *FAS* and *CYCS* genes.

In conclusion, it was observed that combination of FA and gemcitabine affected different genes in apoptosis compared with the single treatments in PC-3 human prostate cancer cell line.

P-02.08.5-055
Antioxidant, antimicrobial and cytotoxic activities of different extracts of *Pistacia lentiscus*

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Pistacia lentiscus Linn. is widely distributed in Mediterranean Europe, Morocco and Turkey. The resin part of its known as Mastic gum and plant called as mastic tree. It has been referred to over centuries as having medicinal properties to treat a variety of diseases. The aim of the present study are to evaluate antioxidant, cytotoxic and antimicrobial activities of heptane, chloroform and methanol extracts from *P. lentiscus* leaves and mastic gum.

The antioxidant activities of chloroform and methanol extracts were assayed with various methods, including TPC, DPPH free radical and ABTS radical cation scavenging activity. Also, cytotoxicity of extracts was evaluated and screened against human MCF-7, HeLa, A549, and HT-29 cancer cell lines and NIH-3T3 cells. The viability of cells in 100 μg/ml concentration of extracts was evaluated using MTT assay. Antimicrobial activity of extracts were investigated against *S aureus, S epidermidis, E coli, P aeruginosa, P vulgaris, K pneumoniae, C albicans, C glabrata, C guilliermondii, C tropicalis, C parapsilosis* test organims by the agar well diffusion and broth microdilution methods .

According to our results, the methanol extract of *P. lentiscus* leaves showed the highest DPPH free radical scavenging activity (IC50 = 0.16 ± 0.02 mg/ml) and ABTS radical cation scavenging activity (1.33 ± 0.06 mg/ml). This study also exhibited that methanol extract of *P. lentiscus* leaves had the highest TPC (126.7 ± 5.7 mg gallic acid equivalents/g extract). The hexane extract of mastic gum showed antifungal activity against all of the tested Candida species (6–16 mm / <0.25–8 mg/ml). The methanol extracts of *P. lentiscus* leaves showed the highest antimicrobial activity against all of the tested bacteria except *K pneumoniae* strain (8–14 mm/>256 mg/ml). On the other hand, *P. lentiscus* showed no cytotoxicity against cancer and normal cells.

The results suggested that *P. lentiscus* may be natural source of antioxidant and antimicrobial activities.

P-02.08.5-056
Antibacterial activity of and chemical composition of alcoholic extract of marjoram against some human pathogens

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Herbs enjoy a unique value and importance in sustaining healthy communities in terms of disease prevention(1). In this regard, Marjoram is a plant of the mint family which has antibacterial properties on microorganisms(2). The current study aims to investigate the anti-microbial activity of the alcohol extracts (i.e., methanol or ethanol) of Marjoram plants on the bacteria of Staphylococcus (atcc: 25923), aureus *E. coli* (atcc: 25922), and *Salmonella enterica* (atcc: 13076) and *P. aeroginosa* through utilizing disk diffusion method. Also, the minimum inhibitory concentration and the minimum bactericidal concentrationwere measured through tube. The measurement of minimum inhibitory concentration and minimum bactericidal concentration of ethanol and methanol extracts on E.coli were equal with 100 and 120 milligrams per milliliter, subsequently. Moreover, the measurement of the minimum inhibitory concentration and of the minimum inhibitory concentration of marjoram ethanol extraction on Staphylococcus aurous was reported to be 90 milligrams and 100 milligrams per milliliter, subsequently. In addition, the amount of ethanol and methanol extracts on Salmonella enteric and *P. aeroginosa* was equal with 80 and 90 milligrams per milliliter, subsequently. The results showed that Marjoram alcoholic extract enjoy antibacterial properties. Also, among the alcoholic extracts, the ethanol extract has demonstrated to be the most effective extract on Salmonella enterica and *E. coli* and *P. aeroginosa.*

P-02.08.5-057
Molecular analysis of serine/arginine rich SC35 splicing factor from olive

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Olive (*Olea europaea* L.) is an evergreen fruit tree adapted to Mediterranean climate and rich in tannins, essential oils and organic acids. Serine/arginine rich splicing factors are essential in seqeunce specific splicing of pre-mRNAs. In this study we report molecular characterization of an serine/arginine rich SC35 splicing factor (*OeSARsc35SF*) that was isolated from a cDNA library constructed from olive pedicels. Nucleotide BLAST and protein BLAST (for comparison of the similarity of the candidate genes from other organisms) were conducted on NCBI web page. Amino acid composition analysis, nucleotide composition analysis, hydropathy analysis, open reading frame determiantion, through, molecular weight and the isoelectric points calculations were conducted using online ExPasy software. Primer3 was used to design forward and reverse primers to amplify the target gene from different olive tissues at different times. Analysis with BioEdit program revealed that A+T ratio was more than that of G+C. *OeSARsc35SF* was a protein consisting of 267 amino acids. As expected, amino acid composition analysis revealed that serines

and arginines were more than other amino acids. Kyte&Doolittle hyddropathy analysis revealed that the protein was hydrophilic. The molecular weight of the protein was 30 kDa with an isoelectric point (pI) of 11.5. The protein was found to have a signal peptide. According to the Predotar analysis results, intracellular localization was found to be in the mitochondrial. The combined results suggest OeSARsc35SF might function as splicing factor as its homologs from the other plants. Confirming this hypothesis with futher experimental characterization including biochemical function analysis continues.

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Keywords: *Olea europaea* L., *OeSARsc35SF*, Alternative splcing, pre-mRNA splicing, BioEdit, Pedicel specific cDNAs.

P-02.08.5-058
Application of three-phase partitioning for the purification of peroxidase from kiwano (*Cucumis metuliferus*)

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Peroxidases are enzymes able to catalyze reduction of H_2O_2 and oxidize various substrates. The kiwano is an oval shaped fruit which has an orange skin with lots of tiny horns. In this study, peroxidase isolated from kiwano is purified with Three-Phase Partitioning (TPP).

Kiwano fruit was homogenized and obtained crude enzyme extract. The extract was saturated with 50% (w/v) ammonium sulfate ($(NH_4)_2SO_4$) and added t-butanol with the ratio of 1:1.5 (v/v). The lower and interfacial layer was collected. The influence of percent saturations of $(NH_4)_2SO_4$ (50, 65, 75, 85, 95%) and t-butanol ratios (1:0.5, 1:1, 1:1.5, 1:2) to the partitioning behaviour of peroxidase were analyzed. After dialyzed, the interfacial and lower phases were measured for peroxidase activity and protein content. The protein pattern of the peroxidases was evaluated by using gel electrophoresis.

Peroxidase activity recovery and the purification fold of interfacial and lower phases were 117.4, 1.7% and 0.84, 0.03. Therefore, other experiments were continued with interfacial phase. At constant t-butanol with the ratio of 1:1.5, the enzyme activity recovery and purification fold of interfacial phase for saturations of $(NH_4)_2SO_4$ (50, 65, 75, 85%) were 40.9, 43.2, 39.2, 135% and 0.37, 0.31, 0.4, 0.91. The interfacial phase was not dissolved in 95% $(NH_4)_2SO_4$. At constant 85% $(NH_4)_2SO_4$, the enzyme activity recovery and purification fold of interfacial phase for t-butanol ratio (1:0.5, 1:1, 1:1.5, 1:2) were 61.6, 48.1, 127.6, 126.1% and 1.79, 0.6, 1.84, 1.41. Finally, at optimum conditions (% 85 $(NH_4)_2SO_4$, t-butanol 1:1.5) after dialyzed interfacial phase, the enzyme activity recovery and the purification fold were 138.8% and 4.46. The results of gel electrophoresis showed that the molecular weight of enzyme was between 30–60 kDa.

The applications of TPP gave the maximum recovery of 138.8% and 4.46-fold purification. As a result, for purfication of peroxidases, TPP is a rapid, simple and economical technique.

P-02.08.5-059
Time-dependent proteome profiling of *Brachypodium distachyon* leaves in response to drought stress

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Accumulating the most robust genes and proteins in elite genotypes without any harmful effect on the potential plant yield is an urgent need to enhance productivity under various stressors. Among the stressors, drought is a major challenge for agricultural productivity. *Brachypodium distachyon*, with its close relationship to agriculturally and economically important crops, is an important model plant species. Although ongoing transcriptomic analyses in *Brachypodium distachyon* available, proteomic analyses are required to obtain an integrated picture of drought response.

In the current study, a comprehensive proteome analysis was conducted on *Brachypodium* leaves under increasing levels of drought stress. To screen gradual changes upon drought stress, *Brachypodium* leaves subjected to drought treatment for 4, 8 and 12 days were collected for each treatment day. The cellular responses were investigated through a proteomic approach involving two dimensional difference gel electrophoresis and subsequent combined tandem mass spectrometry. For the validation of transcriptional expression, the genes encoding selected proteins were examined through quantitative real-time PCR.

Spot detection on Cye-dyed gels revealed a total of 497 distinct spots in *Brachypodium* protein repertoire. A total of 13 differentially expressed proteins (DEPs), with at least 2-fold changes in abundance, were identified by mass spectrometry and classified according to their functions. The biological functions of DEPs included roles in photosynthesis, protein folding, antioxidant mechanism and metabolic processes, highlighting the significant degree of overlapping between metabolic alterations induced by drought stress.

Identified proteins in this study and understanding the molecular mechanisms of drought response and defense mechanisms in plants will contribute to the researches on development of drought tolerant crop species.

P-02.08.5-060
Immunohistochemical and electron microscopy investigation of TMV-based chimeric virus particles carrying conserved influenza antigen in *Nicotiana benthamiana*

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Recently we obtained and partially characterized set of viral vectors based on *Tobacco mosaic virus* (TMV) genome displaying conserved Influenza A M2e epitope from matrix M2 protein. Purified chimeric virus particles (CVP) conferred protection of mice against lethal homologous and heterologous Influenza virus challenge. We revealed significant difference in symptoms of infections caused by TMV-M2e recombinant viruses containing cysteine (Cys) to serine (Ser) or alanine (Ala) substitutions in the human consensus M2e sequence. Accumulation level of M2e-ala

recombinant coat protein was significantly higher than M2e-cys/ser (ratio 5:1). TMV-M2e-ser infection, in contrast to Ala mutant, suspended growth and development of *Nicotiana benthamiana*. Non-inoculated leaves (14 d.p.i.) were fixed with ethanol and histological sections were incubated with mouse serum to M2e and secondary antibodies conjugated with either HRPO or FITC. CVPs of all three mutants were detected in epidermal and stomata cells as well as in sieve elements and minor veins. Electron microscopy analysis of mesophyll cells revealed typical rigid helical particles. Cys/Ser mutants mostly accumulated within ground cytoplasm as aggregates of discrete tubules in parallel arrangement, which were not delimited by lipid membranes. We discovered huge amount of CVPs in the cytoplasm and lesser amount diffused in the central vacuole. Essential part of Ala particles was located in the cytoplasm, but mentioned aggregates were not found and only insignificant number of virions was revealed in vacuole. Unlike wild-type TMV, none of the mutants was revealed in chloroplasts. Diameters of CVPs were as follows: Ser – single particles in cytoplasm 13 ± 1.2 nm, aggregates 5.4 ± 0.5, in vacuole 3.9 ± 0.4; Cys – single particles 14.5 ± 0.7, aggregates 7.3 ± 0.4, in vacuole 7.4 ± 0.3; Ala – single particles 10.7 ± 0.4, in vacuole 5.22 ± 0.2. Therefore, microscopic analysis of CVPs, plant tissue and ultrastructure of mesophyll correlates with the observed differences between infections of plant viral vectors.

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P-02.08.5-061
Potential anti-inflammatory effects of cyanidin in lipopolysaccharide-stimulated RAW 264.7 cells

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Cyanidin is a natural anthocyanidin found in a variety of fruits (grapes, blackberry, blueberry, cherry and cranberry etc.) and vegetables (red cabbage, red onion). This polyphenolic compound is a flavonoid with significant antioxidant activity. Cyanidin and its glycosides have vasoprotective effects and can interfere with inflammation, carcinogenesis, obesity, and diabetes. One important role of the macrophages is the release of pro-inflammatory mediators, such as nitric oxide, various cytokines, in response to activation signals, including chemical mediators, cytokines, and bacterial lipopolysaccharide (LPS). In this study, we investigated the role of cyanidin chloride in inflammation.

Anti-inflammatory effects of cyanidin chloride were examined in LPS-stimulated murine RAW 264.7 macrophages. We observed the level of various inflammation markers such as nitric oxide (NO), inducible NO synthase (iNOS), cyclooxygenase-2 (COX-2), tumor necrosis factor-α (TNF-α) and interleukin-8 (IL-8) under LPS treatment with or without cyanidin chloride.

Cyanidin chloride inhibited not only NO production but also the expression of COX-2 and iNOS, without any cytotoxicity. Cyanidin chloride also attenuated pro-inflammatory cytokines and other inflammation-related markers such as IL-8 in a dose-dependent manner. In conclusion, cyanidin chloride may be beneficial for the prevention and treatment of anti-inflammatory diseases.

P-02.08.5-062
The investigation of *Centranthus longiflorus* plant extacts effects on cell proliferation and apoptosis activity in the cell lines of MCF-7 breast cancer

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Introduction: In the U.S., breast cancer is the second most common cancer in women after skin cancer. Current treatment of cancer can be done by surgery, chemotherapy, and radiation therapy. In addition, there is widespread use of complementary and alternative medicine in developed countries. Plants and plant extracts play a critical role in the research into new anticancerogenic agents. *Centranthus longiflorus* (CL) is used in alternative medicine for sedative and antispasmodic purposes. A plant of Turkish origin, *Centranthus longiflorus* used as traditional Turkish medicine have remained uninvestigated for familial hypercholesterolemia, diabetes, coronary artery disease and cancer for their in vitro biological activity despite their use for sleep disorders. In this study, growth-inhibiting and pro-apoptotic effects of hexane, ethyl acetate and ethanol extracts of CL in MCF-7 breast cancer cell line were investigated.

Material and method: Aerial parts of CL were collected in Erzurum province. Hexane, ethyl acetate and ethanol extraction were done by Soxhlet extractor. The plant extracts obtained from CL was analyzed using a GC-MS system. Dose-and time-dependent cytotoxic and apoptotic effects of CL were evaluated by MTT Cell Proliferation Kit and Cell Death Detection Elisa Kit, respectively. Manufecturer's protocol was followed for analyses. Then, apoptotic genes; caspase3, Bax and p53 and antiapoptotic genes; Bcl-2 and PI3 expression levels were determined by RT PCR.

Results: According to our results, cytotoxic effect on MCF-7 cell was only observed in 20 and 50 μg/ml doses of CL. However, any of the application doses showed an apoptotic effect on MCF-7 cells. They exhibited a necrotic effect rather than the apoptotic effect. Although alterations in expression levels of these genes were determined, this alterations was statistically insignificant.

Discussion and conclusion: Consequently, we can say that CL have a cytotoxic effect on MCF-7 breast cancer cell lines.

P-02.08.5-063
Reduction of the chloroplast genome and the loss of photosynthetic pathways in the mycoheterotrophic plant *Monotropa hypopitys,* as revealed by genome and transcriptome sequencing

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Genomes of parasitic plants represent interesting model systems to study effects of relaxed selective pressure on photosynthetic function. Previous genomic studies of nonphotosynthetic plants revealed reduction of their chloroplast genomes, but the corresponding changes in their nuclear genomes are less known. Here we present the data on the transcriptome and the chloroplast genome of the non-photosynthetic mycoheterotrophic plant *Monotropa hypopitys*.

The chloroplast genomes were sequenced for two specimens of *M. hypopitys*, collected in different regions of Russia. The

cpDNAs are 34,800 bp (MON-2KALR) and 35,336 bp (MON-1VOLR) long and rearranged with respect to each other. Both genomes contains genes encoding ribosomal proteins, *infA, matK*, and 4 ribosomal RNA genes. 17 and 19 tRNA genes were predicted in two cpDNA. Genes encoding NADH dehydrogenase, plastid RNA polymerase, all genes related to photosynthetic apparatus, *clpP*, *ycf1*, *ycf2, accD,* and some genes for ribosomal proteins are missing or became pseudogenes. The reduction of gene content is associated with extensive gene order rearrangement and the lack of inverted repeats. Overall, the size and gene content of *M. hypopitys* cpDNA indicates that it is close to the end of plastid genome degradation process.

In order to get insights into the changes in the nuclear genome associated with the transition to nonphotosynthetic lifestyle, we sequenced and assembled the transcriptome of *M. hypopitys*. As expected for holoparasites, we did not found transcripts for the nuclear genes encoding the components of photosynthetic machinery, including photosystem I and II, cytochrome *b6f* complex, and ribulose bisphosphate carboxylase. Contrary to the holoparasitic plant *Phelipanche aegyptiaca*, almost all genes of chlorophyll biosynthesis pathway from protoporphyrin IX were not found in the *M. hypopitys* transcriptome. This work was supported by the RSF grant 14-24-00175 and RFBR grant 14-14-00749 (MON-1VOLR cpDNA sequencing).

P-02.08.5-064
Chemical constituents of endemic species grow in Turkey: *Centranthus longiflorus* and its new cholesterol-lowering plant sterol ester: beta-sitosterol

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Introduction: Beta-sitosterol is a substance found in plants. Chemists call it a plant sterol ester. It is found in fruits, vegetables, nuts, and seeds. It is used to make medicine. Beta-sitosterol is used for heart disease and high cholesterol. The federal Food and Drug Administration (FDA) allows manufacturers to claim that foods containing plant sterol esters such as beta-sitosterol are for reducing the risk of coronary heart disease (CHD). *Centranthus longiflorus* (CL) is used in alternative medicine for sleep disorders. A plant of Turkish origin, CL used as folk medicine have remained uninvestigated for familial hypercholesterolemia, coronary artery disease and preventing colon cancer for their in vitro biological activity despite their use for sleep disorders. We investigated of the chemical constituents from dried aerial parts of *Centranthus longiflorus*.

Material and method: Aerial parts of CL were collected in Erzurum province. Hexane, ethyl acetate and ethanol extraction were done by Soxhlet extractor. The plant extracts obtained from the aerial parts of CL was analyzed using a Perkin-Elmer GC-MS system.

Results: Ten compounds were obtained and identified as Butanoic acid, hexadecanoic acid (palmitic acid), 7-Methyl-Z-tetradecen-1-ol acetate, octadecanoic acid (stearic acid), *diisobutyl Phthalate*, 9-octadecenamide, octacosane, nonacosane, alfa amyrin and beta sitosterol. The latter two were obtained in all extraction (hexane, ethyl acetate and ethanol).

Discussion and conclusion: All of these compounds are isolated from *Centranthus longiflorus* for the first time. These findings may shed light on the design of new drugs, the cholesterol-lowering effect.

P-02.08.5-065
Role of lutein for the high light-induced inhibition of photosystem II related reactions in thylakoid membranes of *Arabidopsis thaliana,* wt and lut2

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Photosynthetic reactions taking place in thylakoid membranes of higher plants are extremely sensitive towards different environmental stress conditions such as high and low temperature, high light intensity, UV radiation etc. Carotenoids are intrinsic component of photosynthetic pigment-protein complexes and are involved in performing multiple important functions. Their role of accessory pigments in absorbing sun light, participation in photoprotection via dissipation of excess absorbed light, deactivating of stress-induced reactive oxygen species and structural role are well documented and recognized. The role of lack of lutein in high light-induced alterations in structural organization and functional activity of the main pigment-protein complexes was evaluated using isolated thylakoid membranes of *Arabidopsis thaliana*, wt and mutant lut2, deficient in lutein, subjected to photoinhibitory treatment for different periods of time. Alterations in photochemical activity of photosystem I and photosystem II were determined by a Clark-type electrode in the presence of exogenous electron donors and acceptors. Activity of oxygen-evolving complex and of the grana and stroma situated photosystem II reaction centers was evaluated by determination of flash oxygen yields and initial oxygen burst under constant light without donors and acceptors. Low-temperature (77K) fluorescence was applied for unraveling of light-induced alterations in energy transfer and interaction between the main pigment-protein complexes. Maximal quantum efficiency of PSII was registered by Pulse Amplitude modulated fluorescence method. Results obtained are discussed in respect to the importance of lutein for the organization and sensitivity of photosynthetic apparatus towards high light intensity treatment.

P-02.08.5-066
Expression of bacterial phytase in *Arabidopsis thaliana* plants

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Modern agriculture relies heavily on phosphate rock fertilizer to improve phosphorus availability in many soils, but this approach is not sustainable long-term. Phytate (myo-inositol hexakisphosphate) is an organic phosphorus compound often present in many soils. However, phytate can not be utilized by most plants, and its accumulation in soil leads to substantial ecological problems. Phytases are enzymes that hydrolyze phytate and release inorganic phosphate. Many microorganisms such as bacteria and fungi synthesize highly diverse phytases which are suitable for plant biotechnology. Generation of transgenic plants expressing phytases of bacterial origin has been proposed as one option to improve plant phosphorus nutrition.

In this study, we generated and characterized transgenic *Arabidopsis thaliana* plants expressing a modified phytase gene *PaPhyC* from *Pantoea sp.* under strong CaMV35S promoter. Three individual transgenic *A. thaliana* lines expressing the bacterial phytase gene, as well as negative control plants harboring the CaMV35S promoter alone were identified. Expression of phytase in plants was verified at both transcription and translation levels.

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Phytase-expressing plants grown on media with phytate as the sole source of phosphorus demonstrated better than wild-type growth rates, shoot dry mass, shoot phosphorus content, as well as higher phytase activity in cell-wall extracts. Overall, we show that plants expressing bacterial phytase are capable of better growth on phytate as the only source of phosphorus in laboratory conditions. Further research investigating the applicability of using bacterial phytase expression to improve plant growth in soil is necessary to evaluate the different routes of solving the phosphorus deficiency problem in agriculture.

P-02.08.5-067
The role of elevated temperature in photoinhibition and recovery of photosystem II in thylakoid membranes from *Arabidopsis thaliana*

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Photosynthesis of higher plants is the principle process to transform light energy into biochemical usable energy. In nature, plants are exposed to the environment where light, temperature, UV-B radiation varied and very often their extreme values that are unfavorable for effective performance of photosynthetic reactions. Plant are developed various strategies to cope with stress including radical scavenging enzyme system, accumulation of protective compounds, etc. Pigment-protein complexes of photosystem I and photosystem II and their light harvesting antenna, situated within thylakoid membranes, are involved in the primary reactions of photosynthesis - absorption of light, charge separation and electron transport. Photosynthetic process is sensitive towards higher than optimal temperatures, the photosystem II and oxygen evolving complexes being extremely sensitive to elevation of temperature. In present work PAM fluorescence was applied to evaluate the effect of long term action of elevated temperature (38/30 °C) on the quantum yield of photosystem II, non-photochemical quenching and Rdf, the latter quantifying the photosynthetic process. In addition, the activity of oxygen evolving complex was determined polarographically in the presence of exogenous electron acceptor 1,4 - benzoquinone. SDS-PAGE electrophoresis and Western blot were applied to determine the damage of D1 - reaction center protein of photosystem II. Alterations of mutual organization within photosystem II complex and its antenna and of energy interaction between them were followed by analysis of 77K steady state chlorophyll fluorescence spectra. The simultaneous application of high temperature and high light intensity resulted in a well pronounced reduction of non-photochemical quenching that restore to the initial values after recovery for 5 days at optimal conditions. D1 was also restored while quantum efficiency of photosystem II did not recuperate to initial values.

P-02.08.5-068
Reorganization of the main pigment-protein complexes in thylakoid membranes from tomato (*Solanum lycopersicum*) during long term exposure to elevated temperature

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The changes of Earth climate resulted in unfavorable environment for plants. Depending on the duration of influence of stress factors, the response of plants includes short and long term acclimation. The population, structure and organization of pigment-protein complexes within thylakoid membranes are dynamic and flexible, thus providing for the acclimation of the photosynthetic apparatus to the changed environment. The main pigment-protein complexes, involved in energy transduction, are photosystem I, photosystem II and light harvesting complexes. They are separated in grana and stroma regions of thylakoid membranes but it is well established that they can rearrange as a result of alterations of light intensity, temperature increase and decrease in order to balance the perception and utilization of excitation energy. In present work the effect of long term action of elevated temperature on organization and stoichiometry of main pigment-protein complexes in the thylakoid membranes from tomato plants (*Solanum lycopersicum* cv. M82) was investigated. Three weeks old tomato grown at optimal conditions (22/20 °C day/night temperature and light intensity 250 μmol/m^2/s) plants were exposed for 2 and 6 days to elevated temperature at 38/30 °C. By means of blue-native electrophoresis the effect elevated temperature on the populations of PSII (dimmer and monomers) and LHCII (monomers and trimers) was estimated and compared with the same parameters for control plants. The ability of plants to recover from this treatment was checked after 5 days under optimal conditions. The changes of content of chlorophylls and carotenoids were determined at every stage of treatment. Based on the results obtained it can be concluded that one of the mechanisms for regulating the energy balance and maintenance of efficient photosynthetic process involves a change in the organization and stoichiometry of the photosystem II and oligomer state of light harvesting complex II.

P-02.08.5-069
Anti-tumoral effects of silymarin, curcumin and propolis on leptin induced breast cancer cells

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Silymarin, Curcumin and Propolis are natural origin product have possess anti-cancer activities via its effect on biological pathways.

Aim of the study, was to evaluate the anti-tumor effects of Silymarin, Curcumin and Propolis on leptin-induced MCF-7 cells.

MCF-7 cells were incubated various concentrations leptin (physiological, obesity and pharmacologically doses; respectively 10, 100 and 1000 ng/ml) . Then different doses of Silymarin (10, 20, 40, 80 μM), Curcumin (10, 20, 40, 60 μM) and Propolis (0.063, 0.125, 0.25, 0.5 mg/ml) were added. After 24, 48, 72 and 96 hours incubation periods 3 different area images were taken digital camera. Then using dye release reagent we determined the

intensity of apoptosis via colorimetric determination by ELISA reader. Absorbance was directly proportional the number of apoptotic cells (Biocolor Cell-Apo percentageApoptosisAssay). Also, we examined the effect of these natural products on proliferation rate of leptin-induced MCF-7 cells for 1, 2, 3 and 4 hours (Biovision Cell Proliferation Kit) All experiments were carried out 3 different days, at least 3 times.

All of three compounds were stimulate the apoptosis at all time points and all different doses of leptin. The differences was statistically significant at the level of $p < 0.05$ between 24 and 48 hours. It was found that there were not seen much cells at 72 and 96 hours time points. We thought that most of the cells were gone necrosis instead of apoptosis. The best effective doses on apoptosis of propolis was 0.063 mg/ml, silymarin and curcumin were 20 μM. Also, we evaluated the effects of on proliferation rate the MCF-7 cells, we found that only propolis was effective of inhibiting proliferation at all doses of leptin induced MCF-7 cells in 1 hour.

We hope this study will be a guide for the further studies in anti-cancer agent development field and show that the natural origin substances cause cancer cells apoptosis and provide targeted treatment for cancer therapy.

P-02.08.5-070
Investigation of some lichen-derived substances' cosmetic potential for skin protection against ultraviolet B

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Due to the depletion of the stratospheric ozone layer and chronic exposure, the occurrence of various skin diseases have been increased in recent decades. Thence, people and cosmetic companies have progressively given more importance natural sunscreen products for the protection from harmful sun rays, especially ultraviolet B rays. We, therefore, isolated some lichen-derived substances; 3-hydroxyphysodic acid and protolichesterinic acid from *Hypogymnia tubulosa* and *Cetraria aculeate*, respectively. Chemical characterization and identification of the isolated lichen substances were accomplished by using FTIR, ^{1}H-NMR and melting point analyses. The theoretical UV-vis spectra and 3D conformations of the isolated compounds were determined by using the Gaussian 03 software with HF theory at the B3LYP/3-21G level. The dark toxicities and ultraviolet B protection capacities of the substances were lighted up as previously described [1,2] on HaCaT human keratinocyte cell line by using MTT cell viability and LDH cellular membrane degradation assays. The obtained results from the assays showed that protolichesterinic acid has a more dark toxic activity on keratinocyte cells than 3-hydroxyphysodic acid, and the toxic activities were found sufficient as much as 80% at the highest doses of the substances; 400 μM. However, it was observed that the cytotoxicity of the substances were reduced at the rate of approximately 15% by the irradiation. Consequently, we think that the substances block the ultraviolet B rays but their cytotoxic feature is an important limitation to their usage in cosmetic industry.

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P-02.08.5-071
Blockage of ultraviolet B-induced damage in human keratinocytes by a lichen compound norstictic acid

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A great effort and financial supports have been consumed to explore and design novel sun protection factors due to the unhindered increase of malignant and non-malignant skin diseases caused by the chronic exposure and depletion of the stratospheric ozone layer. The testing of naturally produced compounds seems to be the best and inexpensive way to search for potentially photoprotective substances. On the other hand, as photo-resistant species, lichens are still poorly exploited. Norstictic acid was, therefore, isolated from the acetone extract of *Pleurosticta acetabulum*. FTIR, ^{1}H-NMR and melting point analyses were performed to identify the chemical features of norstictic acid. Gaussian 03 software with HF theory at the B3LYP/3-21G level was also performed to determine the theoretical UV-vis spectrum and 3D conformation of the isolated compound. The dark cytotoxicity and ultraviolet B-protection capacity of norstictic acid were comparatively tested as previously described [1,2] by using MTT cell viability and LDH cellular membrane degradation assays. As a result of the experiments, it is observed that norstictic acid has a dark-cytotoxicity as less as 25% at the highest dose of the substance; 400 μM. However, ultraviolet B-induced damage on human keratinocytes was blocked by the lower concentrations of norstictic acid as 25, 50 and 100-μM, and 20% of cells were protected according to the control experiments of irradiated cells. Consequently, we think that norstictic acid might be employed as a sun protection factor at the low concentrations, and further studies should be performed.

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P-02.08.5-072
Cytotoxic and proliferative effects of Fe3O4@VA and vulpinic acid on MCF-7 cell line

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Nowadays, over 1000 different lichen secondary metabolites are identified that have lots of biological activities such as antimicrobial, antimutagenic, antiproliferative, and anticancer. In recent

years, researchers have focused on the lichen acids because of their biological activities. It is also suggested that lichens can be used as anticancer agents. Vulpinic acid, an important lichen seconder metabolite, has antimicrobial activity and strong antimutagenic, anticancer and antioxidant capacity. Nanotechnology has the potential to offer solutions to current obstacles in cancer therapies, because of its unique size (1–100 nm) and large surfaceto-volume ratios. So, in this study we aimed to determine the cytotoxic and proliferative effects of vulpinic acid and magnetic nanoparticles loaded with vulpinic acid (Fe_3O_4@VA) on MCF-7 cancer cell line. The cytotoxic and antiproliferative effect of vulpinic acid and Fe_3O_4@VA was evaluated by LDH (lactate dehydrogenase leakage) and MTT ((3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) (water-soluble MTT to an insoluble purple formazan) assays on MCF-7 (Breast cancer: Michigan Cancer Foundation-7) cancer cell lines. LDH assays results showed that high concentration (100 μM) of Fe_3O_4@VA acid has the most strong cytotoxic effects on MCF-7 in 24 hours. On the contrary, low concentration (25 μM) of vulpinic acid has the most strong cytotoxic effects on MCF-7 in 48 hours. According to the MTT results; high concentration (200 μM) of Fe_3O_4@VA has the most antiproliferative effect in 24 hours and 48 hours. It can be said that Fe_3O_4@VA were inhibited the cell proliferation on MCF-7 cancer cells and may be a potential candidate for the new advanced cancer treatment methods.

Key words: Vulpinic acid, lichen, Fe_3O_4

P-02.08.5-074
Proteomic comparison between Chinese wild rice (*Zizania latifolia* (Griseb) Turcz) and Indica rice (Nagina22)

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Chinese wild rice (Zizania Latifolia (Griseb) Turcz) (CWR) is an ancient plant. It belongs to the genus Zizania, family Poaceae and has a history record for more than 3000 years in China. There are four species of wild rice known around the world. Zizania aquatica L., Zizania palustris L. and Zizania texana Hitche are found in North America, whereas Zizania latifolia (Griseb) Turcz) is native to East Asia. CWR mainly grows in the areas along the Yangzi River and the Huai River in China without any cultivation and domestication. CWR was an ancient grain that has been used in Chinese herbal medicine to treat a variety of ailments associated with nutrition, including gastrointestinal disorders and diabetes. Our previous studies have demonstrated that consuming Chinese wild rice can significantly improve blood lipid profiles and ameliorate high-fat/cholesterol diet-induced insulin resistance. However, compared to the well studied common dietary white rice, active composition and the associated proteomic information of Chinese wild rice have yet to be investigated. In this study, we compared and analysed the different proteins between Chinese wild rice and white rice by proteomics method. Our study provides insights and experimental evidence for further exploration of this ancient medical food in disease prevention and therapy.

The homology between CWR and N22 is 64%, but significant differences also exist between the two. We gained new insight by analyzing the biological function of the high reliability (credibility score 67 or higher, $p < 0.05$) peptide mass fingerprint of CWR2-DE electrophoresis revealed differences in protein composition between CWR and N22. Information obtained from the PMF indicates that glutelin precursor, caffeoyl coenzyme A (CoA) O-methyltransferase and putative bithoraxoid-like protein can provide gene evidences for its biological function.

P-02.08.5-075
Mir396 and growth-regulating factors interaction during maize leaf growth under low-temperature stress

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MicroRNA (miRNA) genes are a class of non-coding small RNAs about 21 nucleotide-long which are revealed as regulators of plant growth and stress responses. The miRNA miR396 targets and regulates Growth-Regulating Factors (GRFs) which are plant specific transcription factors family and this regulation machinery is conserved among plant species. Plant growth is a result of cell division and expansion which took place as spatial gradient zones throughout maize leaf which are meristem, elongation and mature zones. Cells proliferate in meristem, migrate to elongation zone and finally reach to mature zone to get its final size. It has been shown that miR396 affects cell division by regulating GRFs and changes leaf size which are determined by cell number and cell size of leaf. This study aims to investigate the role of miR396 and GRFs interaction during maize leaf growth under low-temperature stress.

Maize seedlings were grown under low-night temperature for stress treatment to generate growth retardation and control conditions as well to make comparative analysis. Length of the third and fourth leaves of seedlings was measured every day and leaf elongation rate was calculated to observe stress effects on the leaves. Growth zones of fourth leaves were harvested during steady-state growth phase for determining expression level of miR396s and their target by q-RT-PCR.

We mined 8 miR396 genes sharing sequence similarity and 8 GRF targets. The expression analyses of mir396s and GRF5 are proceeding for different growth zones.

In conclusion, this is the first study investigating the regulation network between miR396s and GRF5 in different developmental stages of maize leaf under low-temperature stress.

P-02.08.5-076
Molecular characterization of an olive imidazoleglycerol-phosphate dehydratase

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Imidazoleglycerol-phosphate dehydratase (IGPD) catalyzes the 6th step in histidine biosynthesis in various organism including fungi, bacteria and plants. There are two different isoforms of IGPD; IGPD1 and IGPD2. By finding specific inhibitors of IGPD in plants, many herbicides have been designed. Although there are numerous reports on various plant *IGPD*s, there are no studies reporting olive IGPD. The aim of this study is to conduct characterization of an olive *IGPD* (*OeIGPD*).

OeIGPD cDNA was isolated from a cDNA library we constructed from olive pedicels. Homology searches for nucleotide, amino acids and alternative open reading frames were conducted utilizing BLASTn, BLASTp, and BLASTx, respectively. Nucleotide sequences of homologous genes from other plants were aligned using BioEdit and the number of SNPs were detected. The alignment was then used to generate a phylogenetic tree using MEGA7 program. Another alignment with amino acid sequences of the homologues proteins was also generated to construct a phylogenetic tree displaying OeIGPD's position among other plants.

Various aspects of OeIGPD including amino acid composition, hydropathy analysis, isoelectric point (pI) and three dimentional structure of the protein were determined using online

software at ExPASY. Multiple primer pairs to amplify the full length open reading frame of the gene, to clone the gene into the expressing vector pLATE51, and to detect expression through real-time PCR were designed using Primer3. Amino acid composition analysis revealed that OeIGPD contained serine, arginine and isoleucine predominantly while hydropathy analysis suggested it was an hydrophilic protein. Isoelectric point (pI) of the protein was calculated as 4.97. The molecular weight of the protein was calculated as 11 kDa. Analyses continue to determine the polymorphism of *OeIGPD* among olive cultivars, and biochemical function of the gene in olive.

P-02.08.5-077
Cytotoxic effect of fractionated triterpenoid glycosides from *Holothuria polii* in human cancer cells

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Sea cucumbers are the members of class Holothuroidea and they have more than 1100 described living species all around the world. Sea cucumbers secrete special secondary metabolites from their body walls and they are called triterpene glycosides (TGGs). In this study, cytotoxic activity of fractionated TTGs from *H. polii* on different cancer cell lines were carried out. *H. polii* Delle Chiaje, 1824 samples were collected from Dikili-İzmir. The semi-purified extracts were fractioned by using HPLC. Four different fractions (fraction A-D) were obtained. In order to characterize the fractions, MALDI-TOF/MS was used. The cytotoxic activity of the fraction A-D were tested on HT-29, T84 and UPCI-SCC-131 cell lines by using xCELLigence RTCA SP system. The cells were treated with three different concentrations of the fractions for 48 hours. The cell index data were compared with the control group. IC50 values of the fractions for three cell lines were calculated. According to the results, the fractions have Holothurin A (1243.50 m/z), 24–dehydroechinoside A, Scabraside A or Fuscocinerosides B/C isomer (1227.50 m/z). The fraction D was the most effective on all cell lines with IC50 value of 10.46 ± 0.18 mg/l, 11.24 ± 0.57 mg/l and 11.13 ± 0.29 mg/l for HT-29, UPCI-SCC-131 and T84, respectively. In conclusion, sea cucumber TTGs are promising agents for colon adenocarcinoma, oral squamous cell carcinoma and colorectal carcinoma (metastatic) treatment.

P-02.08.5-078
Effect of Horse-chestnut (*Aesculus hippocastanum*) seed extract on matrix metalloproteinases during diabetic wound healing

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Impaired wound-healing in diabetics is a major public health problem. The expression and activation of matrix metalloproteinases (MMPs) are also impaired in diabetic wounds according to previous studies. Their main function is to degrade the various components of the extracellular matrix. Also, they participate physiological processes such as inflammation, angiogenesis, tissue remodeling. Horse-chestnut seeds (HC) are rich in saponins and flavonoids. It has been shown that HC has anti-inflammatory, antioedema, vessel protective, and free radical scavenging properties. The aim of this study is to determine with molecular signs on cutaneous wound healing effects of the ethanol (50%) extract of HC (HCE) seed in rats by excision wound model.

This study was conducted on diabetic Wistar albino rats, which were injected by a single dose (50 mg/kg i.p.) streptozotocin. Diabetic treatment rats were applied topically 15% (w/w) ointment with HCE and control rats were applied topically simple ointment, once a day during the experimental period. The gene expression levels of MMP-1, MMP-9 by qPCR and levels of nitric oxide (NO), hydroxyproline and malondialdehyde in wound tissue investigated at the end of 3rd, 7th, and 14th days. Wound closure was also measured.

The hydroxyproline and NO levels were significantly increased in the HCE treated group versus control after the 3rd and 7th days. The malondialdehyde levels were significantly lower in the treatment group. MMP1 gene (associated with collagen processing and reepithelialization) expression levels in HCE treated rats were increased in the 7th day while it was reduced in 14th day. MMP 9 gene (associated with inflammation and gelatinase) expression levels in HCE treated rats were decreased in 7th, and 14th days compared to the control.

These findings indicate that HCE accelerated the cutaneous wound healing process in diabetic rats via MMP1 and MMP9 regulation.

P-02.08.5-079
Isolation and molecular molecular characterization of VPS39/VAM6 from olive

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VPS39/VAM6 promotes aggregating and fusing of endosomes and lysosomes. It is a component of a protein complex that is found in vacuole membranes. This gene has been studied from various organisms including humans, *Drosophila*, *Arabidopsis* and rice. No studies on olive VPS39/VAM6, however, have been reported. The aim of this study is to report information of olive VPS39/VAM6 including expression pattern and biochemical characterization.

VPS39/VAM6 was isolated from a cDNA library we constructed from fruited olive leaves in July. To determine the putative name of the cDNA, BLAST analyses were conducted for nucleotide, open reading frame and amino acid sequence comparisons. BioEdit program was used to determine the nucleotide and amino acid composition along with its molecular weight and isoelectric point (pI). Hydropathy analysis was conducted using Kyte and Doolittle program. Phylogenetic analysis was done using MEGA7. Cellular localization of the product was predicted using SOSUI GRAMN. The three dimentional structure of the protein was calculated using I-TASSER and compared to previously known structures using Cn3D.

The BLAST and BioEdit analyses revealed VPS39/VAM6 had 836 base pairs coding 120 amino acids with a molecular weight of 13.38 kDa, and pI of 6.39. The AT/GC ratio was very high (1.5) comparing to its homologs from other plants suggesting to expect significant differences of this gene's function from the others. Amino acid composition analysis revealed high rates of

Serine, Leusine and Isoleucine indicating a hydrophobic property of the protein. The hydrophobic feature was confirmed by Kyte and Doolittle analysis while the cellular location was revealed to be extracellular. The hydrophobic nature despite extracellular location suggests it is a membrane associated protein which was confirmed by transmembrane domain analysis. As expected no signal peptide was detected. The 3D structure of the protein was similar to its previously reported homologs.

P-02.08.5-080
Isolation and characterization of the ribosomal L1 protein from olive

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Despite as a ribosomal protein, L1 is known as the inhibitor of the cellular aging gene and it has been reported to have roles in apoptosis. The ribosomal L1 protein is larger than the LSU of ribosome and contains 2 domains as 2-layered alpha/beta domain and 3-layered alpha/beta domain. In ribosomes, it functions in translocation and orientation of tRNAs. Although the ribosomal L1 (RL1) gene has been studied in many plants, reports on olive RL1 (OeRL1) are very rare. This study presents molecular characterization of RL1 gene from olive.

OeRL1 was isolated from a cDNA library we constructed from unfruited olive leaves in July. Homology analyses were conducted using BLAST programs. Nucleotide and amino acid compositions, molecular weight, isoelectric point (pI) and AT/GC ratio were determined using BioEdit and ExPASy programs. Cellular location of the L1 protein was determined using SOSUI-GramN program. Signal peptide detection, transmembrane domain detection, three dimensional (3D) structure analysis, and phylogenetic analysis were conducted using SignalP 4.1, THHMM, I-TASSER/Cn3D and MEGA7, respectively.

OeL1 was found to have an open reading frame of 909 base pairs coding 220 amino acids that constitutes a molecular weight of 24.9 kDA and a high pI of 9.87. Lysine, leucine and valine had higher rates. The hydrophilic nature suggested by Kyte and Doolittle analysis despite high rates of leucine and valine suggests an amphipathic nature of the protein that can bind to both hydrophilic and hydrophobic proteins and / or function in both media. A 1.59 AT/GC rate is significant comparing to that of its homologs from other plants. Sitoplasmic location predicted by SOSUI-GranN is in agreement with the hypothesis suggesting an amphyphatic nature for OeRL1. Likewise, no signal peptide was detected and it was predicted to have at least one transmembrane domain. Further characterization of OeRL1 with respect to expression pattern and biochemical function continues.

P-02.08.5-081
Isolation and characterization of an olive splicing factor 3B subunit 1

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Splicing factor 3B subunit 1 (*SF3B1*) functions in the regulation of translation and gene expression. SF3B1 forms U2 small nuclear ribonucleoprotein complex (U2 snRNP). Splicing factors 3A and 3B binds pre-mRNA at the 5′ site of the intron branching point. This binding joins U2 snRNP to pre-mRNA. Although *SF3B1* from various plants have been widely studied, no studies on olive *SF3B1* (*OeSF3B1*) have been reported. This study reports information on various aspects of *OeSF3B1*.

OeSF3B1 was obtained from a cDNA library we constructed from fruited olive leaves in December. It was putatively identified as a splicing factor using BLASTn, BLASTp and BLASTx. To determine wheter OeSFB1 was a sitoplasmic protein, SOSUI GramN was used. TMHMM was used to detect any transmembrane domains while signal peptide analysis was conducted by SignalP. I-Tasser and Cn3D were used to generate the calculated 3D structure and to compare it with experimentally generated models, respectively. Nucleotide and amino acid compositions along with the calculated molecular weight and isoelectric point (pI) were analyzed using BioEdit and online ExPASy software.

The phylogenetic trees revealed genetic relationship of olive among other plants based on OeSFB1. The ORF contained 1950 nucleotides coding 254 amino acids that produce a 28.2 kDa peptide with a pI of 5.94. Alanine, valine and leucine were found at high ratios suggesting a hydrophobicity which was also predicted by Kyte and Doolittle analysis. The AT rich property of OeSF3B1 is not unusual comparing to most plant genes. Cellular localization of the gene was suggested to be in mitochondria with no signal peptide indicating OeSF3B1 could be synthesizing in mitochondria. The predicted 3D structure of OeSF3B1 was similar to experimentally produced structures while some hydrophobic pockets were predicted. Further characterization of the gene with respect to temporal and spatial expression pattern and biochemical function continues.

P-02.08.5-082
Kafirin profile of Turkish originated sorghum populations

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Sorghum bicolor L. is the fifth important crop in the world with its high photosynthetic activity and resistance to unfavourable conditions as high temperature, drought, salt, and pH changes. Sorghum has attracted great interest due to its intensive usage both as human and animal nutrition, and contribution to resistance against many diseases. Some proteins of Sorghum exerts reducing effect on nutrient digestion through making connetions with other proteins and/or carbohydrates. Kafirin proteins have the highest proportion in grain with a range of 77–82%. They are grouped into α (23–25 kDa), β (18 kDa), γ (28 kDa) and d (13 kDa) subunits depending on molecular weight, solubility and structure.

In the current study, kafirin proteins from Turkey originated 282 sorghum populations were acquired through sequential extraction; first, non-prolamines were removed through application of 5% NaCI concentration, and second, kafirins were obtained using tertiary butanol (60%) and reducing agents. SDS-PAGE was conducted for seperating and visualising the subunits of kafirins.

The α, β, γ, and d subunits of populations were respectively estimated as 97, 85, 95, and 60%. Of the total proteins, 74% was identified as α, 12% β, 12% γ, 1% d, and %1 non-prolamines. Non-prolamin group of proteins were visualised as 18 different bands ranging from 15.6 to 230 kDa.

γ and β group of proteins were only viewed when treated with reducing agents as 2-ME and DTT suggesting that they are connected with complex cross-links. However, α group of proteins visualized without using these agents due to not having intra molecular disulphide bridges and inter molecular cross-links. Non prolamins, except for 47.8, 45.7, 21.7, 20.3 and 15.6 kDa, were able visualised in the presence of reducing agents. Transcriptomic analysis of the genes encoding analysed proteins needs to be elucidated for better understanding of the genetic diversity and biochemical characteristics of Sorghum.

P-02.08.5-083
Untargeted metabolomic profiling of Romanian and UK tomatoes varieties by high performance liquid chromatography coupled with mass spectrometry

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Tomato flesh is a rich source of many phytochemicals of high nutritional value, including a large variety of carotenoid derivatives with health promoting properties. Metabolomics became the most adequate technology for an accurate chemotaxonomic classification and discriminations between different varieties, based on untargeted profiling or targeted, quantitative analysis.

Different varieties of tomatoes (b-carotene-rich, lycopene–rich, ketocarotenoid-rich) cultivated in Romania and UK were comparatively studied using enriched fractions obtained by a preliminary fractionation of the whole pulp homogenate. Two methods were applied for carotenoid extraction: a mixture of hexane/ethanol (1) and chloroform/methanol (2). The dried extracts were dissolved in ethyl acetate and analyzed by UV-Vis spectrometry and HPLC-ESI(+)QTOF-MS (Bruker GmbH). The Base-peak chromatograms were processed by specific biostatistics software (Data Analysis and Profile Analysis) and the molecular identification were determined by comparison with the data base Lipidomics Gateway (www.lipidmaps.org).

The content of carotenoids were significantly higher using extraction (2), ranging from 4.5 to 7.6 mg/100 g. The major carotenoid derivatives, were represented by Lycopene, Hidroxy-lycopene, *all-trans* or *cis*-Beta-carotene, Echinenone, all-trans retinyl palmitate, but also sterols, phospholipids, di/tri glycerides and ceramides. The Romanian varieties were more rich in polar carotenoids and lipids, in general, while the UK tomatoes proved to be enriched in non-polar derivatives, especially esterified carotenoids, keto-carotenoids and glycerides. New molecules were identified, as good discriminatory markers of each tomato variety.

Acknowledgements. This work was supported by a grant of the Romanian National Authority for Scientific Research and Innovation, CCCDI – UEFISCDI, project nr. 16/2015, PNCDI3.

Wednesday 7 September 12:30–14:30

Cell cycle and circadian clocks

P-02.10.4-001
The implication of glycogen in cellular proliferation

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Glycogen is a multi-branched polysaccharide that serves as the main form of glucose storage in the body, where the main reserves are in the liver and muscle. It has been observed that glycogen metabolism is altered in many tumor types, and that glycogen content is inversely correlated with proliferation rate. In addition, it has recently been described that when glycogen accumulation is forced in glioblastoma U87 cells in hypoxia, senescence is induced and tumor growth is inhibited in vivo. Our laboratory has various animal models with different parts of the glycogen metabolism pathway affected. Most notably, we have two animal models lacking glycogen: muscle glycogen synthase (GYS1 KO) and liver glycogen synthase (GYS2 KO) knockout animals. We isolated mouse embryonic fibroblasts (MEFs) from GYS1 KO to perform replicative senescence assays. In addition, we induced hepatocellular carcinomas in GYS2 KO animals via N-nitrosodiethylamine (DEN) injections in order to track tumorigenesis in animals lacking hepatic glycogen. Lastly, we performed partial hepatectomies (PHX), which involves the resection of two thirds of the liver, on GYS2 KOs to evaluate the effect of the lack of glycogen on hepatocyte proliferation. Interestingly, we have observed that glycogen levels are increased in human and mouse fibroblasts under replicative senescence, and that MEFs depleted of glycogen bypass senescence and immortalize faster than WTs. We have also demonstrated that senescence pathways are down regulated in MEFs lacking glycogen. Furthermore, GYS2 KOs treated with DEN show higher tumor burden and mortality than controls. We also evaluated the effect of glycogen on hepatocyte proliferation after PHX. GYS2 KO mice present faster proliferation and liver regeneration rates, when compared to WT counterparts. Collectively, our preliminary data suggest that glycogen metabolism plays a crucial role in the regulation of cell cycle in both physiological and pathological states.

P-02.10.4-002
Pinealectomy alters cyclic guanosine monophosphate- circadian oscillation in rat leydig cells

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It is established that pineal is involved in circadian regulation of testosterone secretion from Leydig cells. However, the precise routes of this regulatory involvement are still unknown. As cGMP has been also regarded as modulator of steroidogenesis we sought to study the effects of pineal removal on the circadian pattern of cGMP variations and expression of the genes that encode elements of NO-cGMP signaling pathway in adult rat Leydig cells.

The analysis was performed on testicular Leydig cells obtained from pinealectomised and shame pinealectomized rats, in six time point during 24 hours. The pinealectomy was confirmed by serum melatonin EIA measurement. The androgen levels were measured by RIA; cGMP by EIA and gene expression was quantified by RQ-PCR. All results were analyzed by cosinor method.

Data revealed circadian transcriptional pattern of *Nos2*, *Nos3* (genes encoded NO producers) and *Pde5a* (gene for cGMP remover) in Leydig cells from adult rats. Pinealectomy significantly increased expression of *Nos2* which lost rhythm and increased and delayed amplitude of *Nos3* expression. Further, pinealectomy initiated cyclic transcription of *Gucy1b3* and non-cyclic transcription of *Gucy1a3* (genes encoded cGMP producers) and increased mesor and amplitude of *Pde5* transcription. The transcription of *Prkg1*, the main effector in this signaling pathway was not affected with pineal abolition. Additionally, pinealectomy did not influence the circadian transcription profile of *Coxi2* or other investigated genes (*Coxi1*, *Nrf1*, *Nrf2a*, *Pgc1a*) related to mitochondrial function and biogenesis. Finally pinealectomy reversed phase of circadian cGMP oscillation in Leydig cells, increased amplitude and slightly advanced peak of serum testosterone oscillation.

Results suggested pineal influence on circadian rhythm of NO-cGMP signaling in Leydig cells. Further studies based on these data are needed to better understand the relationship between pineal and circadian rhythm of testosterone production.

P-02.10.4-003
In vitro genotoxic perspective of Tamiflu

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İntroduction: İnfluenza is a contagious respiratory infection caused by a variety of influenza viruses. Neuraminidase inhibitors is a new class of antiviral drugs that inhibit influenza viruses. The most popular antiviral agents is Oseltamivir, having a commercial name of Tamiflu, within anti-influenza antivirals. As well as Tamiflu is a member of neuraminidase inhibitor group drug. Therefore, this study was performed to determine the effect of Tamiflu on cultured human peripheral blood lymphocytes.
Material and methods: For examining the presence of the indirect mutagenic effect of oseltamivir in iver S9 fraction mix was used. Cells were treated with 0.5, 1 and 2 μg/ml oseltamivir, the Tamiflu capsule ingradient, for 24 or 48 hours in the absence or presence of an exogenous metabolic activation system. The test chemical did not demonstrate any genotoxic effect dose-dependently but it showed a weak cytotoxicity on cells in this study. On the other hand, some concentrations of Tamiflu induced SCE and also decreased significantly the proliferation index ($p < 0.05$) in the absence of S9 mix.
Result: Tamiflu did not induce significant increases of CA or micronucleated cells in vitro in cultured peripheral blood lymphocytes under the treatment conditions used but week SCE induction was observed. On the other hand, the weak cytotoxic effects observed disappeared in the cultures treated in presence of the S9 mix.
Discuss and conclusion: Tamiflu weakly induced SCE at the highest concentration with/without added S9 mix in cultured human peripheral lympocytes. It could be assumed to be a SCE inducer. SCEs can be increased by several agents that attack DNA. Tamiflu decreased the proliferation index and nuclear division index at some concentrations thus interferring it as being weakly cytotoxic, though this effect disappeared in the presence of S9 mix applications. This finding is important for showing the inefficiency of Tamiflu metabolites on the cell cycle.

Wednesday 7 September 12:30–14:30

Single molecule techniques – Applications in biology

P-03.04.4-001
New multibiosensor device for diagnosis of diabetic nephropathy

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Introduction: Chronic renal failure as a result of the progression of diabetic nephropathy is the main cause of mortality in patients with type 1 diabetes. Chronic hemodialysis is a life-saving therapy for patients with strong renal disorders. The main goal of hemodialysis is toxins removal from the patient. The monitoring of hemodialysis is the best way for biomedical evaluation of correctness and efficiency of this clinical treatment.

According to the published data, the markers of development of diabetes complicated with renal failure are increased levels of glucose, urea and creatinine in the patient blood.

Today colorimetric and spectrometric methods are most commonly used for determination of the above metabolites in biological samples. However, these methods are complex in application, have low selectivity, and require pretreatment of samples.
Materials and Methods: We propose for levels of glucose, urea and creatinine detection the potentiometric multibiosensor based on pH-sensitive field-effect transistors and immobilized enzymes developed in our laboratory.
Results: We developed a potentiometric multibiosensor and studied its main analytical characteristics. Linear dynamic ranges of determination of substrates were following: 0.08 – 1 mM of glucose, 0.1–10 mM of urea, and 0.02 – 2 mM of creatinine. It was shown that the potentiometric multibiosensor had good reproducibility, and its bioselective elements were working independently from each other, because test of substrates cross-selectivity was negative.
Discussion and conclusion: Very sensitive, fast and selective multibiosensor for simultaneous measurement of three metabolites in a single cycle based on pH-sensitive field-effect transistors and immobilized enzymes is developed. The developed potentiometric multibiosensor was verified by quantitative analysis of glucose, urea and creatinine in blood serum of patients with diabetic nephropathy.

P-03.04.4-002
pH-dependent interaction of asymmetrically charged peptides with a protein nanopore

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Over the past two decades, the ability to use natural or artificial nanopores to probe at uni-molecular level the structural and kinetic features of various bio-molecules (peptides, DNA, RNA) was successfully achieved. The operating principles of the nanopore-based single-molecule technique are simple: the single macromolecule capture, entry and subsequent translocations through a free-standing, voltage-biased nanopore, depend upon the physico-chemical and topological features of the analyte. The concentration, identity volume and charge of the analyte are then deduced from the analysis of the stochastic current blockade events caused by the trafficked analyte across the nanopore. Herein, we used the α-hemolysin (α-HL) nanopore and set up an experimental model providing efficient control of α-HL-peptide interactions, in the presence of a pH gradient across the nanopore. For this, we engineered a 36 amino acids long peptide containing a neutral asparagines-containing sequence, flanked by oppositely charged aminoacid patches at the N- (glutamic acids) and C-termini (arginines), whose length was set as to span a single α-HL protein. When the pH of the solution in contact to the α-HL's β-barrel opening is changed from neutral to acidic values, the electrostatic interactions between the protein's mouth and either the N- or C-terminus end of the peptide occurs, and this influences strongly the dynamics of a peptide translocating the nanopore. We further proved that during the same experiment, peptide entry into the nanopore can be set to occur with either N- or C-terminus end head on, by simply changing the sign of the transmembrane potential across the nanopore.

P-03.04.4-003
A stop-motion picture of a trapped peptide inside a protein nanopore: electroosmotic flow versus electrophoretic force

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Nanopores are emerging as a powerful and broadly applicable tool in biophysics, which allows one to study the features of charged macromolecules under confinement. A few noteworthy examples are: determining the electrophoretic mobility, effective charge and diffusion coefficients of charged molecules; exploring the folding and unfolding of peptides and proteins; analyzing biopolymers trafficking, protein transport, DNA translocation, RNA and DNA sensing and sequencing.

Herein, we employ single molecule analysis techniques using a wild-type α – hemolysin (α-HL) protein nanopore to study the capture and translocation behavior of a short cationic peptide (20 amino acids in length) at an extremely low pH value.

Our experiments revealed that an effective absorbing field is created by the electroosmotic flow, against the electrophoretic force, which enables the peptide capture inside the nanopore. Furthermore, our findings show that the trajectory of a single peptide can be experimentally visualized and the main steps determined: the peptide capture, reversible translocation across the pore's vestibule and lumen regions, and the peptide release from the nanopore. Also, the kinetic analysis of the main steps observed allowed us to describe the free energy profile of the peptide interactions with the protein nanopore.

The presented work provides evidence for the ability of controlling the dynamics of a single-peptide, its capture and passage inside a α-HL nanopore, that underlie the processes naturally occurring in cells, thus proving a powerful approach for probing single molecule biophysics phenomena, in general.

P-03.04.4-004
Microfluidic cell culture alters the behavior of cancer cell by mimicking dynamic tumor microenvironment

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Changes in the physical conditions of the cancer microenvironment driven by elevated tissue growth and angiogenesis, may introduce exposure of laminar fluid flow, which effect the key factors of cancer, such as progression, immune-escaping and metastasis. Conventional experimental models fail to mimic the physical cues on tumor microenvironment. Microfluidic culture techniques allow precise control of fluids, simultaneous manipulation and analysis of cultured cancer cells. Here, we present a platform that can be used for the investigation of the role of flow mediated mechanical stimuli on cancer cells.

Microfluidic cell culture platform was fabricated using polymethyl methacrylate and double-sided adhesive films with 25 × 41 mm dimensions. Ovary adenocarcinoma cells (EFO-27 and ONCO-DG-1) were used for the optimization of the platform. To understand the fluid and gas distribution patterns, specific modeling was performed. Dynamic microfluidic cell culture and static conventional cell culture conditions were compared for the differences of cancer cell phenotype, such as proliferation, viability, epithelial-mesenchymal transition.

We confirmed that, the proliferation and viability of cancer cells are increasing under dynamic fluid flow. The proliferation rate of ovary adenocarcinoma cells was correlated with the increase of fluid flow rate. Immunocytochemical analysis showed that fluid flow causes decrease in E-cadherin expression, and increase in N-cadherin and vimentin expressions, which indicate mesenchymal phenotype of cancer cells.

Our results showed that, cancer cells present different characteristics due to fluid flow of tumor microenvironment. To understand the role of physical dynamics by using microfluidic culture techniques, is a key to elucidate the mechanisms underlying disease progression, and may lead to new diagnostics and therapeutic approaches. (This study was funded by Turkish Scientific and Technical Research Council (*TUBITAK*-214S334).

P-03.04.4-005
High-sensitive detection of low-affinity antibodies by immuno-PCR with supramolecular olygonucleotide-streptavidin complex

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Detection of low affinity antibodies in blood sera and cell surface outwashes is important both in the study of molecules that bind to cellular receptors (circulating tumor cell masking antibody, for example) and medicine (diagnosis of allergy). Low affinity IgM and IgE antibodies can not sometimes be determined by conventional methods.

We using supramolecular oligonucleotide-streptavidin complex formed from single-stranded synthetic oligonucleotide (60 n) contains biotin on 5'- and 3'-ends, and sterptavidin in molar ratio 1:1. This complex represents a structure with equivalent electrophoretic mobility of 600 bp DNA and preferred "valency" of streptavidin is 2. This universal immuno-PCR approach make it possible to increase a signal by using several oligonucleotides per one antibody.

After the method optimization we achieved 100–1000 times highter sensitivity than ELISA. To reduce the matrix effect we used 100–500 fold dilutions of sera samples. This approach achieved a significant advantage, because it allows working with small-volume samples (need only 2 mkl of serum sample). Antibodies to the disaccharide Galβ1-3GlcNAc (Le^C) are typical of the natural antibodies. The IgM anti-Le^C antibodies are found in almost healthy people without the epitope specificity variation. We have shown that the concentration of IgM anti-Le^C antibodies was higher ($p \leq 0.0005$) for health donor sera ($n = 45$; 31.3 ± 4.3 pg/ml) compared with sera from patients with breast

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cancer (n = 38; 17.0 ± 4.1 pg/ml). Sensitivity of IgM anti-Le^C antibodies detection was 100 pg/sample (50 mkl) ie 6.7×10^7 molecules. Thus for the immuno-PCR detection of antibodies the 10^3–10^4 tumor cells are sufficient. Such amount of cells seems to be a realistic one for detection of antibodies masking circulating tumor cells.

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P-03.04.4-006
Nanopore-based detection of selected Gram-negative bacterial cells

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Bacterial pathogen detection and identification is of crucial importance for disease diagnosis, bacterial contamination surveys and water quality assessment.

We propose herein a novel method for bacterial detection based on the interaction of single Gram-negative bacterial cells (i.e.: *Escherichia coli* and *Pseudomonas aeruginosa*) with an α-hemolysin (α-HL) protein nanopore embedded in a reconstituted lipid bilayer, at neutral pH. As a consequence of an applied voltage, the negatively charged bacteria suspended in saline buffer solution are electrophoretically driven towards the pore opening, inducing reversible blockages in the ionic current through α-HL. Experiments were also performed in the presence of an antimicrobial peptide, CMA3, as well as in acidic environment.

Statistical analysis of the frequency and duration of blockage events allowed us to discriminate between the two types of bacteria. The frequency of interactions was higher for *Escherichia coli* with respect to *Pseudomonas aeruginosa*. Adsorption of CMA3 peptides on the membrane of bacteria increased the frequency of interactions with the pore, contrary to the expected effect induced by lowering the net surface charge of the cells. In experiments performed at pH = 4, the frequency of blockage events was found to be two orders of magnitude higher, with longer interaction life-times.

The net negative charge (7 uncompensated aspartate residues) localized at the entrance of the pore contributes an additional electrostatic repulsion interaction between negatively charged bacterial cells and α-HL. Thus, adsorption of cationic peptides at the interface will reduce this repulsive interaction. The same effect was recorded at pH = 4, when the aspartate residues are partially protonated, confirming our understanding of the previously observed results.

This method could be further developed and integrated with other techniques, making nanopore-based systems a fast and reliable bacterial detection and identification tool.

P-03.04.4-007
Comparative cytotoxic and cytoprotective effects of tauroursodeoxycholic acid on tunicamycin treated human liver epithelial THLE-3 cells

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This study was performed to analyze the effects of tunicamycin (TM) and taurohyodeoxycholic acid (TUDCA) on THLE-3 cells. Cells were treated with TM to induce endoplasmic reticulum (ER) stress and TUDCA was administered as an ER stress inhibitor. Cytotoxicity was evaluated at different times of exposure by incubating cells with increasing concentrations of either TUDCA, TM or both.

THLE3 cells were cultured in fibronectin, bovine collagen I and bovine serum albumin coated plates. Cell lines were grown in BEGM media supplemented with epidermal growth factor, phosphoethanolamine, fetal bovine serum, 100 U of penicillin-streptomycin and maintained in a humidified incubator at 37 °C and a 5% CO_2 atmosphere. Cell viability was measured using the colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay kit. Cells were grown to confluence in 96-well plates and incubated with 1 μl/ml DMSO, 1–10 μg/ml TM, 0.1–5 mM TUDCA, or 10 μg/ml TM + 0.1–1 mM TUDCA for 18–48 hours. Control cells were prepared in plates containing only medium. At the end of the incubation period, MTT was added to each well and incubation was carried out for 4 hours at 37 °C. Formazan production was expressed as a percentage of the values obtained from control cells.

At all hours of incubation neither DMSO or 1 mM TUDCA was cytotoxic. At 24 and 48 hours incubations 5 mM TUDCA and 10 μg/ml TM + 1 mM TUDCA were significantly cytotoxic compared to control, DMSO and 1 mM TUDCA groups. Treatment of cells with 0.5 mM TUDCA 8 hours before administrating 10 ug/ml TM significantly decreased the cytotoxic effect of TM. We conclude that TUDCA may show cytotoxic effects at 1 mM concentration when treated with TM. Therefore 0.5 mM of TUDCA, administered 8 hours before TM treatment should be applied to protect against ER stress. Acknowledgement: This study was supported by a grant from The Scientific and Technological Research Council of Turkey (TUBITAK; 214S223).

P-03.04.4-009
Lipoprotein-associated phospholipase A2 single nucleotide polymorphisms and future cardiovascular risk in patients with preeclampsia

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Preeclampsia (PE) is considered as an inflammatory disease. Recent studies reveals that history of preeclampsia is an independent risk factor for cardiac events and stroke. Lipoprotein-associated phospholipase A2 (Lp-PLA2) is a vascular inflammatory marker associated with cardiovascular diseases (CVD).

We hypothesize that vascular inflammation (Lp-PLA2 mass, activity, index) related genetic variations (PLA2G7) increase the

risk for developing future cardiovascular disease in women with PE.

A group of 150 preeclamptic patients and 50 normal pregnant women were recruited from University of Istanbul, Cerrahpasa Medical School, Department of Gynecology and Obstetrics included into the study. The control group was matched for maternal and gestational age at time point of sampling. Preeclamptic patients were starified into two groups; early-onset and late-onset according to the 32 gestational weeks.

Enzyme-linked immunosorbent assay procedure was used to determine the serum Lp-PLA2 mass level. Lp-PLA2 activity were determined by kinetic method. PLAG7 SNP genotyping performed by using the Sequenom *MassARRAY iPLEX*.

The rs1805017 TT genotype had a higher Lp-PLA2 index ($p = 0.015$) for early onset preeclampsia, CC genotype had a higher Lp-PLA2 mass and Lp-PLA2 index for late onset preeclampsia. No difference were found for control. The rs1809381475 GG genotype had higher Lp-PLA2 mass and index for late onset preeclampsia ($p = 0.008$, $p = 0.012$ respectively). Stepwise logistic regression analysis performed to identify cardiovascular disease related variables that independently and significantly contributed to the presence of alleles of rs1805017 and rs1809381475 snps in early, late onset preeclampsia and control group. Only Lp-PLA2 mass was independently and significantly associated with both snps in early onset preeclampsia.

The association between Lp-PLA2 mass, index and rs1809381475, rs1805017 snps might be useful genetic markers to adress future CVD risk in patients with preeclampsia.

P-03.04.4-010
MPV values in patients with ovarian carcinoma

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Aim: In this study, our aim was to compare the level of Mean Platelet Volume (MPV) levels in ovarian carcinoma patients and healthy control groups.
Materials and methods: Serum samples were collected from 67 healthy control and 20 patients with Ovarian Carcinoma. The mean age for controls and patients were 51.71 ± 14.02 and 59.00 ± 9.31 respectively. MPV levels measured with Abbott Cell-Dyn 3700 Hematology Analyzer. Statistical analysis was performed with SPSS v21 by using independent sample t test.
Results: MPV values in patient with Ovarian Carcinoma [7.31 ± 1.25] were higher compared to control group [7.02 ± 0.81] but it was not statistically significant ($p = 0.69$)
Conclusions: Although MPV is a inflammation marker, our analyses showed that MPV cannot be used for a inflammatuary marker in patients with Ovarian Carcinoma.

P-03.04.4-011
Detection of β-thalassemia IVSI-110 mutation by using piezoelectric biosensor immobilized with a single oligonucleotide

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Introduction: β-Thalassemia is one of the most monogenic autosomal recessive disorder characterized by defective production of the β-chain of hemoglobin. Definition of the β-globin genotype is necessary for genetic counselling in the carriers, and for predicting prognosis and management options in the patients with thalassemia. DNA-based diagnosis of β-thalassemias routinely relies on polymerase chain reaction (PCR) and gel electrophoresis. The aim of this study is to develop a new procedure, a DNA-based piezoelectric biosensor, for the detection of β-thalassemia IVSI-110 mutation, the most common β-thalassemia mutation in Turkey.
Materials and methods: β-globin gene of genomic DNA isolated from whole blood, was amplified by PCR. Bioactive layer was constituted by binding 2-Hidroxymetacrilate Metacriloamidoscystein (HEMA-MAC) nanoploymers on the gold electrode's surface. Single oligonucleotide probes specific for IVSI-110 mutation of β-thalassemia were attached to the nanopolymer via reactive cross-linker glutaraldehyde. The measurements were executed by piezoelectric resonance frequency which is caused by binding of PCR products in media with single oligonucleotide probe on the electrode surface. The results were confirmed by the conventional molecular method as ARMS.
Results: The piezoelectric resonance frequencies obtained by hybridization of the PCR products on bioactive layer were found 216 ± 12, 273 ± 6, and 321 ± 9 Hz for the samples of normal β-globin, heterozygote, and homozygote of IVSI-110 mutation, respectively.
Discuss and conclusion: The developed biosensor serves as a specific result to IVSI-110 mutation. It could accurately discriminate between normal and IVSI-110 mutation samples. Because of low costs, fast results, specificity and high detection/information effectiveness as compared with conventional methods, we can be offered this techique as an alternative to conventional molecular methods.

Wednesday 7 September 12:30–14:30

Molecular mechanisms of inflammation

P-04.04.4-001
Inductions of the inflammatory reactions in human lymphocytes under the influence of titanium dioxide nanoparticles

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The increasing use of nano-sized materials in the last several years has compelled the scientific community to investigate the potential hazards of these unique and useful materials. One of the most widely used nanoparticles is titanium dioxide.

The objective of the research is to investigate the alterations in molecular and cellular responses in culture of primary lymphocytes to TiO2 NPs.

Human lymphocytes isolated from heparinized blood of healthy individuals were exposed to TiO2 nanoparticles. Viability, ROS generation, the changes in the expression of genes encoding proinflammatory mediators TNF-α, Il-1β and Il-8 and DNA damage were assessed.

Human lymphocytes were incubated with nanoparticles of different concentrations and viability was determined in 24 and 48 hours after treatment, respectively. Cell viability was decreased by a treatment with nanoparticles in both a time- and concentration-dependent manners.

The ability of TiO2 to induce ROS formation in lymphocytes was evaluated using DCF fluorescence as a reporter of oxidant production. The fluorescence intensity of oxidized DCF was

increased in cells treated with NPs. This means that ROS generation occurred in response to the treatment with TiO2.

To investigate the expression level of mRNA related to the inflammation responses in human lymphocytes real-time PCR was performed. The expression of Il-1β, Il-8 and TNF-α genes were increased by the exposure to nanoparticles of 10, 20 and 40 μg/ml for 24–48 hours.

TiO2 nanoparticles were shown to induce the dose-dependent fragmentation of DNA strands.

Much evidence of hazardous health effects of Nps has been reported. In this study, viability was reduced under the exposure to TiO2. Oxidative stress was elevated by the treatment with TiO2 NPs. Oxidative stress can also trigger inflammation signals. Induced by exposure to nanoparticles they may cause the translocation to the nucleus of transcription factors, which regulate pro-inflammatory genes, such as TNF-α, Il-1β, Il-8.

P-04.04.4-002
Expression of inflammation and neoangiogenesis markers in two- and three-dimensional cultures of the Ea.hy926 endothelium-derived cell line

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Background: Endothelial cells (EC) represent one of the primary targets of the major pro-inflammatory cytokine – tumor necrosis factor (TNF). Development of the new approaches for the treatment of acute and chronic inflammatory conditions, including the strategies aimed to TNF neutralization, requires the usage of the adequate cellular models closely resembling the properties of the endothelium. The endothelium-derived Ea.hy926 cell line expresses several inflammation and neoangiogenesis markers in response to activation factors however their expression can differ from the patterns demonstrated by primary EC. The aim of the current study was to compare the expression of the known endothelial cellular markers including receptor of vascular endothelial growth factor-2 (VEGFR2) and $\alpha_v\beta_3$-integrin on 2D and 3D cultures (spheroids) of Ea.hy926.
Methods: The Ea.hy926 cell line was used with permission from Dr. Edgell. The cells were cultivated in the presence of TNF (25 ng/ml) or VEGF A (25 ng/ml) for 5 hours. mRNA was isolated using RNeasy kit from Qiagen and reverse-transcribed with RevertAid kit (Fermentas). RT-PCR was performed with specific primers. Expression of VEGFR2 and $\alpha_v\beta_3$-integrin was visualized by confocal microscopy using specific monoclonal antibodies and previously developed fluorescent hybrid proteins.
Results: The expression of $\alpha_v\beta_3$-integrin and VEGFR-2 increased on the 3D culture compared to 2D according to confocal microscopy and RT-PCR. The aforecited methods revealed elevated expression of $\alpha_v\beta_3$-integrin in the 3D culture of the Ea.hy926 cell line activated with TNF. Also increased expression of VEGFR2 in the 3D culture activated with VEGF A. Then by confocal microscopy, we analyzed our fluorescent hybrid proteins that bind $\alpha_v\beta_3$-integrin and VEGFR2 on the surface of 2D and 3D cultures as well as antibodies with fluorescent label.
Conclusions: 3D cultures of the Ea.hy926 cell line represent a promising model for the inflammation studies.

P-04.04.4-003
Discovery of TNF small molecule inhibitors as potential drugs for rheumatoid arthritis therapy

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Tumor Necrosis Factor (TNF) is a trimeric cytokine associated with the inflammatory response to tissue injury and found to possess a key role in Rheumatoid Arthritis pathogenesis. SPD 304 is a highly toxic recently discovered TNF inhibitor that promotes trimer dissociation and lead to the inactivation of the protein.

According to the traditional anti-TNF treatment of RA, we aim at extracellular inhibition of this pro inflammatory cytokine as an effective therapy. The project plan comprises design, synthesis and validation of candidate inhibitors (measurement of dissociation constant and aqueous solubility). Because of the elevated percentage of insoluble compounds a solubility enhancement protocol has been developed.
The experimental procedure was the following:: A. Drug design. Identification of novel drug compounds are based on two approaches: i) structure based drug design using the 3D structure of TNF and ii) design of more potent and less toxic SPD304 analogues.

B. Drug synthesis. A series of SPD304 analogues were in house synthesized while novel candidates discovered by in silico approaches were commercially available. The purity of the majority of the compounds exceeded 90%.

C. Solubility measurement and enhancement. Samples were incubated under specific conditions that can enhance aqueous solubility and solubility measurement with a direct UV method pursued.

D. Measurement of the dissociation constant. A fluorescence binding assay was used in order to evaluate the inhibitory activity of the compounds.

From our results it can be concluded that DMSO, PEG3350 and β-cyclodextrin can be used for solubility enhancement without interfering with fluorescence assay. However PEG3350 –in contrast to DMSO- is not suitable for Isothermic Titration Calorimetry measurements. Dissolution procedure also plays a crucial role in the levels of solubility reached. Finally, it has been shown that some of the studied SPD304 derivatives have better dissociation constants than SPD304.

P-04.04.4-005
The effect of exercises on serum Bmp-6 levels of knee osteoarthritis

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The treatment approaches for osteoarthritis (OA) aims to suppress the inflammation by antagonizing proinflammatory

cytokines. More complicated approaches are expected to focus on molecular proteins as bone morphogenetic proteins (BMPs) of the transforming growth factor (TGF)-beta superfamily. BMPs associated with many cellular functions, such as proliferation, differentiation, and apoptosis. BMP-6 is significantly important for the endochondral bone formation. Inflamation can induced serum BMP levels in OA patients. The aim of this study is to evaluate the clinical findings of OA patients after the isokinetic exercise together with the serum levels of BMP-6 to sustain the molecular approaches for treatments. A total of 36 patients were included in this study. The groups are formed as follows: Group 1, OA patients before the exercise; Group 2, OA patients after the exercise; Group 3, OA patients before the isokinetic exercise; Group 4, OA patients after the isokinetic exercise Clinical and biochemical findings were evaluated before and after 3 weeks of the exercise programme. Self reported severity of pain was measured using the 100 mm visual analog scale (VAS), WOMAC scores were calculated and isokinetic knee muscle strength testing was measured using Cybex dynamometer that a standardized protocol previously described was applied in a subject-specific range of motion. Serum BMP-6 levels of all patients were studied by ELISA method. Results represented a better VAS and WOMAC scores for all exercise groups after treatment. The serum BMP-6 levels were significantly decreased in Group 2 compared to Group 1 (222.94 ± 44.66; 246.49 ± 38.15 respectively, $p < 0.01$) and in Group 4 compared to Group 3 (246.74 ± 32.26; 256.23 ± 33.11 respectively, $p < 0.05$). There is not any statistically differences between Group 2 and Group 4 ($p > 0.05$). As a conclusion, the decreased serum levels of BMP-6 may be suggested as a biochemical marker for OA patients during exercise programmes.

P-04.04.4-006
TNF-α blokade efficiently reduced severe intestinal damage in necrotizing enterocolitis

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Objectives: To ascertain the beneficial effects of infliximab an inhibitor of tumor necrosis factor alpha (TNF-α) on the development of NEC in an experimental NEC rat model.
Material and methods: Thirty newborn Sprague-Dawley rats were randomly divided into three groups as NEC, NEC+ infliximab, and control. NEC was induced by enteral formula feeding, exposure to hypoxia-hyperoxia and cold stress. Pups in the NEC+ infliximab group were administered infliximab at a dose of 10 mg/kg daily by intraperitoneal route from the first day until the end of the study. All pups were sacrificed on the 5th day. Proximal colon and ileum were excised for histopathologic, immunohistochemical (TUNEL and caspase-3), and biochemical evaluation, including, total antioxidant status (TAS), total oxidant status (TOS), malonaldehyde (MDA), and myeloperoxdase (MPO) and TNF-α activities.
Results: We observed better clinical sickness scores, weight gain, and survival rate in the NEC+ infliximab group compared to the NEC group ($p < .05$). Histopathological and apoptosis examination (TUNEL and immunohistochemical evaluation for caspase-3) revealed lower damage in the NEC+ infliximab group compared to the damage in the NEC group ($p < .01$). Tissue MDA, MPO, TNF-α levels, and TOS were significantly decreased in the NEC+infliximab group, whereas TAS was significantly increased in the NEC + infliximab group ($p < .01$).
Conclusion: TNF-α blockade with infliximab efficiently reduced the intestinal injury and preserve the intestinal tissues from severe intestinal damage by its complex mechanisms on NEC. Therefore, it may be an alternative option for the treatment of NEC.Keywords: TNF-α; infliximab; necrotizing enterocolitis; newborn; protection; rat; treatment

P-04.04.4-007
Short-term diabetes causes cardiovascular inflammation: anti-inflammatory effect of resveratrol

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Introduction: Diabetes is a metabolic dysfunction and has been associated with various disorders including inflammation, cardiomyopathy and coronary artery disease. Inflammation is a protective mechanism elicited by the host in response to infection, injury, and tissue damage. The aim of this study was to investigate the effect of intraperitoneally resveratrol administration on cardiac and vascular function in diabetic rats.
Materials and methods: Diabetes was induced in Sprague-Dawley rats by using injection of streptozotocin (55 mg/kg, i.p.). Rats were divided into group I: control, II: control/20 mg/kg resveratrol; III: diabetic/vehicle; and IV: diabetic/20 mg/kg resveratrol. Histopathological examinations with Masson's trichrome and Verhoeff-van Gieson staining were carried out to reveal cardiac and vascular tissue damage and inflammation. In addition to plasma glucose and cardiac & vascular MDA levels were measured by standard enzymatic kits while TNF-α, IL-1β, IL-18 (MBL) were analyzed by ELISA kit.
Results: Final body weight decreased in all groups compared to control. In the diabetic rats, plasma glucose and vascular MDA levels were enhanced while cardiac MDA was unchanged compared to control. Vascular TNF-α, IL-1β and MBL and cardiac MBL were increased in the diabetic groups compared to control.
Discussion and conclusion: It has been found that resveratrol has greatly normalized altered parameters. Taken together, resveratrol partly improved cardiac and vascular inflammation induced by diabetes. This may be due to the healing activity of resveratrol on pro-inflammatory markers.

P-04.04.4-008
Cytokine network is critical in growth hormone-induced resistance mechanism against curcumin which modulates JAK/STAT/SOCS pathway in MDA-MB-231 and MCF-7 breast cancer cells

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Curcumin (diferuloylmethane), a polyphenolic compound that triggers apoptotic cell death in various cancer cells such as prostate, colon, melanoma and breast cancer. A pituitary-derived hormone, Growth Hormone (GH) play role in elongation and differentiation of ductal epithelia into the breast terminal and buds. In this study, our aim is to determine the role of

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inflammation in curcumin induced apoptotic cell death *via* acting on JAK/STAT/SOCS pathway in wt and GH+ MDA-MB-231 and MCF-7 breast cancer cell lines. According to MTT cell viability assay curcumin triggers cell viability loss in time and dose dependent manner in MDA-MB-231 wt and MDA-MB-231 GH+ breast cancer cell lines, respectively. Selected concentrations of curcumin as 20 μM (for MCF-7) and 25 μM (for MDA-MB-231) decreased cell proliferation and induced apoptosis through causing JAK2 dephoshorylation, STAT1, 3, 5 dimerization and acting on SOCS proteins expression in each cell lines. In addition, activated JAK/STAT/SOCS pathway, via forced GH expression has been suppressed following curcumin treatment for 24 hours. 20 μM curcumin-induced apoptotic cell death via dephosphorylating JAK2 at Tyr1007/1008 residues and decreased phospho-STAT1, 3 level in both breast cancer cell lines. Although curcumin dephosphorylated STAT5 in both MDA-MB-231 and MCF-7 wt cells, no significant effect has been observed in MDA-MB-213 GH+ and MCF-7 GH+ cell lines. In consequence, although forced GH expression induced cell proliferation in MCF-7 and MDA-MB-231 breast cancer cells, curcumin overcame GH-mediated resistance mechanism via acting on JAK/STAT/SOCS signaling, which is related to PPARg-induced inflammation.

Acknowledgment: This study was supported by TUBITAK 1001 research project (Project No: 113Z791).

P-04.04.4-009
Growth hormone mediated resistance against curcumin induced-apoptosis via modulating of JAK/STAT/SOCS pathway and inflammatory cytokines in MDA-MB-453 breast cancer cells

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Breast cancer is one of the highest cancer type among women worldwide. Various enviromental and genetic factors such as age, gender, family history, metabolic diseases and gene mutations are involved in the breast cancer pathogenesis. Growth hormone (GH), a pituitary derived hormone, has essential role on postnatal growth and development. It is also established that signalling route of GH and its receptor (GHR) activity is increased in different cancer types. Curcumin, a nutraceutical deriatives from rhizomes of turmeric (*Curcuma longa*), has potential therapeutic activity against cancer cells, including breast cancer. Curcumin inhibits proliferation of cancer cells such as prostate, colon, melanoma, cervical and breast cancer via induction of apoptosis and inflammation. STAT5, a major downstream target of GH/GHR signalling, is related to survival, proliferation and differentiation. In this study, our aim was to investigate curcumin-induced apoptotic cell death in GH overexpressed MDA-MB-453 breast cancer cells via JAK-STAT/SOCS signalling and inflammatory response profile. According to MTT cell viability assay, curcumin decreased cell viability in time and dose dependent manner in wt and GH+ MDA-MB-453 breast cancer cell lines. We found that 20 μM curcumin-decreased in apoptotic cell death through inactivity at JAK2 which led to dimerization of STAT1, STAT3, STAT5. Concomitantly, curcumin affected STAT regulating SOCS proteins in MDA-MB-453 breast cancer cell line. In addition, we demonstrated that 20 μM curcumin induced PPARg expression and altered inflammatory cytokine signalling cascade. Consequently, although GH overexpression led to agressive profile in MDA-MB-453 breast cancer cells, curcumin overcame this resistance.

Acknowledgment: This study was supported by TUBITAK 1001 research project (Project No: 113Z791).

P-04.04.4-010
Molecular mechanisms involved in inflammation modulated by vegetal active principles

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Inflammation is involved in many systemic disturbances, including osteoarthicular or skin diseases, coordinating the signaling network that contributes to tissue injuries. The aim of our study is to reveal pro-inflammatory messengers at the cutaneous barrier (keratinocytes, fibroblasts, endothelial cells), simulating the dermal impact of active principles, especially polyphenols and flavones from vegetal sources: *Salvia officinalis, Asculum hippocastanum and Calendula officinalis*. We focused on IL6 and IL8 cytokines as main mediators of inflammation progression, correlated in keratinocytes with IL1α as skin irritation indicator and VEGF as pro-angiogenic factor, as well as in endothelial cells with ICAM-1 and VCAM-1 adhesion molecules expression. In order to in vitro mimic the inflammatory conditions, we used targeted stimuli for each type of cells: *for fibroblasts and endothelial cells* – TNFα, a systemic stimulus, single or combined with PMA that activates protein kinase C and up regulates NADPH oxidase, which lead to superoxide anion production; *for keratinocytes* – controlled UV-A and UV-B radiation, simulating the solar damages or potential UV interactions with active principles in light exposed skin. The main analysis technique was flow cytometry: beads bases assay for soluble factors and fluorescent antibodies staining. Our results prove the different involvement of polyphenols and flavones in the anti-inflammatory mechanisms, depending of the vegetal source: active principles from *Salvia officinalis* induce a strong inhibition of IL6 and IL8 in TNFα stimulated keratinocytes, fibroblasts and endothelial cells, reduce the ICAM-1 over-expression but have no effects on irradiated keratinocytes; biocomplexes from *Asculum hippocastanum* inhibit only IL8 release in stimulated fibroblasts, but protect keratinocytes from UV-A and UV- B radiation; compounds from *Calendula officinalis* are active on IL8 signaling in fibroblasts and counteracts only UV-B inflammation.

P-04.04.4-011
Protective effects of *Salvia* L. extracts in a rat model of renal ischemia/reperfusion injury

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Ischemia and/or reperfusion injury is one of the most common causes of acute renal failure. Ischemia–reperfusion associated with thrombolytic therapy, organ transplantation, coronary angioplasty, aortic cross-clamping, or cardiopulmonary bypass results in local and systemic inflammation. Within the endothelium, ischemia produces expression of proinflammatory gene products (e.g. cytokines) and bioactive agents (e.g., endothelin), while preventing other "protective" gene products (e.g., thrombomodulin) and bioactive agents (e.g. nitric oxide). Therefore, ischemia induces a proinflammatory state that increases tissue vulnerability

to further injury on reperfusion. This experimental study was designed to investigate the protective effect of *Salvia L.* extracts on kidneys from I/R injury. *Salvia Lamiaceae* have been used for treatment of some illnesses in Turkish folk medicine.

Forty *Spraque Dawley* rats were divided into 5 groups (n = 8). Right nephrectomy was performed to all groups. Group I: control group; Group II: I/R group; Group III: I/R + 50 mg/kg *Salvia L.*group; Group IV: I/R + 100 mg/kg *Salvia L.* group; Group V: I/R + 50 mg/kg *Rosmarinic acid.* group. *Salvia L.* and *rosmarinic acid* for 7 days was given single dose as a gavage. 60 minutes ischemia, 60 minutes reperfusion were applied to groups except control. Intracardiac blood samples were taken, high sensitive CRP (hsCRP), Tumor Necrosis Factor-α (TNF-α), Interleukin (IL)-6 and Interleukin Iβ (IL-1β) levels were detected. Serum hsCRP levels were also determined in our clinical laboratory using routine standard methods. Serum TNF-α, IL-6 and IL-Iβ levels were evaluated using an enzyme-linked immunosorbent assay technique. Mean values were evaluated by statistical analysis.

Serum hsCRP, TNF-α, IL-6, and IL-1β concentrations were significantly increased after renal I/R as compared to the control group. Our treatment group 50 mg/kg *Salvia L.* and 50 mg/kg *rosmarinic acid* especially 100 mg/kg of *Salvia L.* were found to show a protective effect against renal structure and function.

We concluded that *Salvia L.* extracts could be beneficial in the treatment of renal ischemic injury. But 100 mg/kg *Salvia L.* extract were more effective than 50 mg/kg *Salvia L.* extract and used as synthetic 50 mg/kg rosmarinic acid.

P-04.04.4-012
Elevated lipoprotein lipase and secretory phospholipase A2 activity in acne vulgaris patients

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Acne vulgaris is a common chronic inflammatory skin disease of unknown etiology. Excess levels of secretory phospholipase A2 (sPLA2) contributes to inflammatory diseases and studies indicate that lipoprotein lipase (LPL) has differential effects on several inflammatory pathways. The aim of the present study was to assess serum activity of sPLA2, LPL and evaluate changes in circulating protein levels of angiopoietin-like protein 3 (ANGPTL3), ANGPTL4, cyclooxygenase (COX) and prostaglandin E2 (PGE2). Serum from 21 control subjects and 31 acne vulgaris patients with moderate and severe disease was evaluated for levels of sPLA2, COX, PGE2, LPL, ANGPTL3 and ANGPTL4. Disease activity was determined according to the National Health Service (NHS) Lambeth and Southwark Clinical Commissioning Group Guidelines for the management of acne. Lipid profile, routine biochemical and hormone parameters were assayed by standard kit methods using autoanalyzers (Beckman Coulter AU5800 Clinical Chemistry and UniCel DxI 800 immunoassay systems). Serum levels of sPLA2 and LPL were significantly increased in acne vulgaris patients compared to age and gender matched controls. No significant differences were found for COX, PGE2, ANGPTL3 and ANGPTL4 levels between acne vulgaris patients and controls. The results of this study reveal the presence of a proinflammatory state in acne vulgaris as shown by significantly increased serum sPLA2 activity. Increased LPL activity in serum of acne vulgaris can be protective in patients through its anti-dyslipidemic actions. To our best knowledge, this is the first study investigating sPLA2, LPL, ANGPTL3 and ANGPTL4 levels in acne vulgaris. Future studies are aimed to understand the regulation of sPLA2 and LPL expression in acne vulgaris patients. Acknowledgement: This study was supported by a grant from The Scientific and Technological Research Council of Turkey (TUBITAK; #115S940).

P-04.04.4-013
8-OHdG and hOGG1 levels are as an oxidative DNA damage markers in acne vulgaris treated with isotretinoin

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Acne vulgaris is a skin disease that characterized by comedones, papules, pustules, nodules and cysts at face, back and body skin. Isotretinoin is one of the treatment agents in acne vulgaris. About 4 weeks after drug treatment, the amount of sebum which is produced by sebaceous gland reduces keratinization disorder and the number of Propionibacterium acnes normalizes. However, isotretinoin is known that has a wide range of side effects. In recent studies, isotretinoin treatment has been shown to increase the oxidative stress. 8-hydroxy-2′-deoxyguanosine (8-OHdG), an important indicator of oxidative DNA damage, hydroxyl ion is bound at the 8th carbon of guanine. This structure is repaired through a base excision repair mechanism and the human 8-oxoguanine DNA glycosylase 1 (hOGG1) plays a key role in this processes. In this study we aimed to evaluate the DNA damage and it's repair in acne vulgaris before and after 6 months of isotretinoin treatment by measuring 8-OHdG and hOGG1 levels.

The current study includes 43 acne vulgaris patients who are diagnosed in Mustafa Kemal University, Department of Dermatology. 8-OHdG and hOGG1 levels were measured by Enzyme-linked Immunosorbent Assay (ELISA) method for before and after 6 months of isotretinoin treatment. The commercial ELISA kits (Cloud-Clone Corp; USA and Cell Biolabs; USA) were used for the assessment of hOGG1 and 8-OHdG, respectively.Both 8-OHdG (p

As a conclusion, isotretinoin increases DNA damage and high serum 8-OHdG and hOGG1 levels as a result of isotretinoin treatment may effect on the amount of reactive oxygen species.

P-04.04.4-014
The role of signal transducer and activator of transcription 3 (STAT3) in the rat pineal gland

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The pineal gland is a circumventricular organ which serves as a major neuroendocrine gland in the brain. Its primary function is the production of melatonin which is controlled by signals from the suprachiasmatic nucleus. Melatonin codes the length of the night and it is well recognized for its anti-inflammatory effects. Lipopolysaccharide (LPS) is the essential component in the outer surface membrane of gram-negative bacteria and act as a strong stimulator of natural and innate immunity in all eukaryotic species. Furthermore, LPS reduces melatonin synthesis and induces the expression of the serine protease inhibitor 3 (SPI-3) in the STAT3-mediated manner in pinealocytes. However, the precise function of STAT3 in the cell signaling in the pineal gland is not yet known.

Here we investigated the effect of inhibition of STAT3 on LPS-induced changes in melatonin levels, expression of

arylalkylamine N-acetyltransferase (AA-NAT) and SPI-3 in the pineal gland.

Experiments were performed in vitro using organotypic and primary cultures prepared from the rat pineal glands. Levels of melatonin and SPI-3 were determined from tissue homogenate by enzyme-linked immunosorbent assay (ELISA). The pinealocytes were used to carry out siRNA STAT3 transfection. The successful transfection and subsequent decline in STAT3 expression levels were proved by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). The changes in synthesis of AA-NAT and SPI-3 were studied by RT-PCR.

In conclusion, lipopolysaccharide can affect the immunomodulators secreted by the pineal gland. The clarification of the effect of inhibition of STAT3 on those immunomodulators is important from the clinical point of view because inhibitors of STAT3 are nowadays used as tumour suppressors.

P-04.04.4-016
Amorphous silica nanoparticles induced inflammatory and cytotoxic effects in human lung fibroblast cells

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Silica nanoparticles have a great potential for a variety of industrial, diagnostic and therapeutic applications. In this study, we have evaluated the in vitro effects of amorphous silica nanoparticles (7 nm) using human lung MRC-5 fibroblast as model. Cells were exposed to 62.5 μg/ml silica nanoparticles for 24, 48 and 72 hours. The cytotoxic and inflammatory response, and matrix metalloproteinase expression were examined.

The pro-inflammatory cytokine IL-1β, IL-6, IL-8, tumor necrosis factor (TNF-α), matrix metalloproteinases (MMP-2, MMP-9, MMP-1) and tissue inhibitor of metalloproteinase-1 (TIMP-1) were analyzed by Western Blot method. Cytotoxicity was evaluated by lactate dehydrogenase (LDH) released into the culture medium by damaged cells.

The level of LDH activity was increased after exposure to silica nanoparticles, in a time-dependent manner compared to control. The protein expression of IL-1, IL-6, IL-8 and TNF- α as well as of MMP-1 and TIMP-1, was up-regulated whereas those of MMP-2, MMP-9 was down-regulated after 48 and 72 hours respectively.

In conclusion, our data indicate that amorphous silica nanoparticles generate a cytotoxic and inflammatory response, as well as an imbalance in extracellular matrix due to the differential regulation of MMPs and tissue inhibitor of metalloproteinase-1 in MRC-cells after 48 and 72 hours.

P-04.04.4-017
Association of FTO gene variant (rs8050136) with markers of T2DM and obesity in population from Bosnia and Herzegovina and Kosovo

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FTO (fat mass and obesity-associated gene), recently discovered in a genome-wide association study for type 2 diabetes (T2D) encodes a 2-oxoglutarate-dependent nucleic acid demethylase and is mainly expressed in the hypothalamus. This gene may play important role in the management of energy homeostasis, nucleic acid demethylation, and regulation of body fat masse by lipolysis. The aim of this study was to analyze the association of this single nucleotide polymorphisms (SNPs) with clinical and biochemical parameters of obesity, T2D, prediabetes and at the level of healthy population from Bosnia and Herzegovina (BH).

The study included 638 patients with T2D and prediabetes and 360 healthy controls both sexes, aged from 40 up to 65 years. Patients were recruited at the Clinical Centre University of Sarajevo, University Hospital of Clinical Centre in Banja Luka, General Hospital in Tešanj and Health Centre in Prizren. Genotyping of analyzed polymorphism was performed by RT-PCR method in cooperation with the Department of Clinical Chemistry, Faculty of Pharmacy, University of Ljubljana (Ljubljana, Slovenia) and University Hospital of Charles University (Hradec Kralove, Czech Republic).

Our results did not show significant differences in genotype frequencies of the analyzed polymorphisms between patients with T2D, pre-diabetes and healthy population Also, results of logistic regression analyses did not show significant association of risk A allele of *FTO* gene polymorphism - rs8050136 with increased risk of T2D (OR = 1.084, 95% CI 0.758–1.551, p = 0.659). A allele was significantly associated with higher values of HbA1c, insulin, HOMA IR index, diastolic blood pressure and higher levels of inflammatory markers (fibrinogen and leukocytes). Interestingly, a tendency of association of A allele with higher values of obesity markers (BMI, waist and hip circumference) was noted. Further studies are needed on a larger population in order to confirm these results.

P-04.04.4-018
Beneficial actions of dichloromethane sub-fraction of the COWE extract for multiple sclerosis: a potential therapeutic role

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The water extract of *Capparis ovata* (COWE) has been shown to be used as an alternative medicine for the treatment of Multiple Sclerosis (MS). COWE was further fractionated and studied for additional anti-neuroinflammatory effects in SH-SY5Y cells. For this purpose, the dichloromethane sub-fraction of the COWE extract was tested for its anti-inflammatory effects on selected anti-inflammatory genes believed to be important in MS pathophysiology using SH-SY5Y cells. Cell viability was assessed using lactate dehydrogenase (LDH) activity in the media conditioned by the crystal violet cell staining. In these cells, levels of the tumor necrosis factor-α (TNFα), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB1), glial fibrillary acidic protein (GFAP), C-X-C motif chemokine 9 and 10 (CXCL9, CXCL10), matrix metalloproteinase 9 (MMP9), chemokine (C-C) motif 5 (CCL5) and tyrosine-protein phosphatase non-receptor type 11 (PTPN11) were determined by quantitative reverse transcriptase-PCR assay (qRT-PCR). We have found out that the dichloromethane sub-fraction of COWE effectively inhibited the expression of all of the genes given above in SH-SY5Y cells. Thus, phytochemicals present in the dichloromethane sub-fraction of the COWE extract could be beneficial in preventing/treating neurodegenerative diseases in which neuroinflammation is part of the pathophysiology. Studies are underway to identify the individual compound(s) in this subextract of the COWE extract contributing to these effects.

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P-04.04.4-019
Apigenin and luteoline were identified as active anti-inflammatory constitutents of *Lavandula stoeachas* by bioassay guided fractionation

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Introduction: *Lavandula stoechas,* in the genus of lavender, has distinct therapeutic uses among Anatolian people. Rather than worldwide use of its essential oil in aromatherapy, specifically the aqeous portion as decoction has been traditionally used in Anatolia against the components of metabolic syndrome, all of which share a state of chronic inflammation as an underlying cause. The anti-inflammatory constiutents of *L. stoechas* were isolated using a bioassay guided fractionation in lipopolysaccharide (LPS) inflammed RAW 264.7 macrophages.

Materials and methods: An aqeous extract was partitioned into ethyl acetate (EAE) and n-butanol fractions. The EAE, determined as bioactive extract was seperated into 12 subfractions by column chromatography. E6 was identified as active subfraction subjected to sephadex column to get pure compounds which were then applied to NMR, IR, and UV analyses for structure determination. In RAW 264.7 cells, the effects of extracts/fractions/subfractions/compounds on LPS induced NO production was determined by using Griess method. The potential inhibitory effects of each compound on LPS induced iNOS expression were determined by qPCR and Western blot.

Results: p-coumaric acid, apigenin and luteoline were found in the E6, and the first two compounds appeared to be primarily responsible for the anti-inflammatory activity. Apigenin and luteoline at 50 μM decreased NO production 66 and 80%- $IC_{50:}$ 56 and 26 μM-by inhibiting iNOS gene expression 84 and 88% as well as protein expression 94 and 99%, respectively ($p < 0.05$).

Conclusion: This is the first time that luteoline and apigenin have been found in EAE of *L. stoechas*, and the anti-inflammatory properties of the EAE can be attributed, at least in part, to the presence of these two compounds. We are on the way to gain further insight for the action mechanism of these two active principles as anti-inflammatory agent.

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P-04.04.4-020
The role of Tip60 in the inflammation process

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Immun response generates the first line of host defense during inflammation and plays an important role inducing pro-inflammatory response by generating early response against pathogens. IL-6 (Interleukin 6) is one of the pro-inflamatory cytokines and its expression increases during the infection to activate the JAK/STAT pathway. JAK/STAT pathway is regulated by Hamp (hepcidin antimicrobial peptide). Our previous study, we reported that Hamp gene expression was decreased in liver-specific Tip60 conditional knockout mice, so we thought that TIP60 may have a direct or indirect role on inflammation mechanism. TIP60 (Tat interacting protein, 60 kDa) is a member of the MYST enzyme family of histone acetyltransferases (HATs) and plays an important role in multiple function including cellular signaling, DNA repair, cell cycle and apoptosis.

In this study, the quantitative gene and protein expression of IL-6 were investigated by using TaqMan real time PCR, western blot and immunohistochemistry analysis in control group, LPS-induced inflammation group and liver-specific Tip60 conditional knockout group mouse liver. According to our preliminary results, the gene and protein expression of IL-6 was increased in LPS-induced inflammation group ($p < 0.001$, $p < 0.001$) and

liver-specific Tip60 conditional knockout group mouse liver ($p < 0.05$, $p < 0.01$).

Our initial data suggest that Tip60 may be essential for the inflammation process. This work was funded by grants from the Scientific and Technological Research Council of Turkey (TUBITAK) (Grant number: 114Z277).

P-04.04.4-021
Stimulation of gene expression and activities of some renal antioxidant enzymes during inflammation

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Although intracellular reactive oxygen species (ROS) level is necessary to maintain cellular homeostasis, elevated intracellular ROS level with the impact of unfavorable environmental conditions leads to oxidative stress that may cause damage to DNA, proteins and lipids. In case of inflammation, organism seeks to provide cellular homeostatis by increasing ROS levels via antioxidant molecules and enzymes. Therefore, it was thought that there can be a direct or indirect relation between inflammation and oxidative stress.

In this study, inflammation was performed by *intraperitoneal injection* of *lipopolysaccharide* (*LPS*). The gene expression and activity of antioxidant enzyme including Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPX), Glutathione S-transferase (GST), Glutathione reductase (GR) and Glucose 6-phosphate dehydrogenase (G6PD). Additionally, any change of reduced glutathione (GSH), oxidized glutathione (GSSG), malondialdehid (MDA), and hydrogen peroxide (H_2O_2) level are accepted as an indication for the accumulation of ROS, the relative levels of them were also studied.

To show our inflammation model was performed in mouse kidney with LPS treatment or not, the expression of interleukin (Il6), which is accepted as a inflammation marker, was investigated by real time PCR. The expression of Il6 was significantly increased in LPS treated group. While the level of MDA and H_2O_2 was elevated in LPS treated group, GSSG was decreased. No changes was seen for GSH level. The correlation was observed between enzymatic and molecular levels. While the gen expression and the enzyme activity of SOD, CAT, GST, GR, and G6PD were decreased, GPX was increased with inflammation.

In conclusion, increasing ROS level was observed in the inflammation process and, the antioxidant system was affected at the molecular and protein level. This work was funded by grants from the Scientific and Technological Research Council of Turkey (TUBITAK) (Grant number: 114Z277).

P-04.04.4-022
The effect of vitamin D levels in Behcet patients on hemogram parameters

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The aim of our study is to evaluate effect of vitamin D levels on hemogram parameters including neutrophil %, lymphocyte %, neutrophil % / lymphocyte % ratio (NLR) and mean platelet volume (MPV) in Behcet's patients.

Fifty eight patients with diagnosis of Behcet that applied to Selcuk University Faculty of Medicine Department of Dermatology are recruited to the study. Clinical and laboratory characteristics of the patients were obtained from hospital automation. T test was used to examine the differences between the parameters. $p < 0.05$ was taken to be statistically significant.

There was a statistically significant difference between vitamin D values and age ($p = 0.036$) whereas difference was not significant between vitamin D and neutrophil %, lymphocyte %, NLR, MPV values.

According to the literature, there are a lot of studies that show the relationship between vitamin D and hemogram parameters. However, contrary to the previous studies, we were unable to find any significant relationship between vitamin D and these hemogram parameters. These results serve the idea that the effects of vitamin D on the hematopoietic system should be further investigated experimentally and clinically.

P-04.04.4-023
Role of trace elements in the Crimean-Congo hemorrhagic fever disease

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Crimean-congo hemorrhagic fever is a tick-borne disease caused by the arbovirus and characterized by a sudden onset of high fever, severe headache, dizziness, back and abdominal pains. The exact pathogenesis of CCHF has not been clarified yet. The aim of this study, clinical cases of CCHF in Cu, Se and Zn is to examine the relationship between the concentration of trace elements.

The study sample consisted of 30 patients which have been diagnosed with CCHF. Matched for gender, 30 healthy volunteers were similar to the control group according to age. The patients and control groups, serum Cu, Zn and Se levels were analyzed using Atomic Absorption Spectrophotometer.

CCHF patients in the group, Cu Zn and Se serum levels were significantly lower compared with the control group.

In our study, the cofactor of the antioxidant enzyme Cu, Zn and Se elements were lower. This shows us in CCHF disease, a decrease in antioxidant enzyme activity, and suggest that they contribute to the immune system's degradation.

P-04.04.4-024
Inhibitors of MDM2 ubiquitin ligase as prospective modulators of autoimmunity

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Ubiquitin-proteasome system is seen as a pool of promising protein targets for therapeutic impact in many human diseases. MDM2 is an E3 ubiquitin ligase widely studied due to its well-known role in cancer – it negatively regulates p53 oncosuppressor that mediates apoptosis in tumour cells. Inhibitors of p53/MDM2 interaction have long been known as potential anticancer therapeutics. However, recent advances in the field suggest that both MDM2 and p53 might be playing a substantial role in autoimmune processes.

We used a small molecule p53/MDM2 inhibitor Nutlin-3a to test the effect of p53 activation on peripheral blood mononuclear cells (PBMCs) from both healthy volunteers and patients diagnosed with multiple sclerosis. In our study we employed a variety of molecular biology methods, such as immunoblotting, real-time PCR, MTS cell proliferation assay, fluorescence flow cytometry and confocal microscopy. We demonstrated that disruption of p53/MDM2 interaction by Nutlin-3a alters the p53 levels and also affects the lymphocyte subpopulations within PBMCs. Our findings suggest that p53/MDM2 interaction inhibitors can potentially be used as prospective modulators of immune response in autoimmune diseases such as multiple sclerosis, systemic lupus erythematosus and other. The study was funded by RFBR research grant 16-34-60213 mol_a_dk.

P-04.04.4-025
Can YKL-40 be an inflammatory biomarker in vitamin D deficiency?

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Background: Vitamin D deficiency is associated with several conditions and/or diseases like inflammation, atherosclerosis, cardiovascular disease and mortality. Several studies showed that lower vitamin D levels were associated with high serum levels of inflammatory biomarkers. YKL-40 is a glycoprotein, secreted by macrophages, neutrophils and different cell types. It is also associated with inflammation and pathological tissue remodeling. In this study, we aimed to evaluate relationship between the vitamin D deficiency and YKL-40 levels.
Methods: Our study group includes 45 subjects with vitamin D deficiency (Group 1) and 40 age and sex-matched healthy subjects with normal serum levels of vitamin D (Group 2). Plasma 25 (OH) vitamin D levels were measured with liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. Plasma YKL-40 analysis was performed by ELISA. Serum hs-CRP levels were measured with nephelometric method.
Results: Plasma vitamin D levels below 20 ng/ml were accepted as vitamin D deficiency. Although we could not find any significant differences by means of serum hs-CRP *levels between groups* ($p > 0.05$), plasma YKL-40 levels were significantly higher in group 1 than group2 ($p < 0.05$).
Conclusions: In literature, vitamin D deficiency is associated with inflammation. In our study, we found similar hs-CRP levels between groups and higher YKL-40 levels in group 1. Vitamin D deficiency may be related to increased YKL-40 levels in terms of causing chronic inflammation.

Keywords: Vitamin D deficiency, YKL-40, inflammation.

P-04.04.4-026
Evaluation and comparison of TNF-family ligands and receptors genes in mice and humans by bioinformatics techniques

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Object: The tumor necrosis factor (TNF) was found to be cytotoxic to tumor cells and to induce tumor regression in mice. Except for one member, all receptors to the TNF superfamily bind TNF-related ligands and act mostly on inflammatory system. There are currently 19 TNF superfamily ligands. TNF superfamily ligands share several common features. TNF ligands are generally type II transmembrane proteins whose extracellular domains are be divided by enzyme to create cytokines. The TNF superfamily currently consists of 29 receptors. TNF family receptors are type I or type III transmembrane proteins that contain multiple extracellular domains. In this study, we investigated to presence, differences and effects of TNF superfamily and receptors genes in human and mice by using bioinformatics techniques.
Methods: The nucleotide and amino acid sequence of each protein in human and mice was determined using T-BLAST- N for homologous sequences. Homologous sequences of human TNF family genes found an automated procedure by using Psi-BLAST. The secondary structure of and three-dimensional of the protein were analyzed by Psipred and FFAS server. NetCGlyc 1.0 and NetPhos 2.0 program were used for post-translocation modifications. The web apoptosis database was also used for the lists of domains, proteins containing these domains and their associated homologs.
Results and conclusion: Humans TNF ligands have 17 genes encoding proteins that contain a conserved carboxy-terminal domain. This family of proteins is highly conserved between humans and mice. Humans contain 29 genes encoding TNF-family receptors. Sequence data from the NCBI databases demonstrated the presence of 24 mouse TNF-family receptors with orthologs in humans and one additional receptor found only in mice. The differences and similarities in the TNFs genes in humans and mice will provide information for understanding the utility and limitations of the mouse models of disease and comparing of immunology outcomes.

P-04.04.4-027
Relation between angiotensin-converting enzyme I/D gene polymorphisms and modified shock index in patients with a first acute anterior myocardial infarction

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The development of left ventricular remodeling after acute myocardial infarction is a predictor of shock. The genetic influence on cardiac remodeling, and shock in the early period after acute myocardial infarction are unclear. The aim of the present study was to investigate the relationship between angiotensin converting enzyme (ACE) gene polymorphism and Modified Shock Index (MSI) in the early period in patients with acute anterior myocardial infarction.

Overall 140 patients with a first acute AMI were included in this study. DNA was isolated from peripheral leukocytes. The ID status was determined by PCR. Based on the polymorphisms of the ACE gene, they were classified into 2 groups: Deletion/Deletion (DD) genotype (Group 1, n = 57), Insertion/Deletion (ID), Insertion/Insertion (II) genotypes (Group 2, n = 83). Blood pressure and pulse measurements were performed in all patients within 10 minutes admitted to coronary care unit. MSI was defined as heart rate (HR) divided by mean arterial pressure (MAP). Echocardiographic examinations were performed in accordance with the recommendations of the American Echocardiography Committee. One-way analysis of variance (ANOVA) and Chi-square analyses were used to compare differences among subjects with different genotypes. The study was approved by the local Ethics Committee, and each patient gave a written consent.

There were no significant differences among clinical parameters of patients. MSI was significantly higher in patients who have ACE DD genotype than in patients who have ACE ID / II genotypes (1.13 ± 0.52 and, 0.85 ± 0.37, $p < 0.05$).

Presentation time hypotension or developing hypotension during admission was reported to be an important predictor of intensive care unit admission besides other vital sign measurements.

Our results suggested that, ACE Gene I/D polymorphisms D allele may affect modified shock index in patients with a first acute anterior MI.

Wednesday 7 September 12:30–14:30

Functional genomics and proteomics

P-08.01.4-001
Vitamin D3 protects against impairments of cytokine RANK/RANKL/OPG system in rat bone marrow associated with long-term prednisolone administration

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Glucocorticoids (GCs) are widely used in medicine, despite their side effects, e.g. osteoporosis. However, precise molecular mechanisms of GC action, especially on bone marrow (BM) cells, remain controversial. Given the osteoprotective role of vitamin D_3, the aim of our study was to examine prednisolone-induced changes in the RANK (receptor activator of nuclear factor kappa-B)/RANKL (RANK ligand)/OPG (osteoprotegerin) pathway of rat BM depending on the state of vitamin D endocrine system.

Female Wistar rats received prednisolone (5 mg/kg b.w.) with and without 100 IU of D_3 (for 30 days). The levels of RANK, RANKL, OPG, 1α-hydroxylase (CYP27B1) in BM were determined by western blotting. Vitamin D_3 receptor (VDR) and RANKL mRNAs were measured by quantitative RT-PCR. $25OHD_3$ content in the serum was assayed by ELISA. RANK- and VDR-positive BM cells were quantified using flow cytometry and visualized by confocal microscopy.

Prednisolone induced a marked increase in RANKL and RANK levels, while OPG level was shown to decrease. This reflects disturbances in cytokine-mediated regulation of BM progenitor cell function. Data from flow cytometry indicated a significant growth in the number of RANK-positive cells (hematopoietic osteoclast precursors) compared to control. These changes were accompanied by a decrease in the levels of VDR and CYP27B1, which is responsible for $1,25(OH)_2D_3$ synthesis, in BM and $25OHD_3$ content in serum. Co-localization of VDR and RANK in mono- and multinuclear BM cells was observed, indicating a close relation between vitamin D_3 and RANK/RANKL/OPG pathway. Vitamin D_3 co-administration prevented prednisolone-induced changes in BM cells through restoration of vitamin D_3 bioavailability and VDR signaling that resulted in a reduction of the osteoclast progenitor pool in BM.

Thus, prednisolone-induced imbalance in RANK/RANKL/OPG system components is associated with impairments of vitamin D endocrine system in BM and can be ameliorated by vitamin D_3 treatment.

P-08.01.4-002
Heat shock pathway in response to different stress factors

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The heat shock response is an emergency pathway of the cell, which mediates repair and protection from cellular stress and therefore guarantees the survival of the cell. This stress can range from heat or hypoxia to chemicals and heavy metals. It is highly conserved in all eukaryotic cells and plays an important role during atypical conditions. Due to its high complexity, the pathway is not yet completely understood. Most important, after

activation of the pathway, is the refolding of proteins or, in case of severe misfolding, the depletion of proteins to maintain proteostasis. Heat Shock Factor 1 encoded by the HSF1 gene is known as the main switch point in heat shock regulation. After activation it trimerizes and binds to heat shock elements in target gene promoters.

One of these promoters is the HSPA1A promoter (HSP72 promoter). The promoter was analyzed by dismantling it to its functional parts. Especially three elements, the heat shock elements, were in the focus of this work. In first place parts of the promoter were multimerized and combined with different reporters, like luciferase, by cloning. Also mutations in the natural promoter were designed by cloning. The focus now is on the heat shock elements, where HSF1 can bind as a trimer. The idea is that these different elements have various effects on different stressors like heat, chemicals (geldanamycine as HSP90 inhibitor, MG132 as proteasome inhibitor) or heavy metals (cadmium, arsenic, zinc). This was tested on cells transiently transfected with those promoter variants. For promising variants stable cell lines were created. In these stable cell lines further experiments on mRNA level can be conducted. In the last months experiments with the CRISPR/Cas9 system were started. Furthermore, experiments on transcriptional (qPCR) and translational (dual-luciferase assay) levels were done as well. In the end we hope to get a clear picture on the regulation of the HSPA1A promoter by different stress factors.

P-08.01.4-003
New macromolecular complexes in invadopodia formation

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Invasive cancer cells form membrane protrusions, invadopodia, that facilitate cell invasion and metastasis. Key players invadopodia include the adaptor proteins TKS4 and TKS5, the actin regulators cortactin, WIP and N-WASP, the kinase Src and others. In spite that in the last two decades significant advances in our knowledge of the structure and development of invadopodia have been made, detailed mechanisms they are functioning is not yet available. We have identified a series of new TKS4 binding partners including adaptor proteins ITSN1, ITSN2, CRK and GRB2, kinase SRC, AMPH1, BIN1, PLCg1 and also another member of the TKS family – TKS5. It may indicate the possible role of TKS4 in transport and sorting of cell vesicles. Current data are supported by interaction with the proteins of AMPH1 and BIN1, as their main functions are membrane trafficking and remodeling. Adaptor proteins CRK, GRB2 and ITSNs are important for the actin cytoskeleton rearrangements, endocytosis and signal transduction. Moreover, we have identified and characterized new TKS4 isoform – TKS4-beta. We suggested that an active state of TKS4 is regulated via intramolecular interactions between its proline-rich motifs and own SH3-domains. We have shown the interaction between ITSNs and other prominent component of invadopodia WIP. Data from immunofluorescent analysis revealed co-localization of ITSN1 and WIP at the sites of invadopodia formation and in clathrin-coated pits. We have also demonstrated that the key protein ITSN1 and WIP and N-WASP can form a complex in cells. Together, these findings provide insights into the molecular mechanisms of invadopodia formation and identify ITSNs as scaffold proteins involved in this process. We have shown the interaction between ITSNs and other verprolin family members CR16 and WIRE which play an important role in the reorganization of the actin cytoskeleton. We have demonstrated that CR16 and WIRE interact with SH3-domains of ITSNs in complex with actin.

P-08.01.4-004
Correlation between proteomic and phenazine profile of *Pseudomonas* sp.

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Phenazines are widely known compounds with huge variativity of biological activities which are produced by Pseudomonas sp. and some other bacteria species. The results of our work shows the correlation between the changes of proteomic profile of *Pseudomonas aeruginosa* caused by a mutagenesis and the secondary metabolism of antibiotics (phenazine) profile.

Different strains of *Pseudomonas aeruginosa* were obtained using mutagenesis, after that bacterial cells were destroyed by ultrasound. Protein-containing fractions were isolated using methanol-chloroform method as well as phenazines compounds were extracted from culture media using liquid phase extraction. Obtained proteome was analysed by shotgun-proteomics technique. As the result of the liquid phase extraction phenazine compounds were mainly extracted to the organic phase. This phase was evaporated and re-dissolved in 100% methanol. After sample preparation obtained solutions were analyzed by HPLC-Agilent 1290 with quadrupole TOF mass-detector. Results of the analysis were compared with the library of known phenazine compounds mass-spectras generated by CFM-ID online resource. Obtained phenazine profiles were compared with each other and correlation with the changes in proteome was analyzed.

Received results promote better understanding of mechanisms of phenazine production. This data opens possibilities for targeted changes in the methabolic pathway in order to obtain phenazine compound with required biological activity.

P-08.01.4-005
Structural determinants of interaction between Drosophila CP190 BTB-domain and CTCF protein

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Insulators are genomic elements which block enhancer-promoter interaction and prevent spreading of heterochromatin. CP190 protein is an integral component of most known Drosophila insulators, it interacts directly with CTCF and Pita DNA-binding insulator proteins using dimeric BTB-domain, but function of CP190 within insulators still remains to be elucidated. Recently we described an interaction between CP190 BTB-domain and C-terminal domain of CTCF insulator DNA-binding protein, subsequent deletion analysis allowed us to isolate 50aa fragment within CTCF C-terminal domain sufficient for interaction with CP190 BTB, but deletions of flanking regions also lead to the loss of interaction with CP190 in vivo. At the same time crosslinking experiments suggest that a dimer of BTB interacts with one molecule of CTCF, presuming that it could recognize two peptide fragments within CTCF C-terminal domain. We solved crystal structure of BTB-domain from CP190 insulator protein at 1.3 Å resolution. Overall structure is similar to other BTB-domains. CP190 BTB-domain has peptide-binding groove similar to that previously found in Bcl6 BTB domain. Inspection of BTB-domain surface revealed several possible binding sites for

polypeptide fragments from CTCF protein. Based on these observations a set of point mutations within peptide-binding groove of BTB-domain has been designed and we tested CTCF-interaction abilities of these mutants using GST pull-down assay and yeast two-hybrid assay. The most significant impact was found with alanine-substitutions of hydrophobic residues whereas substitutions of hydrophilic amino acids were less effective. Therefore our results support that CP190 BTB-domain recognizes CTCF protein using peptide-binding groove. This study was supported by the Russian Science Foundation (project №14–24–00166).

P-08.01.4-006
Comparative study of the fatty acid composition of lipids in the raw meat samples obtained from hybrid sheep

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One of the most important tasks in the animal biology and husbandry is to clarify the role of animal genetic diversity in providing nutrients to the diversity of animal products. The objective of our work was to study the chemical compositions of raw meat samples obtained from domestic (group I - purebred Romanov sheep) and hybrid sheep (group II - F_3 hybrids of Romanov sheep with 12.5% of argali blood). The significant changes in fatty acid composition of the lipid fraction from the fat and muscle tissue of the hybrid sheep as compared to the control were found. The content of saturated fatty acids (SFAs) in the fat samples of the hybrid animals was by 12.16% lower ($55.12 \pm 2.30\%$, $p < 0.01$), but polyunsaturated (PUFAs) or monounsaturated fatty acids (MUFAs) contents were by 0.64% and 9.43% higher (10.10 ± 0.43 and 28.78 ± 1.77 ($p < 0.01$), respectively) as compared to purebred Romanov sheep. The most pronounced changes were found for palmitic acid (decreased from 27.70% to 14.24%) and for oleic, linoleic, arachidonic acids (increased from 20.37%, 5.58%, 0.35% to 29.98%, 6.10%, 0.70%, respectively). The last two acids together with the linolenic acids belong to the so-called essential acids and very important for the animal metabolism. A similar trend was observed on the composition of the lipid fraction of muscle tissue. SFAs, PUFAs and MUFAs content in muscle tissue of hybrid sheep was 53.38 ± 0.34, 9.39 ± 1.00 and $34.16 \pm 0.39\%$, that was 11.17% lower ($p < 0.001$), and 4.13% and 4.67% higher ($p < 0.01$) compared to purebred Romanov sheep. These results emphasized the difference of the PUFAs/SFAs ratios in fat and muscle tissues, respectively) and characterized the biological value of the lipid fraction of fat and muscle tissue. The obtained data gave evidence of the positive changes in the fatty acid compositions of the lipid fractions for the hybrid animals as compared to the purebred sheep. Supported by the Russian Scientific Foundation, No. 14-36-00039.

P-08.01.4-007
Low-temperature enterobacteria phage vB_EcoS_NBD2 isolated from agricultural soil

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Foodborne illnesses resulting from the consumption of agricultural commodities contaminated with enteric pathogens are an increasing problem around the world. While various possibilities of produce contamination with pathogens exist, the global warming combined with a widespread use of animal manure in agriculture will likely contribute to an increased number of such outbreaks. Thus, phages isolated from different agroecosystems may prove to be useful in detection/biocontrol of enterobacteria in produce.

During the investigation of the impact of global warming on the diversity and co-evolutionary dynamics between microorganisms and viruses in Lithuanian agroecosystems, a novel enterobacteria phage vB_EcoS_NBD2 (NBD2) was isolated from agricultural soil using *E. coli* NovaBlue for phage propagation. NBD2 genomic DNA was isolated from CsCl-purified phage particles, and was subjected to Illumina DNA sequencing.

NBD2 is a virulent siphovirus that has a low-temperature plating profile (fails to form plaques at a temperature >30 °C). The genome of NBD2 is $\sim$52 kb long, and has a total of 87 probable protein-encoding genes as well as 1 gene for $tRNA^{Ser}$. The genome analysis revealed that 20 NBD2 ORFs encode unique proteins that have no reliable identity to database entries. Among the ORFs that encode proteins with matches to those in other sequenced genomes, 64 are similar to proteins from phages that infect different members of Enterobacteriaceae, while 3 NBD2 ORFs are most similar to those from Bacteria. Based on the similarity to biologically defined proteins, 32 NBD2 ORFs were given a putative functional annotation, including 15 genes coding for morphogenesis-related proteins, as well as 14 associated with DNA replication, recombination, and repair.

Phylogenetic analysis revealed that enterobacteria phage NBD2 is distantly related to phages belonging to the subfamily *Tunavirinae*. This research was funded by a grant (No. SIT-7/2015) from the Research Council of Lithuania.

P-08.01.4-009
The antibiotic novobiocin affects the composition of the *Escherichia coli* proteome

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Novobiocin (NOV) is an aminocoumarin which competitively inhibits the ATP binding site in the gyrase-β subunit of prokaryotic topoisomerase II. NOV remains a therapeutic choice for treating infections with bacterial pathogens that are resistant to more commonly used drugs. The aim of this study is assess the proteomic response of *E. coli* strain upon NOV treatment.

Minimum Inhibitory Concentrations of NOV were measured by standard assays. Three different *E. coli* strains (AS19, AS19-*rlmA::aph* and B) were grown aerobically in nutrient rich LB media at 37 °C during one hour. The whole cell proteome (five biological replicates in each sample,) was assessed by LC-MS by using TMT labelling protocol. Raw files were imported to Proteome Discoverer (Thermo Fisher Scientific) and searched together with Mascot against the Uniprot E. coli reference proteome.

MICs for NOV were determined to be >1000-fold higher the wild-type B-strain of *E. coli* than for the hypersusceptible AS19 strains (1 μg/ml). Whole genome comparison of the B and AS19 strains were characterized by an increase in proteasome components (6 proteins), chaperones (3), error-prone DNA polymerase components (4), ribosomal hibernation factors (3), heat shock response (2), electron transport coupled proton transport (8), pentose phosphate pathway (9), flagellar assembly (4), oxidative phosphorylation (10) and TCA cycle (8). Whereas ribosomal proteins (45), aminoacyl-tRNA synthetases (7), RNases (6), ABC transporters (17), mismatch repair (3) and Sec secretion pathway (4) were significantly down-regulated upon NOV treatment.

The three *E. coli* strains respond similarly upon NOV treatment and their proteomes showed upregulation of heat shock response with changes in the components of translation and transcription, the proteasome and ATP biosynthesis. The changes observed can be used to define the processes that are required for antibiotic tolerance and survival of *E. coli* against aminocoumarin antibiotics.

P-08.01.4-010
The potential role of polyamine metabolism in different growth hormone mutations (E33G, N47D, T-24A, A13S, W-7X, IVS1+GAAA, IVS1+83C-1, F166Del) on Epithelial mesenchymal transition (EMT) in HEK293

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Postnatal growth is under control of pituitary derived hormone, growth hormone (GH) that triggers bone, fat tissue growth and development via acting on protein, carbohydrate and fat metabolism. GH functions on postnatal development by JAK2/STAT5 signaling following GH:GH receptor (GHR) dimerization. Isolated Growth Hormone Deficiency (IGHD) is a medical condition of insufficient production of growth hormone (GH) that is caused by mutations on GH-N gene in different ethnic origin children. Various mutations within GH has been determined in different populations so far, and Glutamic acid to Glycine (E33G), Asparagine to Aspartic acid (N47D), Threonine to Alanin (T-24A) missense mutations, Alanine to Serine (A13S) substitution, Tryptophan to stop codon (W-7X), GAAA insertion in intron 1 of GH-N gene and both intron 1 (+83C) and deletion of 166. amino acid of GH protein phenylalanine (F166Del) mutations were detected in Turkish IGHD children. The potential role of these mutations on cell growth, proliferation, EMT via acting on GH signaling pathway has not been observed yet. All these mutations were performed on wild type GH- N gene inserted PC3.1 vector by site-direct mutagenesis and stable cell line of each GH gene mutations were generated by neomycin selection. Although W-7X, E33G, F166Del, A13S and N47D mutations suppresses GH signaling *via* acting on either JAK2 dephosphorylation or STAT5 downregulation, T-24A, GAAA insertion and deletion of +83C mutations have no significant effect on GH signaling. In addition, each mutation lead different growth suppression effect and colony formation potential and intracellular polyamine levels and ODC expression profiles were essential role in EMT potential of HEK293 cell lines. As a result, W-7X, E33G, F166Del, A13S and N47D mutations prevented GH signaling and cell growth and differentiation via polyamine metabolism.

P-08.01.4-012
Mass spectrometric detection of collagen alpha-1 (III) chain nitration in patients with acute pulmonary embolism

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Pulmonary embolism (PE) is a common cardiovascular emergency and affects a large number of patients. Acute PE-induced oxidative stress can lead to the accumulation of specific nitroproteins that may play a role in disease progression. The impact of nitration of a single tyrosine residue often has broad implications on the activity of biologically critical proteins, which has become increasingly related to pathological conditions. In this study, we used a proteomic approach to analyze nitrated serum proteins in patients diagnosed with acute PE and healthy controls. Nitrotyrosine (NO2Tyr)-containing proteins were immunoprecipitated from serum with a NO2Tyr affinity sorbent. Precipitated proteins were separated by SDS-PAGE and visualized by Coomassie Blue staining and Western blotting with mouse monoclonal anti-NO2Tyr antibody. Among the numerous immunoreactive bands observed in disease patients, the 138 kDa protein band was in-gel digested and analyzed by MALDI-TOF mass spectrometry (MS). Mass fingerprint data sets obtained from the peptide fragment ions matched human collagen alpha-1 (III) chain (CO3A1_HUMAN) with Mascot algorithm analysis giving a score of 65 ($p < 0.05$). Collagen alpha-1(III) chain is a fibrillar collagen that is found in extensible connective tissues such as skin, lung, and the vascular system. Altered metabolism of collagen and its excessive deposition in the matrix of the connective tissue is a hallmark of chronic interstitial lung diseases. Collagen can be measured in serum and bronchoalveolar lavage fluid from patients with numerous chronic interstitial lung diseases. Given these considerations, future studies are aimed understand the relevance of NO2Tyr modifications in CO3A1 relating to changes in protein structure and function.

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P-08.01.4-013
Association of GCKR and GALNT2 genetic variants with Type 2 diabetes-related traits in population from Bosnia and Herzegovina

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Recent studies have shown that the genes involved in dislipidemia represent potential loci to be associated with diabetes as a disease. Recent genome Wide Association (GWA) studies have associated *rs1260326* in *GCKR* gene and *rs4846914* in *GALNT2* gene with parameters of T2D and diabetic dyslipidemia. In this study, the association of these single nucleotide polymorphisms (SNPs) with T2D and dyslipidemia was tested in the population from Bosnia and Herzegovina (BH).

Our study involved 352 patients with T2D and 156 healthy subjects. Biochemical and anthropometric parameters were measured in all participants. After DNA extraction, Sequenom IPLEX platform was used for the analysis of *GALNT2* polymorphism (*rs4846914*), while polymorphism in *GCKR* (*rs1260326*) gene was analyzed by using Real Time PCR.

Our results demonstrated significant association of *GCKR rs 1260326* variant with waist circumference ($p = 0.003$) and fasting glucose levels ($p = 0.003$) in the control group. No such association was demonstrated for *rs4846914 GALNT2* gene. In the group of diabetic patients, significant association of *GCKR rs1260326* variant with levels of bilirubin ($p = 0.004$) and *rs4846914 GALNT2* variant with HbA1C ($p = 0.013$) and triglyceride levels ($p = 0.043$) was also demonstrated.

Our results suggest an association of variations of *GCKR* and *GALNT2* genes with specific markers of T2D and dyslipidemia. Further studies would be needed in order to confirm these genetic effects in other ethnic groups as well.

P-08.01.4-014
Relationship between SP1 polymorphism and osteoporosis in young osteoporotic women

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Osteoporosis is the most common metabolic bone disorder affecting the normal bone turnover with low bone mineral density (BMD) and risk of fragility fractures. Polymorphisms at the sp1 binding site of the collagen type 1 A1 (COL1A1) gene is associated with low BMD. We examined the distribution of COL1A1 gene polymorphism in 50 young osteoporotic women and in control group in Turkish population.

Patients had low BMD with T score ≤ 2.5 SD and controls was 25 healthy women (35–57 years). Mean age (51.28 ± 5.8) and (46.56 ± 6.15) respectively. The BMD, as g/cm^2, was measured in the hip and the Lumbar spine (L2-L4) with (DEXA). DNA was isolated from blood. COL1A1 gene was analysed with Genomica clinical array system. The X^2 test was used to compare allele and genotype frequences between patients and controls.

Mean of T score in patients was -3.06 ± 0.42. Mean BMD (as g/cm^2) was 0.708 ± 0.073, and (1.009 ± 0.823) Genotype distribution were18(36%) SS, 31 (%62)Ss, 1(%2)ss for patients, and 12(48)SS, 9(36)Ss, 4(16)ss for control . Patients had 33(%66)S allele, 17(34%) s allele, controls had 67(67%)S allele, 33 (33%)s allele. When genotypes and BMD were compared in patients, there was no significant correlation between osteoporosis and genotypes. The allelic distribution was not significant between patients and controls $p > 0.05$. Genotypic distribution in patients were significantly different. Patients had a higher frequency of the Ss(%62) than controls (Ss %36) $p < 0.05$.

This study shows that high prevalences of the Ss genotype at the COL1A1 locus, in osteoporosis . İt is possible that the presence of the s allele causes variation COL1A1 and COL1A2 mRNA's producing abnormal collagene protein. Since collagen protein is major protein of bone, it is to be expected that a defect in this protein will produce bone fragility. COL1A1 gene should be detected early to initiate preventative therapy for bone health.

P-08.01.4-018
Is thymoquinone promising anticancer agent in hepatoma cell line?

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The biological activity of *Nigella sativa* seeds is mainly attributed to its essential oil component which is pre-dominantly (30–48%) thymoquinone (TQ). Therapeutic effect of TQ was exhibited in many diseases including inflammation, cancer, sepsis, atherosclerosis and diabetes. TQ has been reported to exhibit antiproliferative effects on cell lines derived from breast, colon, ovary, larynx, lung, myeloblastic leukemia, and osteosarcoma and inhibited hormone refractory prostate cancer. TQ induces apoptosis in tumor cells by suppressing NF-κB, Akt activation, and extracellular signal-regulated kinase signaling pathways and also inhibits tumor angiogenesis.

The aim of this study was to evaluate the anti tumor effects of TQ on hepatoma cells. These antitumor assays include cell viability assay, clonogenic assay, scratch assay and molecular expression studies of death related genes. Cells were treated with different concentration of TQ in Hep3B for cell proliferation by MTT and clonogenic assay. In addition, the metastatic character of TQ was investigated by scratch assay in Hep3b at 3–6 and 24 hours. The effect of TQ was also evaluated at mRNA level by real-time-PCR. TQ was treated on the Hep3B cells in three different concentration, namely 75–50 and 37.5 μM.

TQ showed the cell cytotoxicity in concentration and time dependent manner. The scratch assay revealed no healing in the scratched area due to the decreased cell viability. Maximum permissible dose was 50 μM. Proapoptotic genes, Bax and Bad, and autophagy genes, Beclin-1 and LC3, were upregulated in Hep3B cells after 24 hours treatment In contrast, antiapoptotic gene, Bcl-2, expression level was decreased for Hep3B cells after 24 hours.

P-08.01.4-019
Association of IRS1 genetic variation with type 2 diabetes and insulin resistance in patients from Bosnia and Herzegovina

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Insulin receptor substrate-1 (*IRS1*) encodes the IRS1 protein, a substrate for the insulin receptor tyrosine kinase and has a critical role in insulin-stimulated signaling pathways. Previous studies showed that *IRS1* single nucleotide polymorphisms (SNPs) were associated with Type 2 diabetes mellitus (T2D). This is the first study performed in a population from Bosnia and Herzegovina (BH) in which we examined the association of rs7578326 (G>A), rs2943641 (T>C) and rs4675095 (A>T) with T2D risk and related traits.

Our study involved 437 T2D patients and 252 healthy subjects. Biochemical parameters, including but not limited to insulin, HOMA-IR, HbA1c, glucose, and lipoprotein levels, were measured in all participants. Genotyping analysis was performed by Mass Array Sequenom iPlex platform in cooperation with Lund University Diabetes Centre, Malmo, Sweden. Statistical analysis was done by SPSS 23.

Our results demonstrated a significant difference in frequency of rs4675095 ($p < 0.001$) and rs7578326 ($p = 0.021$) SNPs between T2D patients and control subjects. Interestingly, here we showed a significant association of *IRS1* rs4675095 risk T allele with increased insulin levels ($p < 0.001$) and HOMA-IR ($p < 0.001$) in T2D patients. Similarly, rs7578326 variant was also associated with the same markers of insulin resistance in diabetic patients, i.e. insulin levels ($p = 0.029$) and HOMA-IR ($p = 0.025$). No such association was demonstrated for rs2943641. However, this *IRS1* variant was associated with changes in lipoprotein levels, where risk C allele increased VLDL ($p = 0.006$) and decreased HDL levels.

Our results suggest that *IRS1* variants are associated with T2D susceptibility in BH population, thus confirming similar findings in other population cohorts. Furthermore, the associations of these variants with markers of insulin resistance and dyslipidemic metabolic changes point to their role as potential T2D biomarkers.

P-08.01.4-020
The effects of hemoglobin variants on measurements of HbA1C by HPLC

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Aim: Glycosylated Hemoglobin (HbA1c) is routinely used to monitor long-term glycemic control and provides valuable information for management of diabetic patients. The aim of this study was to invesigated the values of HbA1c in hemoglobin variants presence using High Performance Liqued Chromatography (HPLC).

Materials and methods: Blood samples were obtained from 59 patients with twenty-seven Hb SS, five SS(F), one Hb D İran, one Hb D-Los Angelas/A, two Hb O Arab, one Hb E-Saskatoon, two Hb G-Coushatta/A, twelve Hb AD, six Hb AE, one Hb EE, one Hb SE. Whole blood samples (3 ml) were collected K_3EDTA tubes. Levels of HbA1c of these cases were measured by Agilent 1100 HPLC systems.

Results: We found that the HbA1c values for all hemoglobin variants were below the reference range using a HPLC method. Especially, HPLC method gave a zero HbA1c result for all patients with Hb SS, Hb SS(F), Hb D Iran, Hb EE and Hb SE.

Discussions: Accurate and precise measurement of HbA1c is extremly important. We observed that measurement of HbA1c in patients with hemoglobin variants may give misleading HBA1c results.

Conclusions: HLPC method can be adversely affected by the presence of hemoglobin variants.

P-08.01.4-021
Effects of ADRA2A genetic variants on type 2 diabetes-related traits

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The *ADRA2A* gene encodes alpha-2A adrenergic receptor which mediates adrenergic suppression of insulin. A genetic variant in *ADRA2A* was recently associated with defective β-cell function. The objective of this study was to analyze association of two *ADRA2A* polymorphisms (rs553668 A>G and rs10885122 G>T) with Type 2 diabetes (T2D) and its related traits.

In this study we have included 437 T2D patients and 252 healthy subjects from Bosnia and Herzegovina (BH). Biochemical parameters, including but not limited to insulin, HOMA-IR, HbA1c, glucose, and lipoprotein levels, were measured in all participants. Genotyping analysis was performed by Mass Array Sequenom iPlex platform in cooperation with Lund University Diabetes Centre, Malmo, Sweden. Statistical analysis was performed by IBM SPSS Statistics 23 software.

Our data showed that frequencies of both, rs10885122 and rs553668, variants were not significantly different between T2D and control subjects. However, rs553668 risk A allele appear to increase insulin levels ($p = 0.0002$) and HOMA-IR index ($p = 0.00001$). Furthermore, this variant also seems to affect VLDL levels ($p = 0.004$) and waist circumference ($p = 0.02$) in diabetic patients. The genotype analysis of rs10885122 variant demonstrated that risk G allele decreased HDL ($p = 0.0004$) and increased LDL levels ($p = 0.014$), as well as affected the waist circumference ($p = 0.02$) in diabetic patients. Interestingly, haplotype analysis demonstrated the association of rs553668A / rs10885122G with higher HOMA-IR index.

Here we demonstrated that although both, rs10885122 and rs553668, *ADRA2A* polymorphisms were not associated with T2D risk in our cohort, they were associated with markers of dyslipidemic perturbations and insulin resistance in diabetic patients. Further studies in larger cohorts are needed in order to explore these possible interactions and confirm our findings.

P-08.01.4-022
SIP1 regulates ROR1 in hepatocellular carcinoma cells

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Smad-Interacting Protein 1 (SIP1), also known as ZEB2 is a member of ZEB family transcription factors and was shown to regulate epithelial-to-mesenchymal transition, cell cycle, cellular senescence and cancer stemness. Bipartite zing finger motifs at amino and carboxyl termini of the SIP1 mediate its binding to E-box sequences in the genome. However, there are only limited data about SIP1 target genes. By using a home-made anti-SIP1 monoclonal antibody, clone 6E5, we conducted ChIP-seq in three hepatocellular carcinoma cell lines, namely SNU398, PLC/PRF/5 and SK-HEP-1 and found Receptor Tyrosine Kinase-Like Orphan Receptor 1 (ROR1) as one of the targets. SIP1 DNA-binding consensus motif CACCTG was found at +1 kb, +30 kb and +50 kb from ROR1 transcription start site. ChIP experiments validated SIP1 binding to all consensus motifs in the ROR1 gene region. Interestingly, the strongest enrichment was at +50 kb suggesting that long-range interactions play an important role in the regulation of ROR1 by SIP1. SIP1 knockdown by shRNA in high-SIP1 expressing SNU398 cells resulted in the repression of ROR1 expression. ROR1 is expressed in embryogenesis and fetal life, and is absent within most of adult normal tissues. However, overexpression of ROR1 was observed in many human cancers, from hematological malignancies to solid epithelial tumors. ROR1-positive cancer cells have enhanced proliferation, invasion and metastasis capacities, show resistance to apoptotic stimuli and display cancer stem cell characteristics. Therefore, SIP1 and ROR1 act in similar pathophysiological processes. Our finding that ROR1 is regulated by SIP1 at least in hepatocellular carcinoma cells adds another level of complexity to the molecular mechanisms of proliferation, invasion and stemness of cancer cells.

P-08.01.4-024
SIP1 and SIX1 are inversely correlated in hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is the most prevalent primary liver cancer and is one of the leading causes of cancer related deaths. SMAD interacting protein 1 (SIP1), a member of the ZEB family of EMT inducers, is involved in cellular proliferation, senescence, invasion and metastasis in human tumors. However, genes regulated by SIP1 in HCC are yet to be identified. We conducted a ChIP-Seq study in high-SIP1 expressing HCC cell line SNU398 by using a home-made anti-SIP1 antibody, clone 6E5. Among 509 annotated genes, we selected SIX1 for further studies because of its increased expression in multiple cancers and its association with poor prognosis. SIP1 DNA-binding motif CACCTG was found at -3 kb from transcription start site of SIX1 gene. ChIP qPCR experiment validated SIP1 binding to this region with 19,7 fold enrichment. Compared to healthy liver, SIX1 transcripts were upregulated in 8 of 9 HCC cell lines included in this study. Knockdown of SIP1 by shRNA in SNU398 cells caused upregulation of SIX1. Immunohistochemistry studies in HCC tissue arrays showed increased expression of SIX1 in tumors and inverse association with SIP1 expression in a tumor grade dependent manner. Therefore, our results strongly suggest an inverse correlation of SIP1 and SIX1 in HCC

P-08.01.4-025
Association of vitamin D receptor gene polymorphism with serum parathyroid hormone and vitamin D level in healthy women

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Bone mineral density (BMD) and bone turnover are under genetic control and variations in the Vitamin D receptor (VDR) are related to BMD. BMD is known to be affected by 25 –hydroxy vitamin D (25 (OH)D) and intact parathyroid hormon (iPTH) levels. We aimed to determine correlation blood levels of vitamin D (VitD), iPTH, and VDR gene effect in healthy Turkish women.

The subjects were 25 healthy women in age 35–57 years. The BMD was measured as a T score in the Lumbar spine (L2-L4) with DEXA. All subjects had normal T score between (-1.0 to 1.4) SD. VitD was measured by LC-20-AT Shimadzu. iPTH was measured by chemiluminescence method, DNA was isolated from blood. The FOK I (VDRF-FOKI) and BSMI (VDRB-BSMI) polymorphisms of VDR gene was analysed with Genomica clinical array system.

The mean VitD level was (21.06 ± 14.54) μg/l, mean plasma iPTH level was (57.96 ± 30.49) pg/ml. Pearson correlation test showed no relation of Vit D with BMD. There was moderately negative correlation between iPTH and BMD ($r = -0.3062$). Genotype distribution and allele frequency of subjects were as follows: 21(84%) FF), 1(4%) Ff, 3 (12%) ff genotype in VDRF - FOK1 gene, 7 (28%) BB, 14 (56%) Bb, 4 (16%) bb in VDRB-BSMI gene. Allele frequencies were F: 86%, f:14%; B:56%, b:44%. When FOK1 and BSMI were combined, 52%(FF-Bb) and 20% (FF-BB) were found as the most frequent genotypes. BSMI frequency was in Hardy Weinberg equilibrium ($p > 0.5$). But FOKI was not ($p = 0$).

It was found that Vit D, iPTH levels and BMD were in normal levels in all carriers of FF genotype and in combined (FF-Bb) type carrying healthy women (52%). The association VDR genotype and BMD may be different in various ethnic and geographical groups. Therefore it is worthwhile to assess VDR polymorphism among Turkish population. These type of distribution studies of VDR in healthy and in osteoporotic women may enlighten to earlier diagnosis and treatment planning.

P-08.01.4-026
Determination of Hb A1c values in beta thalassemia

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Introduction: Hemoglobinopathies are most commonly seen hereditary blood diseases worldwide. Our aim was to compare the HbA1c values measured on cation-exchange high performance liquid chromatography (HPLC) in beta thalassemia cases.
Materials and methods: We collected 3 ml of whole blood K_3EDTA containing tubes from forty-nine cases. ARMS, RFLP and DNA sequence analysis methodologies were carried out for

determination of beta thalassemia mutations. Hb A1c values were measured using the Agilent 1100 HPLC system.

Results: Forty-nine diabetic and non-diabetic patients were diagnosed with beta thalassemias: twenty-one IVS1-110/IVS1-110, one IVS1-1/IVS1-1, one IVS1-5/IVS1-5, two IVS1-6/IVS1-6, two IVS2-1/IVS2-1, one Fsc5/Fsc5, one Fsc44/Fsc44, two -30/-30, two Cd8/Cd8, one Cd36-37/Cd36-37, one Cd8-9/Cd8-9, two Cd82/Cd83-G/ Cd82/Cd83-G, two IVS1-110/ IVS1-6, one IVS1-110/ IVS2-1, one IVS1-110/Fsc44, one IVS1-110/Cd39, one IVS1-110/Cd8, one IVS1-110/Cd8-9, one IVS1-110/IVS1-5, one IVS1-6/ IVS2-1, one IVS1-6/ IVS1-25, one IVS2-745/Cd8 and one Fsc5/Cd37. Cases were classified as diabetic (6), prediabetic (11) and non-diabetic (32) according to Hb A1c values.

Discussions and conclusions: Disorders causing shortened red cell survival may result in false values for Hb A1c. This study highlights the potential erroneous valuations of Hb A1c in the population having high prevalence of beta thalassemia mutations.

P-08.01.4-027
Aurora A, Aurora B, Aurora C and BRAF kinases genes expression in urine samples from patients with prostate cancer

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Introduction: Members of Aurora kinase family Aurora A, B and C are conservative kinases of cell cycle which are encoded by genes *AURA*, *AURB* and *AURC* respectively. Overexpression of *AURA* and *AURB* was found in human cancers, especially in prostate cancer. Moreover, there is the evidence that AURB interacts with one of the major oncogenic kinases - BRAF. Little is known about implication of AURC in cancer, but it was demonstrated, that it can overlap AURB function and shares its location. We studied expression of genes of these kinases in urine of prostate cancer patients aiming to evaluate their involvement in this disease and their potential as tumor markers.

Materials and methods: 22 urine samples from patients with prostate cancer were gathered after prostate massage before surgical invasion. We used urine samples from 6 healthy men as control. We obtained cells from each urine sample by centrifugation and isolated RNA using standard approach with phenol and guanidine thiocyanate. cDNA was synthesized and taken to qPCR reactions. Data was statistically analysed.

Results: Expression of all studied genes was detected in urine of patients with prostate cancer and of healthy men. Expression of AURB and AURC in cancer samples each was higher than expression of AURA. The cumulative expression AURB and AURC was higher than expression of *AURA* in 13 samples from 22. We observed positive correlation between expression of AURC and BRAF ($rs = 0.548$, $p = 0.01$).

Discussion and conclusion: Previous investigation showed, that for normal prostate tissue 90% of Aurora family expression was presented by *AURA*. We suppose that presence of AURB and AURC cumulative overexpression means presence of cell cycle deviations in prostate tissue of these patients and might be further studied as prognostic marker. In this study we first showed the correlation between AURC and other carcinogenic kinase BRAF expression, which opens the perspective for investigation of role of AURC in carcinogenesis.

P-08.01.4-028
Understanding alkaliphilic adaptation of *B. marmarensis* sp. nov.

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Bacillus marmarensis sp. nov. is an extreme obligate alkaliphile isolated from mushroom compost near Marmara Region of Turkey. It can survive at extreme pH values up to 12.5. Based on its genome sequence, metabolic pathways for 7 proteases, 6 amylases, 2 cellulases, 1 lipase, n-butanol and a biodegradable plastic poly-β-hydroxybutyrate were annotated. In addition to being a potential extracellular hydrolase producer, its ability to survive in the high pH range of 8.0 to12.5 makes it an attractive microorganism for different industrial applications. In the current study, the adaptation strategy of *B. marmarensis sp. nov.* to alkaline conditions was investigated using proteomic tools. The organism was grown at two different pH values, 10.0 and pH 12.0. For extraction of whole cell proteins, cells were disrupted with MP Bio Fast Prep device. Protein extracts were treated with protease inhibitors and a nuclease mix. Salts were removed using a clean-up kit. Obtained proteins were separated based of their isoelectric points in the first dimension and then based on their molecular weights in the second dimension. Proteins maps of cells grown at these two extreme pH values showed significant differences in protein expression for alkaline adaptation.

P-08.01.4-029
Biochemical and proteomic analyses of normal human astrocytes and glioblastoma exposed to dichloroacetate treatment

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Glioblastoma (GBM) is an aggressive malignant tumor composed of astrocytes in brain tissue. GBM cells utilize glycolysis rather than oxidative phosphorylation to support rapid growth rate which is called Warburg effect. Dichloroacetate (DCA) is an antiglycolytic agent that inhibits pyruvate dehydrogenase kinase (PDK) activity and induces apoptosis via normalizing the mitochondrial activity. This study aimed to demonstrate the metabolic alterations between the normal human astrocytes (NHA) and GBM cell lines which are exposed to DCA, and to identify the differentially expressed proteins by MS-based proteomic analyses.

NHA cell line, U87MG and U373 as GBM human cell lines were examined through analyzing the alterations in the glycolysis metabolism upon DCA treatment by measuring the variations in the pyruvate levels, lactate dehydrogenase A, PDK3. MTS was performed to investigate the effect of DCA treatment on cell viability. Immunoblotting of PGC1-a, oxphos complexes, and mitotracker green staining was employed to reveal the mitochondrial differences between normal and the cancer cells, and upon DCA treatment of these cells. Proteomic analyses were utilized for the identification of candidate proteins depending on the acetylation status.

In this study, compared to NHA, the pyruvate and LDHA levels were elevated and PDK3 levels in U87MG were reduced by 14%. Due to MTS results, ≤10 mM DCA treatment showed significant decrease in GBM cells compared to NHA cells.

Immunoblotting and mitotracker green staining results showed increase in mitochondrial mass.

Elevation in the pyruvate and LDHA levels and reduction in PDK3 level in U87MG and U373 cells indicates glycolysis dependent metabolic switch in energy metabolism. Proteomic analyses demonstrate that most of the differentially expressed proteins comprised of metabolic enzymes. This study provides novel information about metabolic alterations existing between NHA and GBM, which can inspire further studies for therapeutic applications.

P-08.01.4-030
Kidney stone formation: gene expression profiling of vitamin D and calcium sensing receptors in rats after using ethylene glycol

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Kidney stone is a complex disease resulting from environmental as well as hereditary factors and principally composes of approximately 75% calcium oxalate (CaOx) crystals, which are formed through a multi-step process. Vitamin D receptor (VDR) gene encodes the nuclear hormone receptor for vitamin D3; downstream targets of this gene are chiefly contributed in mineral metabolism though the receptor regulates a variety of other metabolic pathways. Calcium sensing receptor CaSR plays an important role in sustaining mineral ion homeostasis. The aim of this study is to profile the expression level of VDR and calcium sensing receptor (CaSR) genes and to unravel their role in rat kidney stone induced by ethylene glycol, in order to explain the underlying molecular mechanisms.

Total RNA were extracted from paired sample before and after ethylene glycol treated of 50 rats. The mRNA expression level of VDR and CaSR gene were measured employing quantitative RT-PCR (qRT- PCR).

The mRNA expression levels of both genes were significantly down-regulated according to before treated.

In conclusion, our data suggest reduced mRNA expression in VDR and CaSR genes might be a risk factor for kidney stone formation. Further studies are necessary to verify these findings in different ethnic groups.

P-08.01.4-031
APJ receptor A445C gene polymorphism in Turkish patients with coronary artery disease

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Coronary artery disease (CAD) is a disease in which a waxy substance called plaque builds up inside the coronary arteries. Apelin is a novel endogenous peptide with inotropic and vasodilatory properties and is the ligand for the angiotensin receptor-like 1 (APJ) receptor. We aimed to determine genotype and allele frequencies of APJ receptor A445C gene polymorphism in Turkish patients with CAD and healthy controls by RFLP-PCR. This study was performed on 159 unrelated CAD patients and 62 healthy controls. We obtained AA, AC and CC genotype frequencies in CAD patients as 41.5%, 49.1% and 9.4%, respectively. In the control group, frequencies of genotypes were found as 35.5% for AA, 48.4% for AC and 16.1% for CC. We did not observe difference in APJ receptor A445C polymorphism between CAD patients and healthy controls ($\chi^2 = 2.178$; df = 2; $p = 0.336$). The A allele was encountered in 66% (210) of the CAD and 59.7% (74) of the controls. The C allele was seen in 34% (108) of the CAD and 40.3% (50) of the controls. Allele frequencies were not significantly different between groups ($\chi^2 = 1.57$; df = 1; $p = 0.225$). The frequencies of APJ receptor A445C genotype were not significantly different between control and patients. Individuals with CC genotypes had significantly higher weight, systolic and diastolic blood pressure levels and systolic blood pressure than other genotypes, $p \leq 0.05$. In addition, HDL-C level was found decreased, but this reduction was not statistically significant. Contrarily, the low levels of weight, SBP, DBP and TC were statistically significant in the subjects with AA genotype in CAD. In conclusion, CC genotype carriers may have more risk than other genotypes in the development of hypertension in CAD. We suggest that this polymorphism may not be a marker of CAD, but it may cause useful in function of the apelin/APJ system and may be a genetic predisposing factor for diagnostic processes and can be helpfull in finding new treatment strategies.

P-08.01.4-032
Comparative genomics/proteomics analyses of single amino acid repeat containing proteins across different vertebrate taxa

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Consecutive runs of single amino acids lead to overrepresentation of certain physicochemical properties in protein sequences. Researchers also demonstrated a link between single amino acid repeat (SAAR) containing proteins and neurodegenerative diseases as well as biological functions. Moreover, SAAR frequencies were shown to vary across species based on selected orthologous proteomes and/or proteins. Hence, analysis of whole proteomes across multiple vertebrate taxa may provide additional species- and sequence-specific trends for SAARs. In addition, there is a need for testing the observed SAAR occurrences

against different models of expected frequencies/counts to understand the evolutionary dynamics of SAARs in proteins.

We obtained from Ensembl the assemblies of genomes/proteomes of human and nonhuman primates (chimpanzee, gorilla, and rhesus monkey), rodents (mouse and rat), and birds (chicken and zebrafinch). The expected probabilities for the occurrence of SAARs based on their nucleotide frequencies in coding regions and amino acid frequencies in individual protein sequences or across the whole proteome were compared with the observed repeat occurrences.

We found that with all three methods and in all eight species the correlation between observed and expected repeat counts decreased above a SAAR length threshold. The percentage of SAAR proteins for each amino acid also exhibited variability among species when both the repeat length and counts were taken into account. However, clustering based on SAAR characteristics generally reflected the known phylogenetic relationships between species.

Our comprehensive bioinformatics analyses reveal that SAARs show amino acid-specific occurrence patterns with respect to species as well as SAAR length.

P-08.01.4-033
Comparison of gel-based proteomic analysis from fresh and FFPE kidney tissue

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Tissue proteins play important roles in biological metabolic processes. The qualitative and quantitative analysis of tissue proteins facilitates the understanding of molecular mechanisms that differentiate between physiologic and pathologic states. Health and research institutions routinely prepare formalin-fixed paraffin-embedded (FFPE) tissue blocks for histopathology. Proteomics on FFPE tissue still requires standardization of tissue solubilization processes to overcome variability in protein extraction results.

Our aim is to compare the proteomic studies of fresh frozen and FFPE rat renal tissues. Fresh frozen and FFPE preparations from renal tissues were included in this study. An adult rat was sacrificed and the dissected kidneys were divided two equal section. One immediately frozen in phosphate buffer, and the other tissue specimen not thicker than 5 mm to allow rapid penetration of the fixative put in 10% buffered formalin for 48 hours. The fresh frozen tissue was dissolved and homogenised in the cold phosphate buffer solution containing protease inhibitors. Paraffin blocks were performed from formalin fixed tissue specimens. We have extracted the protein from the FFPE tissues using our previously verified method.

We have utilized electrophoresis three times to compare protein yield, number, intracellular and intercellular of homogenised samples obtained from FFPE and fresh frozen kidney samples.

The number of proteins identified from fresh frozen kidney tissue has generally been shown to be increased compared with FFPE tissue. Decrease of the qualitative results in electrophoretic bands was found similar in all replicative studies.

FFPE tissues undergo extensive cross linking between protein/DNA/RNA molecules during formalin fixation, which creates inter-molecular crosslinks. On the other hand, FFPE tissues represent a valuable resource to carry out retrospective studies aimed to biomarker discovery in kidney cancer as well as other kidney diseases.

P-08.01.4-034
Comparative transcriptional changes in patients with chronic primary mitral regurgitation: Atrial fibrillation versus sinus rhythm

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Background: Development of atrial fibrillation (AF) during the course of chronic primary mitral regurgitation (MR) is common and represents complex molecular mechanisms. However, the gene expression profile of human atrial fibrillation (AF) in the setting of chronic primary MR remains uncharacterized. In the current study, we aimed to compare the gene expression profiles of patients with severe degenerative MR in sinus rhythm (SR) and AF.

Methods: Left and right atrial tissue samples were obtained from patients with chronic primary severe MR in permanent AF (n = 30) and sinus rhythm (n = 30). We performed a novel micro-dissection technique for thin sections of atrial tissue samples and immediately fresh froze intra-operatively. Transcriptomes of left and right atrial appendages of degenerative mitral regurgitation patients with SR versus AF were compared by microarray analysis on Affymetrix HGU-133 Plus 2 platform. Bioinformatics, data mining and pathway analyses were conducted on Partek GS and WebGestalt. Genome-wide gene expression profiles were compared between AF and SR groups among 54.675 transcripts representing 38.500 well-characterized human genes. Differentially regulated genes were evaluated according to fold change (FC $\geq$ 1.5) with a p-value $\leq$0.05.

Results: Most remarkable pathways altered in AF atrial tissues compared to SR group, were extracellular matrix-receptor interaction; MAPK, adipocytokine, and calcium signaling; apoptosis and cardiac muscle contraction pathways.

Conclusions: This is the first human study of comparative transcriptomics in left and right atrial tissues of patients with AF versus SR associated with severe degenerative MR. The main findings of this multidisciplinary translational research provide novel candidate targets for the treatment and prevention of AF.

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P-08.01.4-035
Genomic comparison of siderophore diversity in clinical and environmental isolates of *Serratia marcescens*

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In order to acquire iron under iron-limiting growth conditions, bacteria employ specific mechanisms such as production and secretion of siderophores. Siderophores are low molecular metal-chelating compounds that contribute not only to iron scavenging, but also participate in other important processes including oxidative stress response and cell signaling. *Serratia marcescens,* gram-negative bacterium, could be found in various environments, including wastewater, plant rhizosphere and hospital setting where *S. marcescens* can cause serious life-threatening infections.

In this study, we performed a detailed characterization of the siderophores of the clinically important pigment-free *S. marcescens* strain SR41-8000 and environmental pigment-producing *S. marcescens* strain SM6. Bioinformatic analysis of these genomes

by AntiSMASH software revealed the presence of several clusters involved in non-ribosomal peptides synthesis (NRPS). We found four NRPS clusters in genome of *S. marcescens* SM6. Cluster 1 has a low level of identity to enterobactin gene cluster typical for bacteria producing catechol-like siderophores. Second cluster has only 4% of identity to xantholipin biosynthetic gene cluster. Clusters 3 and 4 of NRPS genes of *S. marcescens* SM6 did not show any homology to known NRPS clusters. In contrast, the genome of *S. marcescens* SR41-8000 contains only one genetic cluster of NRPS genes. This cluster does not have similarity to any of the known bacterial NRPS genes.

Thus, genetic analysis of two isolates of *S. marcescens* allowed us to identify NRPS genetic clusters and showed that the repertoire of these genes is different between strains. We hypothesized that the strain isolated from environment has competitive advantage over clinical isolate due to genetic diversity of siderophores. On the other side, clinical isolate has specific genetic cluster of siderophores which may promote *S. marcescens* growth and adaptation to the extreme niches present in medical facilities.

P-08.01.4-037
The first glance on the genome's structure and activity in hibernator edible dormouse

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Hibernation is a unique adaptive way of survival in extreme environmental conditions where mammals decrease their metabolic rate and demonstrate physical inactivity for prolonged periods of time (up to 6–8 months). Remarkably, some hibernating animals have a long average lifespan and the ability to avoid muscle atrophy caused by disuse or immobilization. To identify main molecular pathways behind the protective musculoskeletal adaptation and genome structure in hibernator edible dormouse (*Glis glis*), whole-genome analysis of mRNA expression in muscles (m. soleus and m. EDL) and lumbar spinal cord samples was conducted.

Three groups of the dormice: 1) active animals 2) hibernated animals and 3) animals immobilized for 2 weeks in laboratory, were examined. RNA libraries have been sequenced using Hiseq 2500 Illumina platform.

Coupled with genome DNA sequencing provided x10 coverage of the estimated genome, we have assembled *de novo* transcriptome of the dormice. Differentially expressed genes in response to immobilization and hibernation were determined. Transcriptional program of these phenotypes was similar. Pathways enriched by differentially expressed genes were identified. Gene expression of the key muscle proteins and muscle atrophy markers was analyzed. Muscle-specific E3-ubiquitin ligases MuRF1 and MAFbx revealed no changes in mRNA expression.

Our study represents the first attempt to elucidate changes in transcription profiles of skeletal muscles and spinal cord during hibernation and hypokinesia in edible dormice. In corroboration to the gene expression data, they demonstrated minimal morphological evidence for muscle disuse atrophy during physical inactivity. Edible dormice, thus, can be considered as a novel model organisms in investigation of the genetic mechanisms of hibernation and prevention of muscle atrophy.

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P-08.01.4-038
Regulation of biofilm formation in bacilli

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In response to diverse environmental cues bacteria form complex structured communities called biofilms. The metabolic pathways activated by these cues are remarkably different depending on the species studied. However, they all lead to the formation of an extracellular matrix that holds the cells together. Non-pathogenic gram-positive spore-forming soil B. subtilis strain is recognized as a model system for the study of biofilms. To discover the pathways regulating biofilm formation in *B. subtilis*, we studied the natural isolate of *B. subtilis* strain *168*, and constructed the recombinant strains with knocked out genes of following regulatory proteins: AbrB (global transcriptional regulator), DegU (two-component response regulator of signal transduction system DegS- DegU), CcpA (regulator of carbon catabolism) and SpoOA (regulator of sporulation). In the minimal medium broth *B. subtilis 168* wild-type strain forms biofilm with its maximum on 48th hour of culture growth. pH-Optimum for biofilms formation by the wild-type strain is in the range of 7.4–8.0. The temperature optimum is in the range from 22 °C to 45 °C. This corresponds to the natural conditions of the *B. subtilis* habitat in rhizosphere. The level of biofilm formation by regulatory mutant strains with deleted *abrB, degU, ccpA, spoOA* genes is on average 40% lower than by the wild-type strain. This indicates that global regulatory system controlls biofilm formation process. Statistically significant differences in the levels of biofilm formation between regulatory mutants haven't been identified. pH and temperature optima of mutant strains are the same as for the wild-type strain – 7,4-8 and 22 °C – 45 °C respectively.

P-08.01.4-039
The prevention of DNA oxidative damage, inhibiton of acetylcholinesterase, tyrosinase, α-glucosidase and antioxidant activities of leaf, stem bark and fruit extracts from *Crataegus microphylla*

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The *Crataegus* genus which is a member of Rosaceae family, has approximately 200 species worldwide and 24 species in Turkey. All plant species in this genus have the common name "Hawthorn". *Crataegus microphylla (C. microphylla)* C. Koch which is characterised by having erect sepals in fruit and smaller leaves in comparison with the other species, is one of the wild edible fruits in Turkey. *Crataegus* species have been used as food and also in folk medicine for the treatment of various diseases. For this purpose, the potential biological properties of *Crataegus microphylla* were aimed to reveal by the preliminary work.

In this study, prevention of oxidative DNA damage using supercoiled pBR322 plasmid DNA, acetylcholinesterase, tyrosinase, α-glucosidase inhibition and antioxidant effects: 2,2-diphenyl-1-picrylhydrazyl radical scavenging effect, phosphomolibdenum-reducing antioxidant power, ferric-reducing antioxidant power with total phenolic and total flavonoid contents of the *C. microphylla* leaves, stem barks and fruits that extracted with ethanol, methanol and water were investigated.

The experiments of oxidative DNA damage studies and antioxidant activities of *C. microphylla* extracts showed that methanol and ethanol extracts possessed a strong ability to prevent DNA damage and significantly antioxidant activities.

Methanol extracts of stem barks from *C. microphylla* exhibited the highest acetylcholinesterase and tyrosinase activities (48.86 ± 4.06% and 85.57 ± 2.01%, respectively), at 200 μg/ml. In addition, ethanol extract of leaves from *C. microphylla* inhibited the α-glucosidase activity significantly when compared to acarbose.

This study explained significant antioxidant, enzyme inhibitory, hypoglycemic, and neuroprotective activities of methanolic or ethanolic extracts prepared with stem bark and leaf from *C. microphylla* and also strong ability to prevent DNA damage that corresponded to antioxidant potential of methanol extracts of leaf and stem bark.

P-08.01.4-040
Study of physiological regulation of the POR1 gene in the *Yarrowia lipolytica* yeast

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The *Yarrowia lipolytica* species (*Yl*) is nonconventional yeast widely used for recombinant protein expression due to its system of post-translation protein modification, which is the most similar to that of higher eukaryotes. *Yl* appears the promising producer of recombinant proteins with much more complicated molecules compared to those of prokaryotic producers. However, an important feature for a producer strain of recombinant proteins is the genes, the expression of which undertakes under controlled conditions, and consequently, search of new effective inducible promoters in the *Yl* genome is of great interest. Proteome analysis of the *Yl* cells grown at different pH values (4.0, 5.5, 9.0) showed that under alkaline conditions the amount of mitochondrial porine VDAC (voltage dependent anion channel), one of the most abundant protein of the mitochondrial outer membrane, increased significantly. VDAC is supposed to let reactive oxygen species out of mitochondria protecting the cell against oxidative stress. Therefore, the *POR1* gene expression, encoding VDAC should increase in the stress conditions. The promoter of the *POR1* gene was used to construct some new expression systems based on *Yl* W29. A new genetic construct bearing a reporter β-galactosidase gene under control of the *POR1* promoter. β-galactosidase activity was assayed in the cells grown in various pH conditions and exposed to exogenous oxidants such as hydrogen peroxide, menadione, and methyl viologen. It was shown, that in H_2O_2 and methyl viologen treated cells β-galactosidase activity increased 1.5–2.0 –fold reaching its maximum in the cells, grown at pH of 9.0. Thus, we demonstrated high inducibility of the *POR1* promoter, which is essential for effective action of the expression system based on it and potency of application for transformed lines of producers. Acknowledgments: Supported by the Russian Foundation for Basic Research (grant No 16-34-00634 mol_a).

P-08.01.4-041
Aspergillus nidulans is able to detoxify and catabolize the toxic proline analogue, L-azetidine-2-carboxylic acid

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L-Azetidine-2-carboxylic acid (AZC) is a toxic analogue of proline produced by members of the *Liliaceae* family and beat roots. In nature, AZC serves as a plant protectant against infections and consumption. We have obtained evidence that AZC is not only non-toxic for the model ascomycete *Aspergillus nidulans*, but it can be utilized as a poor nitrogen source. In order to elucidate the molecular mechanism underlying AZC detoxification, we have constructed and studied *A. nidulans* strains deleted in the cognate genes involved in AZC detoxification in *Pseudomonas* and *Saccharomyces cerevisiae*. These genes, found by *in silico* analysis, encode a putative hydrolase, *achA*, and an AZC acetyltransferase, *ngn2*, respectively.

Gene deletion was accomplished through double crossover. A cassette containing the ~1500 bp 5' and 3' flanking sequences of each gene, with the *AfpyrG* gene as a selection marker, was contructed. Crossing the *achAΔ* and the *ngn2Δ* strains isolated the *achAΔ ngn2Δ* double mutant strain. RT-PCR was used for gene expression analysis in the wild type strain, AreA-loss of function and CreA-derepressed mutant strains.

Our results clearly show that AZC can be used as a poor nitrogen source by *A. nidulans*. This utilization requires a) *achA*, a putative AZC hydrolase, and b) a fully active GABA catabolic pathway, as lack of either *amdR* or *gatA* abolishes AZC utilization. Most importantly, the double mutant, *achAΔ ngn2Δ*, shows AZC toxicity, suggesting that *ngn2* is a true orthologue of MPR1, able to detoxify AZC, a phenotype that can be observed only in the absence of *achA*. As a final point, *ngn2* was shown to be induced by the presence of AZC and and to be under Nitrogen Metabolite Repression (AreA-dependent), whilst the expression of *achA* is not subject to Nitrogen Metabolite and Carbon Catabolite Repression

P-08.01.4-042
First crystal structure of Serendipity ZAD-domain from *D. melanogaster* proved dimeric state of the protein

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The spatial genome organization plays a great role in the maintenance of the nuclear architecture and regulation of all processes occurring in the nucleus. This system is controlled by a set of special proteins having an architectural function. However, the mechanisms of their action remain unknown. Among these proteins are, in particular, ZAD-domain-containing proteins. Zinc finger-associated domain (ZAD) is a ubiquitous motif of C2H2 zinc finger proteins of *Drosophila*. Genes that encode ZAD proteins are specific for and expanded in the genomes of insects. Only a few ZAD-encoding genes have known functions, and the role of ZAD is being discussed. Up to date there was only one known crystal structure of ZAD-domain from *Drosophila* transcription factor Grauzone (GrauZAD).

Here, we present for the first time the crystal structure of the ZAD-domain of Serendipity-d transcriptional activator of the egg-polarity gene bicoid. ZAD-domain was cloned, overexpressed in *E. coli*, purified and the structure was solved at 3.5Å by MAD technique. Detailed analysis of the structure proved that the protein exists in dimeric form and revealed unique spatial organization of the protein, different from those for GrauZAD. This work is supported in part by Russian Ministry of Education and Science grant (14.616.21.0066).

P-08.01.4-043
Transcription regulation in *Mycoplasma gallisepticum* under different types of conditions

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Mycoplasma gallisepticum is a convenient model object for studying the regulation of transcription because it has a reduced genome, lack of cell wall and many metabolic pathways and also easy to culture and non-pathogenic to humans. Due to the nature of the genomic organization and the loss of many of the known regulators, the effect of disrupting the function of some proteins may be a useful tool for studying the regulation of transcription.

The gene expression study was performed on Agilent one-color microarray with custom design and random-T7 polymerase primer for cDNA synthesis. Microarray represents 3366 probes for 678 ORF including genes and ncRNA.

In this work, we have investigated the effect of changes in the level of gene expression of *M. gallisepticum* for two different types of conditions: a genetic knock-out mutants and the cell response to treatment with sub-lethal concentrations of antibiotics. We characterized transcription of *M. gallisepticum* when the cell responses to dysfunction of proteins with metabolic potential, possible regulators of expression, in violation of permeability of membrane by CCCP, inhibition of ribosomal synthesis by tetracycline, DNA gyrase by novobiocin and ATP synthase by oligomycin.

The data obtained allow to characterize the transcriptional response under different conditions and to identify groups of genes that change expression together. Major transcriptional changes were observed in the response of cells under CCCP treatment due to uncoupling of the proton gradient and further reducing the membrane potential, as well as under novobiocin treatment due to changing the topology of DNA.

P-08.01.4-045
Proteogenomic profile of the new alkane-oxidizing strain Tsukamurella tyrosinosolvens PS2 in relation to the emulsification activity

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Global problem of oil pollution forces scientists to search for a new safe remediation technologies constantly. Careful attention is paid to bacteria, some of which possess additional biotechnologically valuable properties, such as utilization of hydrocarbons and production of biofurfactants. In this regard, we carried out proteogenomic characterization of *Tsukamurella tyrosinosolvens* strain PS2, which was isolated from chemical sludge and capable for alkane degradation and biosurfactant production.

Whole genome of the strain was sequenced on the MiSeq (Illumina) platform, assembled and annotated. Proteome on mineral medium with glucose, sucrose and hexadecane as a sole carbon and energy source was studied. Shotgun proteomics approach was performed on hybrid chromatography-mass spectrometry machine (maXis Impact).

Alkane oxidation genes (alkane-1-monooxygenase, rubredoxin and rubredoxin-reductase) under genome sequence, as well as two pathways of trehalose synthesis and genes for mycolic acids production were found. Emulsification activity of cell-free culture liquid was about four times higher on hexadecane in comparison with sugars. Proteomic profile was different at various culture conditions. All glycolysis genes, beginning with glucose-6-phosphate isomerase to pyruvate kinase, were found on the media with sugar. The medium with hexadecane helped to reveal enzymes involved in the beta-oxidation of fatty acids, for example 2,4-dienoyl-CoA reductase, 3-ketoacyl-CoA thiolase and enzymes of the initial mycolic acid synthesis pathways.

Thus we have established that the strain *T. tyrosinosolvens* PS2 utilizes sugar by glycolysis. Also, the bacterium is capable for alkane oxidation followed by beta-oxidation of fatty acids. Based on the proteogenomic data, we assume that the bacterium is able to synthesize trehalose lipids, namely, trehalose mycolates. Obtained results could be useful to create conditions for increased biosurfactants production.

Wednesday 7 September 12:30–14:30

Personalized medicine

P-08.02.5-001
Serum adiponectin and resistin levels in gestational diabetes

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Gestational diabetes mellitus (GDM) is a glucose intolerance firstly diagnosed during pregnancy. In this study, we aimed to investigate the association between serum adiponectin, resistin levels and insulin resistance in gestational diabetic patients.

A total of 80 patients; 40 healthy pregnant women (control group) and 40 pregnant women diagnosed with GDM (GDM group) were included in this study. Serum adiponectin, resistin, glucose, insulin, HbA1c levels and lipid parameters were measured. Insulin resistance index HOMA-IR values were calculated.

In this study, serum glucose, insulin, HbA1c levels and HOMA-IR were significantly higher in GDM group compared to the control group ($p = 0.038$, $p = 0.011$, $p = 0.001$, $p = 0.008$, respectively). Serum adiponectin levels were significantly lower ($p < 0.001$); whereas serum resistin levels were significantly higher ($p = 0.004$) in GDM group than in the control group.

It can be concluded that resistin contributes to the formation of insulin resistance, adiponectin plays an important role in the regulation of this resistance and they also have effects on GDM pathophysiology.

Key words:*Gestational diabetes mellitus, adiponectin, resistin, HOMA-IR.*

P-08.02.5-002
Determination of chemoterapoetic drug sensitivity subgroups of acute leukemia

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Hematological cancers including Acute myeloblastic leukemia (AML) and Acute lymphoblastic leukemia (ALL) in terms of incidence and mortality, are the second most important cancer type in Turkey. Numerous studies show that cancer patients

respond differently to treatment thus supporting the idea of personalized therapy need for individuals.

Renin angiotensin system (RAS) have key roles in AML and ALL progression and it has been shown by many studies suggests that these system's genes might be good biomarkers for AML and ALL personalized therapy.

We aimed to identify RAS gene based homogeneous subgroups of acute leukemia and determine the most effective chemotherapoetic agent for each subgroup. After validation and verification of the results, more effective drugs can be recommended for the use in clinics for chemotherapy of AML and ALL.

Results of our preliminary studies showed that we are able to identify subgroups of AML and ALL as well as correlating each existing subgroup with FDA approved drugs. Considering the long and highly cost process of developing new drugs for cancer treatment makes the present study all the more valuable. In addition, there is a serious need for change in AML and ALL therapy since there is no highly effective chemotherapy protocol available for their treatment.

Welcome Trust Sanger (WTS) and Cancer Cell Line Encyclopedia (CCLE) databases will be used to determine subgroups of AML and ALL based on RAS genes or whole genome expression using standard deviation and hierarchical clustering analysis. The most effective drugs for each subgroup will be identified using Pearson's r correlation analysis with drug sensitivity data (IC50, IC50, Amax, Aare, etc.) available in same databases. Further validation tests will be performed by in vitro validation using AML and ALL cell lines: drug sensitivity profiles will be determined and gene expression will be shown by Q-RT-PCR.

P-08.02.5-003
Functional polymorphisms of EPHX2 in a Turkish population

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Soluble epoxide hydrolase (sEH; EC 3.3.3.2) is encoded by EPHX2 and catalyses the degradation of endogenous fatty acid epoxides generated by CYP450 epoxygenases. These fatty acid epoxides such as epoxyeicosatrienoic acids (EETs) have been shown to posses vasodilator, anti-inflammatory, anti-platelet, anti-hypertensive, anti-apoptotic, anti-thrombotic and natriuretic effects. It has been reported that EET levels are associated with hypertension, stroke and cardiovascular diseases. Individual differences in the EPHX2 gene that affect the sEH activity may alter the circulating levels of EETs. K55R and R287Q polymorphisms have been known to cause increased and decreased sEH activity, respectively. Therefore we aimed to determine the genotype frequencies of these two polymorphisms in a Turkish population. K55R and R287Q polymorphisms were determined by the real time PCR using double-dye hydrolysis probes or PCR-RFLP method. The observed genotype frequencies for K55R polymorphism were 80.8% wild type (AA) and 19.2% polymorphic genotype (AG+GG) and for R287Q polymorphism 81.4% wild type and 18.6% polymorphic genotype (GA+AA). The genotype distributions for both polymorphisms were in Hardy–Weinberg equilibrium.

P-08.02.5-004
Frequencies of alleles and polymorphic variations of genes for folate cycle in women of Kazakh ethnic group with complicated pregnancy

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Pregnancy is one of manifestations for thrombophilia factors, which in its turn leads to various complications of its course. One of the markers of hereditary thrombophilia is mutations in the folate cycle MTR, MTRR and MTHFR genes. Insufficient intake of folate during pregnancy disrupts the functioning of the genome, leading to miscarriage, violation of embryogenesis and various fetal malformations. However, results of studies on the role of hereditary thrombophilia in the occurrence of complications during pregnancy are rather contradictory.

Aim of this study was to determine the frequency of alleles and polymorphic variants of folate cycle genes MTR A2756G, MTRR A66G and MTHFR C677T in women of Kazakh ethnic group with pregnancy complications. We used Real-time PCR. Blood samples for DNA isolation were obtained from 129 pregnant women. The main group consisted of women (n = 90) which had a history of two or more pregnancy complications in the form of pre-eclampsia, eclampsia, missed abortion, miscarriage, and etc. Control group consisted of women (n = 39) with two or more normal pregnancy outcomes, and had no complications during pregnancy in history. Average age of women in experimental group was 32.0 ± 0.50 years compared with control of the age 33.6 ± 0.33.

The analysis of the frequency distribution of alleles of genes in experimental group of women with complications of pregnancy revealed no significant differences relative to the control group. Analysis of the distribution of polymorphic variants of folate cycle genes showed significant difference between the study and control groups in the occurrence frequency of heterozygotes for the mutant allele G in the gene MTRR A66G (OR = 2.89, CI 95% = 1.25–6.71; $\chi^2 = 6.376$, $p < 0, 05$). No significant differences in alleles between homozygous wild-type and homozygous mutant alleles were observed.

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P-08.02.5-005
A study on the association between rs6918698 polymorphism in connective tissue growth factor gene and pseudoexfoliation syndrome

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Pseudoexfoliation syndrome (PES) is a disorder of the extracellular matrix characterized by the production and progressive accumulation of an abnormal fibrillary material in many ocular tissues. PES prevalence is 11.3% above the age 40 in Turkey. Since PES is characterized by excessive synthesis of elastic microfibrillar components throughout the body, growth factors can have important roles in the pathophysiology of PES. Human Connective Tissue Growth Factor (CTGF) is a protein expressed

in a variety of tissues, including the anterior chamber of the eye. CTGF coding gene has several genetic polymorphisms. rs6918698 G/C single nucleotide polymorphism (SNP) is found at position −945, in promoter region. The presence of a C allele for rs6918698 is critical for transcriptional suppression of the CTGF gene which would reduce CTGF production. Aim of this study was to investigate if there is any association between PES and rs6918698 polymorphism of the CTGF gene.

Study population consisted of 60 patients with PES and 60 controls. Blood samples were collected by Gülhane Military Medical Academy, Department of Ophthalmology, Ankara, Turkey. Genotypes were assigned by PCR followed by restriction fragment length polymorphism analysis. Genomic DNAs were isolated from whole blood samples using manual DNA isolation.

The frequency of CTGF rs6918698 polymorphic allele G was 0.442 in patients, and 0.450 in controls (0.967, p = 1.000). Distribution of genotypes was GG: 31.7%, GC: 48.3% and CC: 20.0% among patients, while GG: 35%, GC: 40.0% and CC: 25.0% (OR = 1.162, p = 0.689) in controls. Statistical analysis showed that there is no significant relationship between CTGF rs6918698 SNP and PES.

These are the preliminary findings of a research project which is the first study analyzing the relationship between CTGF rs6918698 SNP and PES. This work did not point out a role for CTGF rs6918698 in the risk for PES. A significant relationship might be found when the study population is enlarged.

P-08.02.5-006
Evaluation of rs11136000 single nucleotide polymorphism of clusterin gene in pseudoexfoliation syndrome risk

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Pseudoexfoliation syndrome (PES), an age-related systemic disorder, is characterized by production and accumulation of abnormal fibrillar extracellular material in anterior structures of the eye. Clusterin (CLU) is a multifunctional glycoprotein produced and secreted by almost all cell types and is found in all body fluids and in accumulated PES material. Under cellular stress conditions, CLU provides inhibition of stress-induced precipitation and aggregation of misfolded proteins. CLU expression level in PES patients is unexpectedly low and this could be due to single nucleotide polymorphisms (SNP) on the gene coding for CLU. rs11136000 C/T polymorphism has been found to be associated with Alzheimer's disease and pathophysiology of Alzheimer and PES are similar. This study aimed to determine whether rs11136000 SNP of *CLU* gene have a role in the development of PES.

Study population consisted of 60 patients with PES and 60 controls. Blood samples were obtained from Gülhane Military Medical Academy, Department of Ophthalmology, Ankara, Turkey. Genomic DNAs were isolated from whole blood of subjects using manual DNA isolation. Genotypes were assigned by PCR followed by restriction fragment length polymorphism analysis.

T allele frequency of PES patients was 0.425 and that of controls was 0.442 (0.934, p = 0.794). The distribution of genotypes was CC: 30.0%, TC: 55.0% and TT: 15.0% among patients while CC: 28.3%, TC: 55.0% and TT: 16.7% (0.922, p = 0.841) in controls. There was no statistically significant difference between PES patients and controls in terms of TT genotype and T allele frequency.

These are the preliminary findings of a research project which is the first study analyzing the relationship between *CLU* rs11136000 SNP and PES in Turkish population. This work did not point out a relation for polymorphic genotype in the risk for PES. However, a relationship between *CLU* rs11136000 polymorphism and PES can be found when we enlarge the study population.

P-08.02.5-007
The relevance of TP53 mutational landscape and its regulatory network in HPV negative HNSCC patients

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The tumor suppressor TP53 is the most frequently mutated gene in head neck squamous cell carcinoma cancer and represents a known transcription factor and tumor suppressor gene that regulates different microRNA and target genes. The aim of our work is to construct the transcriptional and post-transcriptional network regulated by TP53 and to evaluate the difference at mRNA and protein expression levels of the TP53 target genes in HPV negative head and neck squamous cell carcinoma (HNSCC) patients with distinct TP53 mutation states and to elucidate the molecular mechanism that underlie the poor prognosis of TP53 mutation.

To show the TP53 mutation landscape and its prognostic relevance for survival, we used cbioportal for cancer genomic analysis. We downloaded mutational profiles of 243 HPV negative HNSCC patients. Employing different databases we constructed the TP53 regulatory network. And then, to evaluate the effect on mRNA, protein and microRNA regulated by TP53 we used the mRNA and protein expression profiles of patients from TCGA.

Our results show that hotspot, truncating and missense mutations have statistical significance in the univariate analysis. The TP53 regulatory network show the involvement of important target involved in the progression of HNSCC and the deregulation of protein expression of an important key epigenetic modifier EZH2 was significantly associated with TP53 mutational state.

EZH2 is a member of the polycomb group protein enhancer zeste homolog 2 which is known to be directly repressed by TP53 and indirectly by the activation of miR-200a and miR-200b. We found a significant up-regulation of EZH2 that depend from TP53 mutation.

It is important to understand the difference in mRNA and protein expression of TP53 regulatory network that could depend from its mutational state. This finding suggest that EZH2 might be a potential therapeutic target for HNSCC.

P-08.02.5-008
Next generation sequencing based approach for monitoring of minimal residual disease in acute lymphoblastic leukemia

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Minimal residual disease (MRD) monitoring is widely used to evaluate efficiency of chemotherapy and to choose a strategy for further treatment in acute lymphoblastic leukemia (ALL). The most commonly used approaches for MRD detection are based on flow cytometry and qPCR. These methods have several important limitations including insufficient sensitivity, complicated experimental setup and false positivity. The newly developed next generation sequencing (NGS) approaches could overcome the existing limitations in MRD monitoring.

Here we describe a new MRD monitoring approach based on targeted deep sequencing of malignant rearrangements. First, we identified BCR/TCR rearrangements specific for the leukemic clones in initial bone marrow samples of 10 ALL patients. For this, we used Sanger sequencing of the products of 19 multiplex PCR, performed with BCR/TCR specific primers combined according to the optimal frequency distribution of V/J-genes in healthy donors. Second, we analyzed concentration of malignant clone rearrangements, identified at the first step, in DNA samples obtained from bone marrow after 36 days of treatment. For this purpose, we performed NGS of 10 libraries for each identified leukemic rearrangement. Four libraries were amplicons of BCR or TCR gene rearrangements generated using characteristic V and J segment specific primer combination. Six additional libraries were amplicons of the same primer combination from the same DNA sample which contained initial leukemic DNA spike-in (in concentrations corresponding to 1 per 100, 1000 and 10,000 cells) for a calibration curve generation. Using this approach, we analyzed 7 ALL clone specific rearrangements for three patients and calculated concentration of the leukemic clones by using the calibration curve. For one patient we didn't find any leukemic cells and for two patients we found 1 leukemic cell per 100,000 analyzed cells.

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P-08.02.5-009
Association of variability in the ZNF365 gene with BC in Kazakh population

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ZNF365 is considered as a transcriptional target for *p53* and plays an important role in the homologous recombination, mitosis, centrosome dynamics. As was shown by GWAS some SNPs in *ZNF365* have strong association with the risk of breast cancer (BC). However, it was unclear whether the same SNPs are associated with risk of BC in Kazakhstan. Therefore two polymorphisms (*rs10995190*, *rs10761659*) of *ZNF365* were investigated in Kazakh population in this study.

The present case–control study was carried out with participation of 444 Kazakh females with BC and 390 cancer-free donors. Additionally, subtypes of BC, stratified by estrogen receptor (ER+/−), progesterone receptor (PR+/−) and human epidermal growth factor receptor 2 (HER2+/−) status were estimated. Pearson p-value, odds ratio, 95% confidence interval tests were applied to data analysis.

Significant differences were found in allele frequency and genotype distribution at *rs10995190* locus in *ZNF365* between the patients and control groups ($p = 0.013$ for allele; $p = 0.007$ for genotype). Moreover, significant association with BC was revealed for *rs10995190* after dividing patients according to ER+/−, PR+/− and HER2+/− status of the tumor. The G allele was associated with ER+ ($p = 0.01$, OR = 1.69, 95%CI:1.11–2.56), PR+ ($p = 0.01$, OR = 1.78, 95%CI:1.13–2.79) and GG genotype with HER2- BC carriers ($p = 0.02$, OR = 1.66, 95%CI:1.04–2.63). Also, G allele can be considered as a risk factors in ER+/PR+/HER2- luminal type of tumor ($p = 0.028$, OR = 1.79, 95% CI:1.06–3.05).

Our findings correlate with the data of several GWAS where the association of the *rs10995190* polymorphism with higher mammographic density and the risk of breast cancer have been shown.

The obtained results allow us to consider G allele and GG genotype of *rs10995190* as a marker of BC risk with predictive value, restricted to ER, PR and HER2 status of the tumor in the Kazakh population.

P-08.02.5-010
Polymorphism combinations in four different genes in Kazakhstan population

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Breast cancer (BC) is the most common cancer among women in most of countries. Alternative variants of low-penetrance genes such as *FGFR2* (*rs2981582*), *TOX3* (*rs3803662*), *MAP3K1* (*rs889312*), *LSP1* (*rs381798*) are shown to have high frequency in North America, South-East Asia, Australia, Europe populations and a multiplicative effect on the development of BC. In this study was investigated assosiasion between alleles/genotypes combinations of these genes with increase/decrease of BC risk.

The case–control study included 625 BC patients and 672 healthy women from Kazakh and Russian populations. Genotyping was performed by PCR-RFLP methods. Combined effect of allele and genotype variations in four different genes on BC risk was assessed by APSampler algorithm. The Fisher exact test, odds ratio (OR) with 95% confidence intervals (95% CI) were applied to data analysis.

According to obtained results combinations of allele C of *TOX3 rs3803662* and A of *MAP3K1 rs889312* ($p = 0.006$, OR = 2.2), also allele C of *TOX3 rs3803662* and C of *LSP1 rs381798* ($p = 0.02$, OR = 1.47) associated with increased BC risk in the Russian population. Consequently, combinations with C allele of *TOX3 rs3803662* contribute significantly to BC risk with p-value = 0.04, OR = 1.86. On the contrary, TT genotype of *TOX3 rs3803662* with $p = 0.04$, OR = 0.53 and its combination with allele T of *LSP1 rs381798* with $p = 0.03$, OR = 0.47 determine a BC risk reduction in Russian population. In addition, a risk combination of allele C of *LSP1 rs381798* and A of *MAP3K1 rs889312* was found in Kazakh population ($p = 0.02$, OR = 1.35).

Studies have shown that a genetic predisposition to BC can be determined by the cumulative effect of individual alleles and

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genotypes and possible epistatic interactions of studied genes. Obtained combinations of alleles and genotypes can be considered as complex genetic markers of BC and may be used as predictive.

P-08.02.5-011
Anticancer properties of new metal complexes derived from a polydentate azo-schiff base

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Cancer that is caused by excessive proliferation of cells and reduced apoptosis is a pathological condition. Currently, studies that are comitted with breast cancer are great important early detection and diagnosis of breast cancer. After the discovery of cisplatin as chemoterapy drug, new transition metal based complexes have been developed for treatment of cancer.

In this study, anti-cancer activity of azo-azomethide ligand and its mononuclear metal complexes is studied on human cancer cell lines (MCF-7) and mouse fibroblast (L929) cell lines. Cells were studied four different concentrations (12,5; 50; 75; 100 μM). XTT (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) protocol was applied after 24 and 48 hours. In our study 10% fetal bovine serum (FBS), 1% L-glutamine, 100 IU/ml penicillin and 10 mg/ml streptomycin in DMEM (low glucose) were used. cancer cell lines in DMEM medium is produced in 95% humidity and 5% CO_2 incubator at 37 °C.

Viability of control cells has been accepted as 100%. IC50 values calculated for MCF-7 at 24 and 48 hours. IC50 values for [Ni-as(eda)]. H_20 at 24 and 48 hours for MCF-7 (21.82; 20.56) and for L929 (46.67;44.00); for as-(eda) at 24 and 48 hours for MCF-7 (23.09;21.06) and for L929(44.91;40.50); for Co-aseda at 24 and 48 hours for MCF-7 (45.09;20.08) and for L929 (46.19;39.81); for Zn-aseda at 24 and 48 hours for MCF-7 (20.37;21.31) and for L929(49.46;41.90); for Cu-aseda at 24 and 48 hours for MCF-7 (25.65;62.20) and for L929(49.89;42.24); for as at 24 and 48 hours for MCF-7 (21.63;20.87) and for L929 (46.29;38.50).

Anti-cancer activity of synthesized complexes were determined on MCF-7 and L929. In the biological activity studies, synthesized compounds showed higher anticancer activity than positive control (5-FU). Finally, our new synthesized complexes can be suggested that potent ajan for anti-tumuour for breast cancer drugs.

P-08.02.5-012
Could clinical-pharmacogenetic models facilitate treatment selection in malignant mesothelioma?

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Large interindividual differences in response to chemotherapy present an important issue in cancer treatment. Malignant mesothelioma (MM) is an aggressive tumor with poor prognosis, treated mostly with gemcitabine/cisplatin (GEM/CIS) or pemetrexed/cisplatin (PMX/CIS) chemotherapy. As both clinical characteristics and genetic variability may affect treatment outcome, our aim was to construct and validate clinical-pharmacogenetic prediction models of treatment outcome in MM for both chemotherapy regimens and to develop an algorithm for genotype-based treatment recommendations.

Clinical-pharmacogenetic models were built on 71 GEM/CIS-treated and 57 PMX/CIS-treated MM patients. Pharmacogenetic scores were assigned by rounding the regression coefficients. GEM/CIS model was validated on 66 independent MM patients.

Model predicting outcome of GEM/CIS chemotherapy included CRP level, histological type, performance status, *RRM1* rs1042927, *ERCC2* rs13181, *ERCC1* rs3212986, and *XRCC1* rs25487. Values ranged between 0 and 3.4; cutoff value of 0.75 had sensitivity of 0.62 and specificity of 0.81. Patients with higher score had shorter progression-free and overall survival ($p < 0.001$). In the validation group, positive predictive value was 0.74 and negative predictive value was 0.56.

Model predicting outcome of PMX/CIS chemotherapy included CRP level, *MTHFD1* rs2236225, and *ABCC2* rs2273697 with scores ranging between 0 and 3.9. Cutoff value of 2.7 had sensitivity of 0.75 and specificity of 0.61. Patients with higher score had lower probability of good response and shorter progression-free survival ($p < 0.001$).

Clinical-pharmacogenetic models could enable stratification of MM patients based on their probability of response to GEM/CIS or PMX/CIS and improve treatment outcome. This approach could be used for translation of pharmacogenetic testing to clinical practice as it would facilitate the selection of the best treatment option for each patient.

P-08.02.5-014
Evaluation of anti-diabetic potential of circiliol and circilineol using CACO2 cell line

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Diabetes mellitus is a metabolic disorder, and many people are suffering from this disease in the worldwide. Oral hypoglycemic agents such as sulfonylureas and biguanides are currently used for the treatment. However, studies searching for a more effective anti-diabetic agents are being carried out continıously. Based on that we aim to investigate the potential anti-diabetic effects of circilineol and circiliol *isolated from Teucrium alyssifolium* extract, using in vitro cell culture models. For this purpose, the anti-diabetic actions were investigated by applying model Caco2 (colorectal adenocarcinoma) cell line. We determined the level of AG (alpha-glucosidase), SGLT1 (sodium-glucose transporter-1) and GLUT1-5 and glucose transport. Neither AG activity nor SGLT1 activity was increased with either circiliol or circilineol treatment in CaCo2 cells compared to positive control. Similarly, neither the activity nor the expression level of GLUT1*-5 was increased in Caco2 cell line with either circiliol or circilineol treatment relative to control. In conclusion, these results strongly suggest that circiliol and circilineol do not possess any anti-diabetic potentials.Supported by TUBITAK 114Z640 and PAUBAP 2014FBE029

P-08.02.5-015
Fe3O4 nanoarchitectures functionalized with eugenol modulate virulence, biofilm formation and quorum sensing molecular signaling in *Pseudomonas aeruginosa*

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The purpose of this study was to characterize and assess the impact of a novel magnetite (Fe3O4) nanosystem functionalized with the natural origin compound eugenol (E) on the *Pseudomonas aeruginosa* virulence, biofilm formation and QS signaling in order to advance research aimed to find alternative and personalized therapeutic approaches for severe infections produced by this opportunistic pathogen.

Fe3O4 nanoparticles were obtained by a co-precipitation method and functionalized with analytical purity E. Functionalized nanoparticles (Fe3O4@E) were characterized by IR, SEM, TGA and HR TEM. One laboratory and 9 *P. aeruginosa* clinical isolates were utilized in the study. Growth and biofilm formation were assessed by an adapted microdilution method followed by absorbance reads and viable count analysis in dynamics (6, 12, 24 and 48 hours of treatment). Soluble virulence factors production was assessed by enzyme activity evaluation of bacteria grown on specific media. The expression of QS core genes was analyzed by qRT-PCR and a luminescence assay.

Results demonstrated that the average size of the obtained nanosystem ranges 5–9 nm, particles are relatively homogenous and have a low tendency to form aggregates. Subinhibitory concentrations of Fe3O4@E limited biofilms formation in a time and strain dependent manner, and significantly inhibited the production of toxin pore forming enzymes (haemolysins and lipases) in most strains. The expression of *lasI* and *lasR* genes was three fold downregulated, while the expression of *pqsR* was upregulated in planktonic cultures suggesting that pqs signaling may be involved in virulence modulation after nanoparticle stimulation.

The modulation of bacterial virulence and molecular signaling by functional nanoparticles utilized in subinhibitory amounts offer valuable perspectives to develop personalized antimicrobial approaches based on molecular communication control that clearly modulate pathogenicity and progression of the infectious process.

P-08.02.5-016
Specimen processing and handling for plasma ammonia measurement

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Objectives: Ammonia requires special processing and handling conditions due to its' unstability. In this study, our aim to investigate a preanalytical factor (delayed analyze) affecting plasma ammonia measurement.

Design and methods: Blood samples were obtained from 20 healthy volunteers. For determining different handling and storage conditions, the following protocols were applied: First protocol (a) transportation on ice and separation (centrifugation at 0 °C) within 15 minutes of collection and analyze immediately. Second protocol (b) transportation on ice and separation (centrifugation at 4 °C) within 30 minutes and analyse refrigerated 2–8 °C 2 hours (a1, b1) and 4 hours (a2, b2). All plasma ammonia levels was analyzed enzimatic glutamate dehiydrogenase methods by Abbott Architect C 8000 Clinical Chemistry Analyzer.

Results: There were statistically alterations in all protocols compared to first protocol. Prolonged centrifugation time for plasma ammonia lead to have higher results (38.6 versus 29.75 μg/dL, $p < 0.001$). In all protocols including a1, a2, b1, b2 also cause an elevation in plasma ammonia results (35.7, 37.4, 39.5 and 41.2; $p = 0.032$, $p < 0.001$, $p < 0.001$, $p < 0.001$, respectively).

Conclusions: Ammonia concentration in the blood sample increases over time due to high concentrations in cells as erythrocyte or platelets (three fold). Blood samples collected for ammonia determination should be stored on ice, and measured immediately.

Key words: Ammonia, stability, specimen processing:

P-08.02.5-017
CTNNB1 alterations in tissues of patients with colorectal cancer

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The Wnt/b-catenin signaling pathway has been considered to be a factor in the development and progression of colorectal cancer. Many studies have demonstrated that the presence of mutations or polymorphisms in CTNNB1(gene encoding b-catenin; mostly mutations in exon 3) can lead to aberrant activation of Wnt/b-catenin signaling at the onset of various types of malignancies, including colorectal cancer. The aim of our study was to assess CTNNB1alteration in the patients with colorectal cancer and compared their tumor and normal tissues.

A total of 30 paraffin-embedded colorectal tumor specimens were obtained from department of pathology in Cerrahpasa Medical Faculty. Also a total 30 paraffin-embedded normal tissue was used from same cases as a control group. Ten-micrometer-thick tissue sections were placed on a glass slide and stained with HE. Then the tissue sections were dehydrated in graded ethanol solutions and dried without a cover glass. DNA was extracted from the tissues with 100 μl of extraction buffer at 55 °C over night. The tubes were boiled for 10 min to inactivate the proteinase K. CTNNB1 exon 3 was amplified by PCR. SSCP is used to observe any difference between the 2 groups. Genomic DNA was isolated as described above. 10 μl of each PCR products mixed with 10 μl denaturating buffer and were denatured by heating at 95 °C for 10 minutes in, and then were rapidly cooled on ice. The denatured PCR samples are run on 12% acrylamide/bis gel in 0.5× TBE buffer for 3.5 hours at 200 V at room temperature with water cooling system. The gel was stained with silver staining method.

Migration and adhesion involve continuous modulation of cell motility, beta-catenin play major roles. Beta-Catenin gene alterations frequency range between 0 and 16% in colorectal cancer according to the different published studies. In our study no significant differences were found in the CTNNB1 exon 3 between the tumor group and normal groups.

P-08.02.5-018
Theranostic approach in agressive recurrent meningioma – first experience in Turkey

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Meningiomas arise from the meningothelial cells of the arachnoid membranes of the leptomeninx, which are attached to the inner layer of the dura mater. Meningiomas can be classified into three grades (I–III): grade I meningiomas which are benign, exhibit slow growth; grade II (atypical) and grade III (anaplastic) meningiomas, which have a much more aggressive clinical behaviour. Meningiomas express non-steroid hormones, including somatostatin. In the brain, somatostatin-a cyclic tetradecapeptide neuropeptide-is believed to act as a neurotransmitter and neuromodulator. Somatostatin performs its physiological functions by binding to specific receptors (SSTRI-SSTRV). SSTR2 exhibit high affinity for octreotate (Tate). Tate is a polar, water-soluble peptide that does not penetrate the intact BBB (Brain-Blood Barrier). PET and scintigraphic imagings can only demonstrate somatostatin receptor positive intracranial lesions if the BBB is disrupted.

In this aim, DOTATATE (DOTA-DPhe1, Tyr3-octreotate, Tate) has been labelled with the positron emitter ^{68} Ga and the beta and gama emitter ^{177}Lu.

In this case, we conducted a study to evaluate Peptide Receptor Radionuclide Therapy (PRRT) planning based on PET/CT imaging of meningioma in the Department of Nuclear Medicine and Molecular Imaging at the Amerikan Hospital. [^{68} Ga] DOTA^{0}-Tyr^{3}-OC-PET/CT has been established as the imaging modality of choice for the diagnosis and management of patient with skull-base malign meningioma (rapid progress -to 41 × 48 × 52 mm. from 31 × 37 × 35 mm. in 20 d.-after fifth operation). Due to its high SSTR2 selectivity, [^{177}Lu]-Tate showed significantly lower uptake/dose delivered to normal tissues, GaTate-PET represents the imaging strategy of choice for an accurate assessment of SSTR2 expression levels. Although some studies have not shown a clear advantage over PET/CT, there is some evidence that it will have an advantage in selected body sites such as the head and neck, liver, and the pelvis.

P-08.02.5-019
Cardiovascular diseases can be treated by using 'TetR-ODD-VP16' and 'HRE' hypoxia inducible systems

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Ischemia is an insufficient supply of blood to a tissue or organ, usually due to a blocked artery by a blood clot. Up to now, the number one cause of death worldwide is caused by ischemia and related conditions such as heart attack or stroke. HIF-1α is a transcription activator that functions as a master regulator of oxygen homeostasis. HIF-1α protein levels increase under hypoxic conditions as a result of decreased O2-dependent prolyl-hydroxylation, ubiqutination and degradation.

We aimed to break up clots in blood vessels and to prevent damage caused by ischemia by using hypoxia inducible systems. We added oxygen dependent degredation domain (ODD) of HIF1α between and in front of TetR DNA binding domain and VP16 transactivation domain, so that TetR-ODD-VP16 or ODD-TetR-VP16 could activate transcription of tissue plasminogen activator (tPA) controlled by tetracycline response element (TRE), in a HIF1α independent manner. In addition, we also designed tissue plasminogen activator (tPA) under control of hypoxia response element (HRE) of HIF1α target genes.

Western blotting and immunofluorescence assay results showed the expression and nuclear localization of TetR-ODD-VP16 and ODD-TetR-VP16 constructs under hypoxic conditions, but not normoxic. In addition, using fluorometric reporter systems and tPA enzymatic assay we proved functionality of these constructs under hypoxic conditions. Final apporoach to our project is predicting kinetic enzymatic activity of tPA during break up blood clots by using MATLAB.

The results of the present investigation showed, the developed hypoxia responsible systems that can be engineered into endothelial cells to prevent ischemia related cardiovascular diseases.

P-08.02.5-020
Osteogenic potential assessment of some original scaffolds with magnetic properties

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New advances in bone tissue engineering demand the development of materials that can not only replace bone, but also regenerate the damaged tissue based on external or even internal stimulus. Magnetic materials inside bone scaffolds are known to be a promoting factor for bone healing especially when the therapy is accompanied by application of external magnetic stimulation. Based on a recent report, the presence of iron oxide in hydroxyapatite can improve the radio opacity and osteoblast proliferation. In this view, this study focuses on the development of silk fibroin-magnetite biocomposites for potential uses in bone tissue bioenegineering. Such novel composites possess good mechanical properties, biocompatibility and biomineralization potential by in vitro tests and could become smart arhiectures, able to stimulate bone regeneration.

A new culture model was developed by exposing a 3D cell/scaffold bioconstruct to a continuous magnetic field during 3 weeks of osteogenic induction. In this view, MC3T3-E1 murine osteoblastes progenitor cells were seeded inside the novel silk fibroin-magnetite biocomposites and subjected to osteogenesys in a magnetic field during 21 days. Osteogenic specific markers were evaluated every week in the presence and absence of the field.

Our results showed that the osteogenic marker's expression started earlier when MC3T3-E1 cells were exposed to the magnetic field.

Consequently, in our experimental model, the magnetic field had a benefic effect on the osteogenic differentiation process as MC3T3-E1 cells differentiated more efficiently in its presence.

These results suggest that the bone healing process could be improved in the presence of a magnetic field. Nevertheless, further in vivo studies on animal model should be employed for validation.

P-08.02.5-021
Impact of physical activity performed on different times of day on cardiac and skeletal muscle damage in trained and untrained male subjects

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Introduction: Physical activity elevates creatine kinase (CK) and creatine kinase myocardial band (CK-MB) levels which have been considered to be an indirect marker of skeletal and cardiac

muscles damage. Purpose: Impact of physical activity performed on different times of day on serum levels of CK and CK-MB were investigated in trained and untrained male subjects.
Materials and methods: Trained (n = 10, 18.3 ± 0.1 yr, 61.4 ± 2.3 kg) and untrained (n = 10, 18.6 ± 0.1 yr, 63.2 ± 2.4 kg) subjects performed three soccer matches (60 min) in field (30 m versus 50 m) in morning (M), afternoon (A) and at night (N) on separate days. The study protocol was approved by the Local Ethics Committee. Venous blood samples were taken at onset and at end of match. Serum CK and CK-MB levels are measured using autoanalyser. Data are expressed as mean ± S.E., compared by Wilcoxon-signed rank and Mann–Whitney *U*-tests. $p < 0.05$ was accepted as statistically significant.
Results: CK and CK-MB levels increased in three matches in both groups ($p < 0.05$). Importantly, there were significant increases in CK-MB levels in A and N exercises compared to M exercise ($p < 0.05$) in trained (10.6 ± 1.6 U/l versus 14.2 ± 1.9 U/l, 35% (M) 9.7 ± 1.3 U/l versus 18 ± 2.6 U/l, 66% (A) 9.5 ± 1.1 U/l versus 16.3 ± 2.0 U/l, 103% (N) and also untrained groups (8.6 ± 0.8 U/l versus 11.5 ± 0.8 U/l, 42% (M), 7.3 ± 0.5 U/l versus 11.9 ± 0.9 U/l, 85% (A) 7.9 ± 0.9 U/l versus 13.8 ± 0.9 U/l, 76% (N). Discussion: Increased metabolic stress or muscle damage during physical exercise elevate serum CK and CK-MB levels. However, higher percentage of increase in CK-MB levels in A and N exercise may reflects additional increases in cardiac muscle stress despite the similar skeletal muscle stress as indicated by CK levels.
Conclusion: Considering the observation of higher percentage increase in CK-MB levels in untrained and also trained subjects, the caution should be taken while performing an exercise in A and N time especially in subjects who has cardiac weakness.

P-08.02.5-022
Regional assessment of hematological and discrimination indices of complete blood count for beta-thalassemia screening

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Beta-thalassemia is one of the most common genetic abnormality causing health problems worldwide. Blood count and film of beta thalassemia trait and iron deficiency anemia have similar features. Therefore, several simple screening indices have been developed for differentiating between these diseases. It was to asaimed to assess the hematological parameters and discrimination indices in patients with betathalassemia trait who admitted to our hospital.

The parameters of complete blood count (CBC) in 141 subjects (51 males and 90 females) diagnosed by mutational analysis (PCR, gene amplification, DNA sequencing) between 2013 and 2015, were retrospectively screened and the thalassemia status of patients was assessed in terms of discrimination indices (England&Fraser (EF), Green&King (GK), Mentzer (M), Ricerca (R), Shine&Lal (S-L), Srivastava (S), Ehsani and Sirdah).

The percentages of being above the cut off value were detected by EF 29.72%, GK 30.4%, M 26.35%, R 6.75%, S-L 8.78%, S 35.13%, Ehsani 25.67% and Sirdah 29.72%. The percentages of falsely negatives for the indices of Ricerca and Shine&Lal were lower than others. Morever, when the first three common mutations of our study were considered, 5 out of 23, 7 out of 21 and 4 out of 21 patients were up to the cut off values in terms of E&F, G&K, M, S, Ehsani and Sirdah indices for IVS-I-110 (G>A), IVSII-1(G>A) and heterozygous codon 8 deletion (-AA), respectively.

The molecular diagnosis and prenatal detection for families at risk is important because of the difficulties of treatment in this disease. However, the use of discrimination indices may be valuable for distinguishing of thalassemia trait from iron defiency anemia when the equipment of molecular diagnosis are limited. In our study, Ricerca and Shine&Lal had lower falsely negative results than others. Nevertheless, further studies to detect diagnostic perfomance of discriminant indices should be conducted.

P-08.02.5-023
Novel therapeutic agents in the development of effective drug combinations to treat glioma

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Glial tumors are driven by multiple molecular aberrations that cannot be controlled by a single targeted agent. So, it is possible to expect that the combined multitarget anti-cancer therapy aimed simultaneously at different elements of tumor formation mechanisms will be more effective and will promote the extension of patients' life.

To find out which drug combinations will enable the development of therapeutic regimens with improved effectiveness and decreased toxicity, the cytotoxic effects of several bradykinin antagonists (BA) were analyzed for different glioblastoma (GB) cell lines. Among all the BA under investigation, BKM-570 appeared to be the most effective, with IC50 values of 4 μM and 3.3 μM in rat glioma C6 and human glioblastoma U251 cell lines, respectively. BKM-570 suppressed ERK1/2 and AKT1 phosphorylation in U251 cells. Temozolomide (TMZ), the first-line anti-gliomic drug used in clinics, has only a temporary positive effect and severe side effects in GB patients. We showed that the combination of BKM-570 and TMZ led to significant potentiation of TMZ cytotoxicity at sub-therapeutic concentrations. Recombinant proteins with cytotoxic properties are promising agents for complex therapeutic applications. We revealed that the glioma-associated protein CHI3L2 inhibited the viability of U251 cells more effectively than TMZ. Furthermore, the combination of CHI3L2 and BKM-570 resulted in an additive cytotoxic effect. CHI3L2-mediated decrease of cell viability was associated with a G1/S transition arrest. CHI3L2 provoked the dramatic reduction of pRB phosphorylation and a significant decrease of cyclin D1 expression, as well as a substantial increase in p53 level. In addition to the accumulation of p53, we observed the upregulation of CDK inhibitor p21. Therefore, G1/S arrest in CHI3L2-treated cells could be realized via activation of pRB, downregulation of cyclin D, and activation of p53.

P-08.02.5-024
Mutation analysis in BRCA1 and BRCA2 genes by Sanger sequencing and determination the relation between breast cancer in a small Turkish population

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Harmful hereditary mutations in *BRCA1* and *BRCA2* are one of the most important risk factors of breast cancer. The aim of this study is to determine the mutations which are associated with breast cancer in people which is diagnosed breast cancer and/or

have breast cancer-diagnosed family members by Sanger Sequencing and thus provide predictive and prognostic utility.

Our ongoing study is present the genetic variations in *BRCA1* and *BRCA2* genes in breast cancer-diagnosed 12 patients, that one of them is male, and 1 person yet healthy whose *BRCA2* was sequenced by Sanger Sequencing. The Data were analyzed by using SeqScape Software 2.6 and detected variations were compared with literature.

In *BRCA1*, we determined 16 different benign genetic variations and 1 variation with unknown significance and 1 variation which has not in literature. In *BRCA2* gene of 12 patient and 1 healthy person,14 benign variations,2 variations with unknown significance,7 variations which has not in literature and 1 mutation were determined. This mutation is c.3189-3192delGTCA and is located in OCCR. c.9257-74A>C variation in *BRCA2* gene, was determined in only male patient.c.32T>A variation in Exon2 of *BRCA2*, was observed in only the youngest patient who has no family member with breast cancer and healthy person. While this variation takes place in literature as variation with 'uncertain clinical significance', an *in silico* program Mutation Taster speculated as 'disease causing' for this variation. Also, almost all of variations with 'unknown significance' literature knowledge were determined in only one and different cases. This situation increases the possibility of being pathogenic of this variations. The our findings until now can contribute to variations with uncertain clinical significance in the literature. Also the variations that have not in the literature but we suggest the possible relation with breast cancer as an estimate may be added to literature by expanding the study.

P-08.02.5-025
Inhibition of the recombinant human butyrylcholinesterase with paraoxon and coumarin analog of soman

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Organophosphorus compounds (OP) represent a class of extremely toxic chemicals that are used as warfare agents. Uncontrolled utilization of OP is highly dangerous due to their high potential to be efficient poisons in terrorist attacks. Current therapy of OP poisoning include intravenous administration of atropine and acetylcholine reactivators however, it does not completely eliminate brain damage effects. Alternative experimental therapy against OP poisoning is utilization of bioscavengers that irreversibly react with OP and rapidly inactivate them. Recombinant human butyrylcholinesterase (rhBChE) is one of the most promising candidates as bioscavenger due to its pharmacokinetic characteristics and broad spectrum of OP neutralizing activity. Here we investigated in vitro inhibition of rhBChE with two model OP – pesticide paraoxon (POX) and coumarin analog of soman (GD_C). Both OP lead to rapid and irreversible inactivation of rhBChE that was monitored using Ellman assay and fluorescence measurements. Bimolecular inhibition rate constants dramatically differ between POX and GD_C that could be explained by steric hindrance in soman analog. The next steps forward creation of catalytic bioscavengers based on rhBChE should be done based on mechanisms of OP-rhBChE interactions. This work was performed in frame of grant RFMEFI60414X0069.

P-08.02.5-026
Non-Hodgkin's lymphoma B-cell receptors agonists screening based on reporter cells approach

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Non-Hodgkin lymphomas (NHLs) represent a heterogeneous group of malignancies that arise from the lymphoid system. At the present time exist a lot of drugs for the NHLs therapy, but mostly all of them are unsafe and there is no consensus regarding the best treatment protocol. To increase the efficacy and safety of therapeutic B-lymphocyte depletion in lymphomas and leukemia's it would be preferable to induce the death of pathological B cells without affecting normal B cells to prevent side effects. Similar to other types of cancer, NHLs arise by a multistep accumulation of genetic aberrations that induce a selective growth advantage of the malignant clone. All B-cells of organism have a unique cell surface marker – antigen B-cell receptor (BCR).

We generate novel approach for personalized non-Hodgkin lymphomas therapy based on peptide specific to malignant cells surface receptor. For this purpose we designed new lentiviral peptide library screening technique based on fluorescent reporter cells system. Herein 7aa peptide library was used for screening of NHL's malignant receptor agonist. Patients' lymph nodes biopsy samples mRNA was used as a source of malignant BCR nucleotide sequence. Variable domain of the lymphoma BCR was used for chimeric receptor generation, where BCR VH/VL part responsible for agonist recognition and bottom part of receptor was retranslate signal to the reporter gene. In this embodiment of the method, very large numbers of candidate 7aa peptides expressing lentivirus and eukaryotic reporter cells are packaged together in a format where each is capable of replication, thereby forging a direct link between genotype and phenotype. After four rounds of screening we discover peptides specifically interacted with malignant BCR's. Selected peptide ligands were fused with chimeric antigen receptor for expression on T-cells. Modified T-cells selectively eliminate NHL's malignant cells ex vivo.

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Wednesday 7 September 12:30–14:30

Chemical and biochemical aspects of oxidative stress

P-09.04.4-001
Chlorpyrifosethylene and rose water effects on oxidant and antioxidant parameters in rat kidney tissues

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Introduction: Pesticides are used to prevent damage of unwanted insects, rodents, plant, moss and other pests. Excessive use of pesticides may cause adverse effects in animals and humans. Chlorpyrifosethylene (CPE) is an organophosphate pesticide, used in many agricultural products such as figs, cherries, olives worldwide; caused acute poisoning and chronically oxidative stress. *Rosadamascena Mill* (Rosaceae) is a rose, used for

production of rosewater (RW) and rose oil worldwide. Rosaceae products are consumed in food and cosmetic industries.

Materials and methods: In this study, we investigated that CPE and RW effects on kidney tissues of rats. This study was included 32 adult male rats, divided into 4 groups. Each group included 8 rats. I.group: control (regular feed), II.group: CPE (0.3 mg/kg/day), III.group: RW (100 mg/kg/day) and IV.group: CPE (0.3 mg/kg/day) + RW (100 mg/kg/day). Following 15 days, kidneys were taken after sacrificed. Analyzes were performed that malondialdehyde (MDA), nitric oxide (NO) as oxidant; superoxide dismutase (SOD), glutathione reductase (GR) as antioxidant parameters.

Results: As compared with control, MDA and NO levels in CPE were a significant increase was determined (p

Conclusion: CPE is shown that significant increase on oxidant parameters, but significant decrease on antioxidant parameters. RW occurs opposite situation. Similarly results of CPE, RW + CPE increased oxidant parameters, but decreased antioxidant parameters. These changes are lower than only CPE. These results showed that positive effects both RW and RW + CPE increasing on antioxidant parameters, also decreasing on oxidant parameters.

P-09.04.4-002
Redox state and zinc homeostasis of red blood cells of patients with metabolic syndrome

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We provide the comparative analysis of the reduced glutathione (GSH), reactive oxygen species (ROS), α-tocopherol levels, an intracellular labile Zn^{2+} pool and esterase activity of red blood cells of patients with diagnosed components of the metabolic syndrome (MS) – arterial hypertension and diabetes mellitus type II (Ah^+DM^+). As the comparison groups were selected patients with one diagnosed component of MS – arterial hypertension (Ah^+DM^-) or without any diagnosed component of MS (Ah^-DM^-). Patients of all investigated groups were at the hospital treatment with a diagnosis coronary heart disease (CHD) II degree.

Human blood was obtained from normal donors and patients with CHD II stage. Cytosolic esterase activity was assessed using Calcein-AM test. ROS level was evaluated using CM-H_2DCF-DA. GSH level was estimated using Lowry method. An intracellular labile Zn^{2+} pool was assessed using FluoZin-3-AM. Investigations were performed on the Specord M40, HPLC system LC-20 Prominence (Shimadzu) and FACSCantoII (BD).

A significant decrease of the intracellular level of labile Zn^{2+} in erythrocytes of patients with Ah^+DM^+ compare with Ah^+DM^- and Ah^-DM^- was shown. This fact confirms our assumption concerning the important role of zinc homeostasis in the etiopathogenesis of diabetes mellitus type II. A direct relationship between the intracellular Zn^{2+} level modification and erythrocytes esterase activity of patients with CHD II degree was observed. Moreover, in Ah^+DM^+ group of patients this relation was more marked. The unidirectional alteration in the erythrocytes redox state (GSH and α-tocopherol levels reduction, ROS formation activation) was revealed at the whole of investigated CHD patients groups (Ah^+DM^+, Ah^+DM^-, Ah^-DM^-). However, the pathological erythrocytes response on in vitro action of the different antioxidants (N-acetylcysteine, ascorbic acid, α-tocopherol, quercetin) had a diverse character that can be a significant test under antioxidant therapy prescription.

P-09.04.4-003
Long-lasting stress, quantitative changes in nitric oxide concentration and functional state of heart muscle cells

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It is known that long-term social isolation and the disorder of natural circadian rhythm is considered an important stress factors, which cause a variety of metabolic and mental disorders. It should be noted that the impact of the stresses takes up a larger area, according to, review of the action of their mechanism is one of the topical issues of modern science. It is estimated that as a result of stress the metabolic processes change in the organizm. Because of this, we've studied the functionality of the antioxidant system in laboratory rat heart muscle cells and blood under psycho-emotional stress. It was found that quantitative changes of nitric oxide (NO) was initiated the process of LPO, which caused oxidative stress in the cells and decreased antioxidant enzymes activity, such as catalase,SOD, GPx and GR. The results suggested that psycho-emotional stress was accompanied by oxidative stress, causing a reduction in the intensity of energy metabolism in cardiac muscle cells, which was further strengthened by the fact that the activity of the enzymes involved in ATP synthesis in mitochondria was reduced. Also, we've studied exogenous creatine positive and negative affects on energy metabolism and blood lipid spectrum. Based on this, we proposed that psychological stress is one of the factors contributing to the development of various cardiac diseases.

P-09.04.4-004
Photosensitized destruction of sphingosines and their analogues

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The importance of free radical lipid transformations, which differ from the peroxidation processes, was pointed out for the first time in our laboratory studies. We have found that ROS can induce free radical destruction processes of sphingolipids with C-C-bond cleavage [*Lipids, 2011, 46:271–276; Lipid insights, 8, 1–9*]. In case of acylated sphingolipids, they can undergo decomposition with C-C-bond cleavage upon direct UV irradiation [*Photochem. Photobiol., 2012, 88:899-903*]. It was of interest to establish the possibility of photosensitized decomposition reactions of not acylated sphingolipids, which do not absorb an ultraviolet.

In this work we studied photosensitized reactions of sphingosines containing a free amino group, and low molecular compounds, which simulate their structure, such as aminoalcohols (serinol). As photosensitizers, the salts of transition metals, hydrogen peroxide, and acetone were used. Oxygen was removed by bubbling with argon to reduce the probability of side reactions during photolysis of sphingolipids, such as oxidation processes (including oxygen reactions with alkyl radicals).

We have shown that the action of UV-radiation on aminoalcohols and sphingosines in aqueous solutions in the presence of photosensitizers induces their destruction with C-C bond rupture. The main carbonyl product of sphingosines free radical destruction was an unsaturated aldehyde - 2-hexadecanal. It was found, that 2-hexadecenal possesses a wide spectrum of biological activity: it promotes reorganization of the cell cytoskeleton and modifies the redox state of the cells [*FEBS Journal 282 (Suppl. 1), Abstracts: Mem. Biol. S5, Lipid Signaling & Dynamics, P12-029,*

p. 235.]. The results of this study can expand the frontier of research regarding free radical lipid damage, which could contribute to a better understanding of the origins of diseases associated with the activation of free radical processes in living organisms.

P-09.04.4-005
Structural basis for the 14-3-3 protein-dependent inhibition of apoptosis signal-regulating kinase 1

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Protein kinase ASK1 (apoptosis signal-regulating kinase 1) is a member of the mitogen- activated protein kinase kinase kinase (MAP3K) family that plays a crucial role in immune and stress responses. The activity of ASK1 is regulated through homo-oligomerization and interaction with other proteins including the 14-3-3 protein which binds to the phosphorylated motif located at the C-terminus of the kinase domain of ASK1 and suppresses its catalytic activity through unknown mechanism. Under stress conditions, ASK1 is dephosphorylated at Ser966 and the 14-3-3 protein dissociates. This dissociation is then one of the factors that lead to the activation of ASK1.

We performed low-resolution structural analysis of the kinase domain of ASK1 (ASK1-CD) bound to 14-3-3 using chemical cross-linking, analytical ultracentrifugation and small angle X-ray scattering.

The low-resolution structural analysis shows that ASK1-CD binds to the 14-3-3 protein in two to two stoichiometry through a small binding interface involving surface of 14-3-3 outside its central channel and several regions from the C-lobe of ASK1-CD. The complex is dynamic and conformationally heterogeneous. Phosphorus NMR and time-resolved fluorescence measurements, together with low-resolution structural analysis, indicate that binding of ASK1-CD to 14-3-3 modulates conformation of ASK1's activation segment. These results suggest that the 14-3-3 binding suppresses the catalytic activity of ASK1 through direct structural modulation of its activation segment.

Our study provides new insight into the interaction between the kinase domain of ASK1 and 14-3-3 and offers a plausible structural explanation for the 14-3-3 protein-dependent inhibition of ASK1 kinase activity.

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P-09.04.4-006
Thymoquinone induces ROS-dependent glioma cells growth inhibition through PI3K/Akt pathway

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Introduction: Thymoquinone (2-methyl-5-isopropyl-1,4-benzoquinone, TQ) exerts a great antitumor activity against different types of cancer cells. A growing body of evidence indicates that reactive oxygen species (ROS) generation followed by modulation of the AKT and MAPK pathways is a general mechanisms underlying the TQ antitumor action. However, the data of TQ effects on the MAPK pathway are conflicting. To date, the activation or inhibition of the MAPK protein family seems to depend on the cell type and on the TQ concentration used. In order to elucidate the antitumor potential of TQ against gliomas and the underlying molecular mechanism, TQ influence on C6 rat glioma cells functioning was studied.

Results: It has been shown that the cultivation of C6 cells with TQ in concentrations of 10 – 100 μM during 24 hours strongly inhibits cell proliferation and induces cell death with ID_{50} of 60 μM. At the same time, TQ induces ROS generation and intracellular GSH depletion in a dose-dependent manner, that is followed by the mitochondrial potential decrease. Interestingly, ROS production has only cytoplasmic, but not mitochondrial origin in cells challenged with TQ at the concentrations up to 100 μM. Two-electron reduction of TQ by DT-diaphorase attenuates TQ anticancer efficiency whereby inhibition of DT-diaphorase by dicumarol increases TQ-induced C6 cell death by 25 %.

We analyzed MAPK pathways involvement in C6 cells growth inhibition at TQ treatment. It has been shown that inhibition of the ERK pathway by PD98059 and JNK pathway by SP600125 does not influence on TQ-induced effects. On the contrary, the specific phosphoinositide-3-kinase (PI3K) inhibitor (LY294002) abrogates TQ-induced growth arrest.

Conclusion: Antitumor effects of thymoquinone on C6 glioma cells is a result of ROS generation and intracellular GSH depletion, that is followed by mitochondria disfunction, and growth arrest via PI3K pathway.

P-09.04.4-007
Lipid peroxidation parameters and antioxidant defence in women with HIV-mono and HIV-coinfection

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Assessment of oxidative stress and antioxidant defense activity parameters in patients with HIV-infection is of great importance, especially for HIV-positive women of reproductive age planning to have children.

Data of 53 women of reproductive age with HIV-infection analyzed: patients with HIV-monoinfection (n = 26) and patients with co-infection (HIV and hepatitis B and / or C) (n = 27). As a control we used the data of healthy women (n = 28). Serum and hemolysate used as material for the study. LPO-AOD products were determined by spectrophotometric and fluorometric methods.

Average value of initial LPO products - diene conjugates was significantly increased in the group with HIV-co-infected compared to control (1.57 times; $p = 0.001$) and group with HIV-monoinfection (1.4-fold; $p = 0.027$). The level of secondary products - ketodienes and conjugated trienes increased in patients of both groups compared to control (2.16 times ($p = 0.0002$) and 2.43-fold ($p < 0.0001$), respectively). At the same time isolated double bonds and TBA-active products content showed no significant changes ($p > 0$, 05). Total antioxidant activity parameter decreased 1.28 fold ($p = 0.0273$) in the group with HIV-monoinfection compared to control. Decrease in activity of the primary antioxidant enzyme - superoxide dismutase ($p = 0.0077$, compared to the control and $p = 0.0035$, compared with the group with HIV-monoinfection) and the level of α-tocopherol (1.22-fold to control and 1.25 fold to HIV-monoinfection) was detected in

the group with HIV-coinfection. 1.29-fold higher content of retinol in HIV-coinfection group (compared to the control) revealed.

In women with HIV-coinfection the oxidative stress was significantly higher than in women with HIV-monoinfection. Suggested to include antioxidant supplements in the complex pathogenetic therapy in women with HIV-coinfection (HIV and hepatitis B and / or C), which will contribute to women's ability to bear children.

P-09.04.4-008
Acute different doses of malathion induce cholinesterase inhibition, glucogenic enzymes and histopathological change in rat liver

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Malathion, which is an organophosphorus compound, is a widely used pesticide all over the world. Despite its benefits, Malathion has many toxic effects on many tissues including liver. We designed to evaluate the acute different doses of Malathion on cholinesterase (ChE) inhibition, gluconeogenic enzymes and histopathological change of rat liver. For this purpose 4 groups were formed. Rats in Group 1 served as control group animals which were only given corn oil. Group 2, Group 3 and Group 4 were administered 100, 200, 400 mg/kg of Malathion, respectively, dissolved in corn oil by oral gavage. The rats were sacrificed after 24 hours following administration and the livers of rats were removed. The liver ChE, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) were studied using autoanalyzer. Histopathological investigation was performed using microscope. The liver ChE activities of Group 2, Group 3 and Group 4 were inhibition percentage of 53%, 43%, and 51% respectively, comparison of Group 1's ChE activity. The liver ALT, AST and LDH increased in Group 3 and Group 4 compare to Group 1 and Group 2 ($p < 0.013$). We also observed that there was vacuolar and hydropic degeneration in liver of Group 4. According to our result, acute administrations of Malathion result in hepatotoxic effects with increasing doses.

P-09.04.4-009
Oxidative stress assessment in patients with acute myocardial infarction

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Background and aims: An imbalance between free radicals and antioxidants is closely linked to the onset of an acute myocardial infarction (AMI). The aim of this study was to investigate the antioxidant status and the lipid peroxidation in patients admitted to hospital immediately after AMI.

Methods: The study population comprised 19 patients with AMI and 14 healthy subjects. Patients that had an acute myocardial infarction (AMI) less than 72 hours since onset were selected for this study. Antioxidant status was assessed by lactonase activity of paraoxonase (PON1 DHC), Trolox equivalent antioxidant capacity (TEAC) and plasma uric acid level. Malondialdehyde was used as marker of lipid peroxidation.

Results: Compare with the control group, the levels of MDA and PON1 DHC were significantly higher in group with AMI ($p < 0.005$, respectively $p < 0.001$). Elevated levels of MDA ($p < 0.005$) were found in patients with AMI compare with the control subjects. AMI patients had also statistically significant reduced levels of TEAC ($p < 0.005$) comparative with healthy subjects. No statistically significance was found for plasma uric acid level in subjects from our study.

Conclusion: A high lipid peroxidation correlated with a decreased TEAC activity suggest an exacerbated oxidative stress in subjects admitted to hospital immediately after an AMI.

P-09.04.4-010
Dealing with copper toxicity: New insights into copper detoxification in yeast

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Copper (Cu), an essential metal, is a double-edged sword, as its essential nature is counterbalanced by the toxic effect that it can exert on cells when not properly controlled. As such, organisms have evolved defence mechanisms against Cu toxicity, and in the yeast Saccharomyces cerevisiae, the transcription factor Ace1 orchestrates several of those mechanisms, by activating Cu-detoxification genes.

In S. cerevisiae iron (Fe) uptake is partially dependent on Cu, as the membrane multicopper-oxidase Fet3, which is part of the high-affinity iron uptake system, requires Cu as a cofactor. Aft1, the low iron-sensing transcription factor in yeast, is known to regulate the expression of FET3 gene. However, we found that a strain lacking FET3 is more sensitive to Cu surplus conditions than a strain carrying the AFT1 gene disrupted. This finding suggests that under such conditions another regulator came into play and controls FET3 gene expression. We next evaluated whether Ace1 could be the alternative regulator of FET3 under Cu excess. To test this hypothesis we first constructed the double mutant aft1ace1 and assayed its fitness under Cu surplus. As expected, the double mutant is much more sensitive to Cu than the single aft1 or ace1 mutants. We next monitored the expression of FET3 gene in cells lacking ACE1, using yeast-one hybrid and qRT-PCR approaches. Our data clearly indicates that FET3 expression is dependent on Ace1 when Cu is overly abundant.

Altogether our data unveil a novel mechanism of Cu detoxification relying on the activation of FET3 by Ace1 in an Aft1-independent way. Experiments to understand the consequences of this regulation in terms of Cu detoxification are currently being undertaken.

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P-09.04.4-011
Cellular oxidative processes relevant for articular degenerative pathologies modulated by an active extract from small sea fish

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In joint degeneracy, reactive oxygen species manifest their toxicity both through intrinsic reactivity and the inflammatory process activation, leading to cartilage dysfunctions, injuries of matrix proteins and cytokines stimulation.

The study is focused on the identification of oxidative balance modulation (enzymes and oxygen free radicals) by a bioactive extract obtained from small sea fish. The cellular support was represented by the chondrocyte line CHON-001 derived from human long bones (ATCC® CRL-2846™), that assure the reproducibility of a standardised biological system. The oxidative stress was induced through stimulation with IL1β, a cytokine-factor that promotes the protein catabolism and also with TNFα, the initiator of pro-inflammatory activation, combined with PMA, the activator of protein-kinase C, triggering of oxygen reactive species generating cascades. The antioxidant effect was compared with known antioxidants: Vitamin C, ω3 fatty acid, N-Acetyl –Cysteine.

The acellular antioxidant/antiradical screening was done using two complementary techniques for total antioxidant status evaluation and completed by measuring cellular catalase (CAT) and superoxide – dismutase (SOD) activity, correlated with intracellular hydrogen-peroxide and superoxide anion monitoring through flow cytometry.

The antioxidant properties of the bioactive extract proved in acellular systems are confirmed at cellular level by the involvement of the product in the enzymatic cascade CAT –SOD, potentiating the catalytic action of the enzymes, and by the decline of both intracellular reactive oxygen species (the hydrogen peroxide decrease with 50%, the superoxide anion is reduced with 31% compared with the stimulated control).

The demonstrated antioxidant synergy assures a complete cellular protection induced by the small sea fish extract in human condrocytes.

P-09.04.4-012
DNA susceptibility to oxidation and glutathione redox status in patients with Alzheimer's disease

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Introduction: Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by memory loss, cognitive impairment. Oxidative stress is a contributory factor in AD pathogenesis. Glutathione (GSH) is the main antioxidant cellular defence. The ratio of GSH/GSSG shows the redox status of GSH, and plays important role in maintaining intracellular redox homeostasis. The current study was carried out to determine oxidative DNA damage and ratio of GSH/GSSG which plays an important role in protection of target molecules from oxidation in the patients with AD.

Methods: The study subjects were consisted of patients with AD (n = 67) and age matched control group (n = 42) who were treated and followed in the Cerrahpasa Medical Faculty Hospital, Department of Neurology and Department of Internal Medicine/Geriatrics. DNA strand breaks and H_2O_2-induced DNA damage were determined in lymphocyte DNA with comet assay. The GSH and GSSG levels in the erythrocyte lysates were measured by using a commercial spectrophotometric kit. The ratio of GSH/GSSG were calculated. Statistical analysis was performed with SPSS 22 software package.

Results: DNA strand breaks and H_2O_2-induced DNA damage were found to be higher ($p = 0.000$ for all), the ratio of GSH/GSSG was found to be lower ($p = 0.024$) in the AD group than control group. There was no significant difference between male and female for DNA strand breaks and H_2O_2-induced DNA damage in the AD group, but ratio of GSH/GSSG were higher in male when compared with female ($p = 0.045$). No significant difference was found between the men of AD group and men of the control group for GSH/GSSG ratio whereas women of the AD have a lower GSH/GSSG ration than those in the women of the control group ($p = 0.009$).

Conclusion: Increased systemic oxidative DNA damage and DNA susceptibility to oxidation may be resulted from diminished GSH/GSSG ratio in AD patients. This finding shows the importance of antioxidant support in AD management.

P-09.04.4-013
Validation of a liquid chromatography-tandem mass spectrometry method for the measurement of lipid peroxidation product 8-iso-prostaglandin F2α in urine

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8-iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$) is a reliable indicator of lipid peroxidation resulting from oxidative lipid damage and is postulated as a gold standard biomarker for the evaluation of oxidative stress. The aim of this study was to validate non-invasive and highly accurate stable isotope dilution-multiple reaction monitoring liquid chromatography-tandem mass spectrometry (SID-MRM LC-MS/MS) method for identification and quantification of 8-iso-$PGF_{2\alpha}$.

Twenty four hour urine samples were collected from healthy volunteers at Medical School of Dokuz Eylul University for analytical performance studies. LC-MS/MS analyses were performed on conventional HPLC coupled to a triple quadrupole ion trap mass spectrometer equipped with a TurboIonSpray™ source. Analyst Software Version 1.5 were used for data analyses. MRM transitions used were m/z→ 250/164 for 8-iso-$PGF_{2\alpha}$ and m/z→ 255/169 for 8-iso-$PGF_{2\alpha}$-d_4. Analytical performance of the method was evaluated by linearity, selectivity, sensitivity, precision and accuracy studies using pure standards and samples extracted from urine of healthy volunteers.

The linear calibration range for 8-iso-$PGF_{2\alpha}$ was determined as 20 ng/ml by using standards ranging from 0.25–50 ng/ml. Analytical sensitivity of the method was determined by LOD with S/N of 3 and LOQ with S/N of 10. LOD and LOQ for 8-iso-$PGF_{2\alpha}$ were 2.4×10^{-2} and 38×10^{-2} ng/ml, respectively. The assay stability and reproducibility were assessed by the precision and accuracy of intra- and interday measurements (n = 10). The intra- and interday CVs for 8-iso-$PGF_{2\alpha}$ were 4.5% and 1.2%, respectively. SID-MRM LC-MS/MS method for absolute

quantification of 8-iso-$PGF_{2\alpha}$ was optimized and validated in our laboratory and therefore this highly accurate method can successfully be applied to clinical patient samples.

P-09.04.4-015
Synergistic anticancer effects of sulforaphane and cisplatin through the induction of apoptosis and autophagy following oxidative stress in malignant mesothelioma cells

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Malignant mesothelioma is characterized by poor responsiveness to current chemotherapeutic drugs, usually owing to high resistance to apoptosis. Here, we investigated chemosensitizing effects of phytochemical resveratrol, in combination with cisplatin, on malignant meothelioma cells. Cell viability was evaluated using MTT assay. Cell apoptosis was detected with DAPI staining, caspase 3/7 activity assay and flow cytometry. Cell cycle distributions, ROS levels and mitochondrial membrane potential were determined using flow cytometry. The expression of cell cycle-, apoptosis-, and autophage-related proteins was measured with Western blotting. The combination treatment of cisplatin and resveratrol (Cis/Res) synergistically induced apoptosis, as evidenced by typical cell morphological changes, the appearance of a sub-G_0/G_1 peak, an increase in the Annexin V(+) cells and the cleavage of caspase-3 and PARP. Cis/Res increased ROS production and depolarization of mitochondrial membrane potential with an increase in the Bax/Bcl-2 ratio. These changes were attenuated by pre-treatment with N-acetylcysteine, suggesting that Cis/Res induced apoptosis through oxidative mitochondrial damage. Compared with MSTO-211H cells, Cis/Res was less efficient in killing H-2452 cells. H-2452 cells exhibited an enhanced autophagy to Cis/Res, as observed by an increase in viable cells exhibiting intense LysoTracker Red staining and up-regulation of Beclin-1 and LC3A. Inhibition of autophagy by bafilomycin A1 rendered cells more sensitive to Cis/Res-induced cytotoxicity and this was associated with induction of apoptosis. These data indicate that the increased resistance of H-2452 cells to Cis/Res is closely related to the activation of self-defensive autophagy, and provide the rationale for targeting the autophagy regulation in the treatment of malignant meothelioma.

P-09.04.4-016
Evaluation of the protective effect of Ethyl pyruvate on in vitro fertilization (IVF) & sperm quality in cyclophosphamide treated adult male mice

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Stress oxidative induced by chemotherapy with cyclophosphamide (CP) causes vulnerability in sperm and decline of fertility. This study was aimed to evaluate the role of ethyl pyruvate (EP) in the amelioration of fertility and growth of primitive embryo in animals that received CP.

24 adult male mice (6–8 weeks) were randomly divided into 3 groups: Control group received normal saline (0. 2 ml/day, IP), CP group received CP (15 mg/kg/week,¬ IP), the CP+EP group received EP (40 mg/kg/day, IP) along with CP, were treated for 35 days. 8 mice from each group were arranged for evaluation of sperm quality and in vitro fertilization (IVF) too. Afterward, the separated oocytes from 72 ovulation stimulated mice were conducted to evaluation of IVF and embryo development.

The results revealed that CP caused notable decrease in percentage of fertilization in CP group, but administration of EP along with CP caused an increase in the percentage of fertilization in comparison to CP group. The percentage of the two cell zygotes in CP+EP group, and the percentage of blastocysts in control and CP+EP groups were higher than that in CP group ($p < 0.05$). Results showed that the total number of arrested embryos in control and CP+EP groups was decreased in comparison to CP group ($p < 0.05$). The percentage of arrested embryos type I, II, and III in CP+EP group was decreased in comparison to CP group, but that decrease was significant only in types I and II ($p < 0.05$). The average motility, viability, nucleus maturity and sperm morphology were decreased significantly in CP group in comparison with control and EP groups, whereas EP caused significant increase of these parameters ($p < 0.05$). Also, the percentage of DNA damage was increased significantly in CP group in comparison with control and EP groups ($p < 0.05$).

The results of this study indicated that the ethyl pyruvate was able to suppress free radicals and enhance the IVF and quality of sperm in CP treated animals.

P-09.04.4-017
The effects of acute different doses malathion exposure on insulin level, amylase, lipase activity and oxidant status in rat pancreas

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Malathion, which is a member of organophosphate chemical family, is used to control pests and is a widely used pesticide all over the world. However it is also known to be highly toxic on many tissues including pancreas.

To test this we set 4 groups to administer a single dose of Malathion dissolved in corn oil via oral gavage at the doses of 100 mg/kg (Group 2), 200 mg/kg (Group 3) and 400 mg/kg (Group 4). Only plain corn oil was given to Control group (Group 1). The rats were sacrificed 24 hours after administration of the chemical and the pancreases of rats were removed. In an attempt to evaluate the dose dependent response, we measured amylase and lipase activities, insulin, malondialdehyde (MDA), total oxidant status (TOS) levels in rat pancreases. All of the parameters were measured spectrophotometrically.

We found that pancreas insulin levels significantly increased in Group 4 compare to Group 1, besides the insulin levels of Group 3 and Group 4 were significantly higher than Group 2 ($p < 0.013$). Pancreas amylase and lipase activities significantly decreased in Group 3 and Group 4 compare to Group 1 and Group 2 ($p < 0.013$). However, there was no significant change in pancreas MDA and TOS levels ($p > 0.05$). According to correlation analysis, when pancreas amylase levels declined, lipase levels were decreased simultaneously and there was a strong positive correlation between them ($p < 0.01$). In addition, when the comparison was evaluated as a binary, while pancreas amylase and lipase levels diminished, pancreas insulin levels increased and a strong negative correlation between them was found ($p < 0.01$).

According to our result, acute administrations of malathion leads to alterations of insulin, amylase, and lipase levels with a dose dependent manner, while it does not to change oxidant status.

P-09.04.4-018
Potential toxic effects of mancozeb on catalase (CAT) activity and lipid peroxidation (LPO) on brain tissue of zebrafish, *Danio rerio*

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The aim of this study is to evaluate the potential toxic effects of mancozeb on the stress biomarkers such as catalase (CAT) activity, malondialdehyde (MDA) level and protein levels in the brain tissue of zebrafish (Danio rerio). Mancozeb, is a synthetic fungicide contaminating aquatic environments as a potential toxic pollutants, was investigated in the present study for acute toxicity. Zebrafish groups (M-Low and M-High) were exposed to different doses of mancozeb (5 mg L-1 and 7.5 mg L-1) for 120 hours except the control group. Catalase (CAT) activity, Malondialdehyde (MDA) level and total protein levels were determined by spectrophotometer. The results showed that CAT activity and MDA levels were decreased in all experiment groups. Protein levels were increased in experiment groups when compared to the control group. In conclusion, the changes in the CAT activity and MDA levels were time and as well as mancozeb dose-dependent. Furthermore, the biochemical parameters of mancozeb exposure on zebrafish, showed that mancozeb has significant effect on the zebrafish and/or aquatic organisims.

P-09.04.4-019
Possible protective effects of betaine on liver and kidney tissues of long term therapeutic doses of paracetamol (acetaminophen) administered on pregnant rat's newborn puppies

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Paracetamol (PARA), which is antipyretic and analgesic, is widely used around the world. Paracetamol can be recommended for moderate or mild pains especially in pregnancy as an analgesic. It is known that, paracetamol can cause hepatotoxicity or nephrotoxicity. We were aimed that in this study to show potential hepatoprotective and nephroprotective effect of Betaine against long term paracetamol using at therapeutic doses.

It has been prepared 3 groups, control, PARA and PARA+-Betain groups. Paracetamol and Betaine were administered by gavage to pregnant rats, from first day to the last day of pregnancy (aprox. 21 day). 2 ml physiological saline (%0.9 NaCl solutions), 30 mg/kg/day Paracetamol, 30 mg/kg/day Paracetamol and 800 mg/kg/day Betain was given by orally to control, PARA and PARA+Betain groups respectively. The day of the birth, newborn rats anesthetized by ether and after decapitated. 8 newborn rat's liver and kidney tissues used for biochemical analysis [Malondialdehyde (MDA), Reduced Glutathione (GSH), Nitric Oxide (NO) and Paraoxonase-Arylesterase (PON-ARE)] and 5 rat's liver and kidney tissues used for histological studies.

We showed that, MDA and NO levels was significantly increased, while PON activities decreased. On the other hand GSH levels and ARE activities was decreased but these decline wasn't significant in the liver and kidney PARA group. These biochemical results showed hepatotoxicity and nephrotoxicity in neonates which can be formed in long term maternal paracetamol using at therapeutic doses. Also our histological findings was support these biochemical results.

We used Betaine against potential hepatotoxic and nephrotoxic effect of long term maternal Paracetamol using at therapeutic doses for neonates. Betaine has antioxidant properties and also used as a methyl donor for transsulfuration reactions in the cell. Biochemical and histological examinations showed that Betaine protected the tissue injury relatively.

P-09.04.4-020
Lipid rafts are involved in modulation of Ca^{2+} responses induced by glutoxim and molixan in macrophages

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Pharmacological analogues of oxidized glutathione (GSSG) disulfide-containing drugs glutoxim® (GSSG disodium salt with d-metal nanoaddition, «PHARMA-VAM», St. Petersburg) and molixan® (complex of glutoxim with inosine nucleoside) have found clinical application as a wide range immunomodulators and hemostimulators. However, the cellular and molecular mechanisms of these drugs action are still unclear.

Previously we showed for the first time that glutoxim and molixan cause biphasic intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) increase due to Ca^{2+} mobilization from thapsigargin-sensitive Ca^{2+} stores and subsequent store-dependent Ca^{2+} entry in rat peritoneal macrophages.

It is known that key proteins involved in Ca^{2+} signaling are localized in discrete plasma membrane lipid rafts domains. Lipid rafts are cholesterol and sphingolipids enriched microdomains that function as unique signal transduction platforms. Thus, the aim of the present work was to elucidate the possible involvement of lipid rafts in glutoxim and molixan effects on $[Ca^{2+}]_i$ in macrophages.

$[Ca^{2+}]_i$ measurements were performed with Fura-2AM microfluorimetry. To examine the involvement of lipid rafts in the effect of GSSG-based drugs on $[Ca^{2+}]_i$ we used methyl-β-cyclodextrin (mBCD), widely used to remove cholesterol from membranes, thus disrupting the lipid raft domains.

We have shown for the first time that macrophage preincubation with 10 mM mBCD for 1 hours before molixan addition causes significant inhibition of both Ca^{2+} mobilization (on average, by 77.6 ± 9.2%) and subsequent Ca^{2+} entry (on average, by 82.3 ± 10.5%), induced by molixan. Similar results were obtained in experiments with glutoxim.

Thus, we have demonstrated for the first time that mBCD significantly decreases both phases of glutoxim- or molixan-induced Ca^{2+} responses in macrophages. The results suggest that intact rafts are required to initiate complex signaling cascade activated by glutoxim or molixan and leading to $[Ca^{2+}]_i$ increase in macrophages.

P-09.04.4-021
Pleiotropic anticancer activity of selected nutraceuticals against MCF-7, MDA-MB-231 and SK-BR-3 breast cancer cell lines

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Plant-derived natural substances (phytochemicals) with potent pro-apoptotic activity towards cancer cells in vitro are considered as promising nutraceuticals in anticancer therapy. Nevertheless, due to their relatively low bioavailability, administration of high doses of nutraceuticals that are not achievable in vivo seems to exert potentially negligible physiological effects in clinical trials. Thus, there is a need for revealing novel cytophysiological effects of low doses of phytochemicals towards cancer cells. In the present study, we have considered thirty one nutraceuticals and selected four phytochemicals acting at low micromolar range (5 to 20 μM) against phenotypically different MCF-7, MDA-MB-231 and SK-BR-3 breast cancer cells, namely diosmin, sulforaphane, ursolic acid and betulinic acid. Nutraceuticals inhibited cell proliferation and caused changes in the cell cycle that was accompanied by elevated levels of p53, p21, p16 and/or p27. Apoptosis (Annexin V staining, multicaspase and mitopotential assays) was observed exclusively when nutraceuticals were used at the concentration of 20 μM, whereas at the concentration of 5 μM, stress-induced premature senescence was noticed (SA-β-gal activity). Nutraceuticals diminished the levels of GLUT-1 and selected glycolytic enzymes. Nutraceuticals promoted oxidative and nitrosative stress as judged by increased production of total reactive oxygen species, total and mitochondrial superoxide, nitric oxide and protein carbonylation. Nutraceuticals also stimulated DNA single and double strand breaks that was accompanied by ATM phosphorylation and to a lesser extent by histone H2AX phosphorylation and 53BP1 foci formation.

Taken together, several responses to nutraceutical treatment were observed in breast cancer cells that may reflect the heterogeneous nature of cancer cell populations.

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P-09.04.4-022
Nutraceutical-induced ribotoxic stress in MCF-7, MDA-MB-231 and SK-BR-3 breast cancer cell lines

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Nucleolus is thought to be a stress sensor and oxidative and ribotoxic stimuli may cause the inhibition of rRNA synthesis by the inactivation of transcription factor TIF-IA/RRN3 that is accompanied by the relocation of nucleolar proteins and p53-based cell cycle arrest and/or apoptosis. As nutraceutical-mediated modulation of nucleolar activity may be considered an attractive anticancer strategy, in the present study, we have investigated the effects of three selected nutraceuticals, namely sulforaphane, ursolic acid and betulinic acid on nucleolus state in three breast cancer cell lines (MCF-7, MDA-MB-231 and SK-BR-3). We found that low dose nutraceutical treatment resulted in p21-mediated inhibition of cell proliferation, a decrease in rRNA synthesis, shifts in lamin A/C and B1 pools, changes in the nucleolar protein levels and their carbonylation, and changes in nucleolus size and number. Breast cancer cells differed in ERK activity that resulted in different patterns of ERK activation/inhibition, phosphorylation status of S6 and autophagy induction upon nutraceutical stimulation. Nutraceuticals also affected DNA methylation parameters, namely the levels of DNMT1, DNMT3a and DNMT3b that resulted in both global DNA hypo- and hypermethylation.

Taken together, after nutraceutical treatment, nucleolus-centered cellular response was revealed in breast cancer cells of different phenotypic characteristic that may be considered a potential target of anticancer therapy.

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P-09.04.4-023
Rate of apoptosis in human macrophages infected with *Leishmania tropica* promastigotes

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Infection of the cells with parasites or exposing cells to heat stress induces a cellular stress. In the present study human macrophages are infected with *Leishmania tropica* promastigotes and exposed to heat stress. The measurement of cytoplasmic histone-associated DNA-fragments was carried out using ELISA technique. Visualization of apoptotic cells was performed by the terminal deoxynucleotidyl transferase dUTP nick end labeling staining method (TUNEL). Degree of oxidative stress on cell is evaluated by measuring nitric oxide (NO), malondialdehyde (MDA), reducte glutathion (GSH) levels and superoxide dismutase (SOD) activities. Results of the ELISA technique showed that infection of macrophages with promastigotes induced apoptosis rate significantly ($p < 0.001$), heat stress however decreased the rate of apoptosis in infected macrophages remarkably ($p < 0.001$). High levels of apoptosis rate in infected macrophages and drastic decdrease in apoptosis in heat subjected macrophages infected with promastigotes are confirmed by visualisation of apoptotic cells using TUNEL method. Levels of glutathion (GSH) in infected macrophages decreased significantly ($p < 0.05$), while malondialdehyde (MDA) levels increased notably ($p < 0.05$). However, no statistical significant alterations were detected in the nitric oxide (NO) values and superoxide dismutase (SOD) activities.

Results of the present study indicates that infection of human macrophages with *Leishmania tropica* induces a cellular stress response, characterized by decreased values of GSH and increased levels of MDA. Increased rate of apoptosis in infected macrophages may be due to the increased cellular stress caused by *Leishmania tropica* amastigotes. Decreased rate of apoptosis measured in heat exposed macrophages infected with promastigotes indicates an extention in life span of macrophages.

Furthermore extention in life span of macrophages may enable parasites to implement their differentiation process within these cells.

P-09.04.4-024
Demonstration of new biochemical markers in diabetic patients

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Measurements of the parameters characterizing the redox and inflammatory processes in blood are essential for diagnosis and prognosis of type 2 diabetes mellitus (T2DM), but also for monitoring the effectiveness of medical treatments.

Along with other biochemical effects, hormonal imbalance leads to modified transport function of erythrocytes due to changes in enzyme systems involved in upholding of cellular homeostasis through a rapid degradation of altered proteins, being the second line of defense against the free radicals, which degrade and eliminate the damaged molecules. Some of these enzymes are hemoglobin peroxidase (PA) and esterase (EA).

The aim of this research study was to identify new parameters with a potential role of biochemical markers in T2DM like hemoglobin peroxidase and esterase activity from erythrocyte.

Our data showed that pathological processes involved in T2DM imply an increased enzymatic activity of PA (73.85%), which correlates with increased levels of EA (47.9%) and glycated hemoglobin (HbA1c) (13.51%), compared with control group. The variables HbA1c, PA and EA are correlated: the identified Pearson correlation coefficients $r = 0.637$ and $r = 0.573$ respectively, have an associated probability of $p < 0.001$ and $p < 0.006$ a value that indicates a strong positive correlation between the dependent variables PA and EA and independent variable HbA1c.

Based on these results we concluded that together with glycosylated hemoglobin assay, all the studied parameters can be successfully used as extra test for diabetes associated with oxidative stress and disorders in erythrocyte functions or blood rheology.

P-09.04.4-026
The radioprotective effects of propolis and caffeic acid phenethyl ester on radiation-induced oxidative/nitrosative stress in brain tissue

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Head and neck cancer patients treated with radiotherapy suffer severe side effects during and following their treatment. Efforts to decrease toxicity of irradiation to normal tissue, organs and cells have led to searching for cytoprotective agent. Investigations for effective and non-toxic compounds with radioprotective capability led to increasing interest in antioxidant such as Propolis and Caffeic acid phenethyl ester (CAPE). The aim of this study was to evaluate the antioxidant and radioprotective effects of Propolis and CAPE on radiation-induced oxidative/nitrosative stress in the brain tissue. Fourty Sprague-Dawley rats were randomly divided into five groups. Group 1 (Irradiation (IR) + Propolis) received total cranium irradiation and propolis was given orally through an orogastric tube daily. Group 2 (IR+CAPE) received total cranium irradiation plus CAPE, was dissolved in dimethyl sulfoxide (DMSO) just before giving to the rats, intraperitoneally (IP) every day. Group 3 (IR) received 5 Gy of gamma irradiation as a single dose to total cranium plus 1 ml saline daily. Group 4 received daily plain DMSO. Group 5 received daily plain saline. At the end of the 10 day time period, xanthine oxidase (XO), nitric oxide synthase (NOS) activities, nitric oxide (NO•) and peroxynitrite ($ONOO^-$) levels were significantly higher in IR group compared to all other groups. In conclusion, the results suggest the radioprotective ability of Propolis and CAPE involving prevention of radiation-induced oxidative/nitrosative damage.

P-09.04.4-027
Role of leptin and adiponectin in obesity-associated oxidative stress

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Objective: Increased oxidative stress is one of the major characteristics of obesity and obesity-related complications. Adipokines also induce the production of reactive oxygen species and generating oxidative stress in physiological and pathological conditions of obesity. The aim of this study was to determine the association of leptin and adiponectin levels with body mass index, lipid parameters and oxidative stress biomarkers in obesity.
Methods: The study included 150 obese and 120 non-obese subjects. Plasma leptin and adiponectin levels (ng/ml) were measured using commercially available enzyme-linked immunosorbent assay kits. Serum lipid, superoxide dismutase, malondialdehyde and antropometric parameters were measured.
Results: Obese and non-obese subjects did not differ in age, while plasma glucose, total cholesterol, triglycerides, LDL cholesterol and leptin levels were significantly higher and mean HDL cholesterol and adiponectin levels were significantly lower in obese than non-obese subjects. The plasma leptin ($p < 0.001$) and adiponectin ($p = 0.003$) levels were significantly correlated with BMI in both obese and non-obese subjects. In obese subjects, leptin levels were significantly correlated with superoxide dismutase ($p < 0.01$) and malondialdehyde ($p < 0.01$). Strikingly, adiponectin was significantly correlated with superoxide dismutase ($p = 0.02$) and malondialdehyde ($p = 0.0012$) levels in obese group.
Conclusion: Our results suggest that leptin and adiponectin levels are associated with defective antioxidant status and increased lipid peroxidation which may have implications in the development of obesity related health problems.

P-09.04.4-028
Quercetin ameliorates liver injury secondary to cerulein-induced acute pancreatitis

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The aim of this study is to evaluate the effect of quercetin (Q) on liver injury secondary to cerulein induced-acute pancreatitis (AP). For this reason, rats were randomly divided into four groups (8 rats for each group) Control group received physiological saline, four time and dimethyl sulfoxide, two time, at 1 hours intervals, intraperitoneally (i.p.). Cerulein group received cerulein (50 μg/kg-rat weight, in physiological saline), four times, and dimethyl sulfoxide (1%), two times, at 1 hour intervals, i.p. Quercetin pretreatment (Q+Cer) group received quercetin (100 mg/kg-rat weight, in dimethyl sulfoxide) one time, one hour before cerulein treatment and physiological saline, one time, six hour after cerulein treatment. Quercetin post-treatment (Cer+Q) group received dimethyl sulfoxide, one time, one hour before cerulein treatment and quercetin, one time, six hour after cerulein treatment. Cerulein treatment increased significantly vascular congestion in hepatic cells. Quercetin treatment also decreased significantly vascular congestion. The liver MDA and carbonyl levels in cerulein group were significantly higher than the control group ($p < 0.01$, $p < 0.001$, respectively). The MDA and carbonyl levels in Q+Cer group decreased significantly compared to the Cer group ($p < 0.001$). The MDA, Carbonyl, MPO levels in Cer+Q group were significantly lower than the Cer group ($p < 0.001$). The GSSG/GSH ratio of Q+Cer and Cer+Q groups were significantly lower than the Cer group ($p < 0.05$, $p < 0.001$, respectively). The SOD activity in Cer group was significantly lower than the control group, but the SOD activity in Q+Cer and Cer+Q groups was significantly higher than the Cer group ($p < 0.05$).

This study shows that quercetin treatment was reduced the severity of liver injury secondary to cerulein induced-AP as reflected by changes in the parameters of hepatic oxidant and antioxidant.

P-09.04.4-029
Identification of water extract of propolis components by using different columns in gas chromatography-mass spectrometry

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Propolis is a natural material obtained by honey bees from various plants. Protective effect of propolis against damages of free radicals is due to different compounds within propolis. The aim of this study is to identify qualitatively and quantitatively the chemical composition of water extract of propolis (WEP) provided by Erzurum region using Rtx-1 and Rtx-5 ms column of gas chromatography-mass spectrometry (GC-MS) and to compare with two columns.

In this study, WEP of 25 mg/ml was prepared, cleared by membrane filter of 0.45 μm and freezed at −80 °C. Then, it was lyophilized up to dry form and derivatized to apply for gaseous form. 7 mg of dry extract was reacted with 350 μl pyridin + 700 μl bis-trimethylsilyl trifluoroacetamide (BSTFA) mixture including 1% trimethylchlorosilane (TMCS) and incubated for 30 minutes at 100 °C. All analyses were performed with Shimadzu GCMS-QP2010 Ultra by using a flame ionisation detector (FID). Rtx-1 and Rtx-5 ms capillary columns and helium for carrier gas at a flow time of 1 ml/minute were used. Injection was applied on split mode at 250 °C. Derivatized propolis sample was injected as 2 μl, initial column temperature was adjusted as 60 °C, then increased to 240 °C with increments of 3 °C. Total analysis time was determined as 62 minutes. Relative percent amount of separated compounds was calculated from total ion chromatogram with computerised integrator. All components were defined by using Nist and Wiley libraries.

Peaks obtained from Rtx-1 column were much more than those of Rtx-5 ms. On the other hand, analyses performing with both two columns have similar carbohydrate, aromatic acid and other acid contents.

Consequently chemical constituents of WEP were determined qualitatively and quantitatively with GC-MS. It was concluded that Rtx-1 column among both columns differentiating for polarities may produce more compounds in the propolis analysis.

P-09.04.4-030
Cd, Zn and their combination altered the GST and GSH levels in the liver of freshwater fish *Oreochromis niloticus*

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Introduction: Aquatic environment can be mostly contaminated by mixtures of metals. Biochemical parameters have gain importance to characterize the effects of metals on aquatic organisms. Glutathione S-transferase (GST) and its substrate glutathione (GSH) are important parameters of antioxidant defense system of fish metabolism due to their vital role in xenobiotic conjugation.
Objective: The goal of the current study to evaluate the changes in GST and GSH levels in response to Cd, Zn and Cd+Zn effects after 14 and 28 exposure days.
Materials and methods: Fish were obtained from Cukurova University fish culturing pools (Turkey). Fish were exposed to 1.0 μg/ml of Cd ($CdCl_2.H_2O$) and Zn ($ZnCl_2$), and their mixture, for 0, 14 and 28 days. At the end of the exposure period, liver tissues were dissected and homogenized in a phosphate buffer (pH 7.4). Homogenates were centrifuged at 10,000 g (30 min, +4 °C). Supernatants were stored at −80 °C until the analysis. One-way ANOVA was used to compare data (Mean ± SE) followed by Duncan's test ($p < 0.05$).
Results: GST and GSH changes were recorded as decreases after all metal exposures at day 14. Although 28 day exposure was found as less effective, combined effects caused significant decreases in GST and GSH levels. Also longer exposure durations were appeared to be more effective in that situation.
Conclusions: Significant decreases in GST and GSH levels could be occurred due to increased reactive oxygen species caused by metals particularly their combined effects. Metal type, their single and combined effects and also exposure duration should be also taken into account when considering the antioxidant system response. GST and GSH might be considered as sensitive biomarker in toxicity assessment of metal mixtures.

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P-09.04.4-031
Anthocyanin-rich preparations and their effects against NO degradation products

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Clinical trials of biologically active plant substances show a significant preventive effect in cancer, cardiovascular diseases and peptic ulcer disease in the form of both nutritional supplements and therapeutic intervention. Anthocyanins contained in dark berries show great antioxidant potential, with the most important including a reduction in oxidative degradation of lipids or tyrosine oxidation by peroxynitrite. Our previous studies of the antioxidant properties of extracts from Vaccinium corymbosum, Aronia melanocarpa and Sambucus nigra, however, indicated that their activities largely depend on the method of extraction. While quantitative determination of anthocyanins pointed to a disproportionately larger content of anthocyanins in isolates from lyophylized berries, their scavenging activities against hydroxyl radicals was surprisingly the lowest. Inflammatory processes, vascular damage, atherosclerosis and others are caused by oxidative-nitrosative stress, so we tested their efficiency to scavenge NO degradation products. We found that only purified extracts of lyophilized berries showed the most significant effects against NO degradation products, with efficacy of around 50%. An extract from aronia showed greater than 60% efficiency, and a net ethanol extract from all the berries showed a 30% effect. Cleaned ethanol extracts showed the lowest effects, while aronia initiated production at a concentration of 25 mg/l. Conversely, all acetone extracts consistently initiated NO degradation products. These findings are in complete contrast to their determined action against reactive oxygen species. In summary, it follows that a particularly adjusted lyophilized extract of the berries could be responsible for the increased biological activity of NO and the observed biological and pharmacological activities of anthocyanins in circulatory disorders. The study was supported by grant VEGA 1/0782/15 and 1/0018/16.

P-09.04.4-032
Attenuation of dysfunction, oxidative stress and apoptosis by resveratrol in benzo(a)pyrene exposed INS1-E 832/13 pancreatic beta cell line

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Diabetes is one of the most important problems in the world. This disease is a very important health problem due to affects many different organs and systems. It has been well established that, environmental pollutants had deleterious effects on glucose metabolism, and caused insulin resistance and type 2 diabetes. With this investigation, it was aimed to investigate the effects of benzo(a)pyrene on pancreatic beta cells and treatment affects of resveratrol.

In this study, rat INS-1E beta cell line was used. After reaching the appropriate number of cells culture operations by cell culture, benzo(a)pyrene (20 μM, 24 hours) application have been made after resveratrol (80 μM, 48 hours) application. After incubations oxidative stress, insulin secretion (in cell and in medium), cell proliferation and apoptosis were analysed in cells by biochemical and molecular techniques.

B(a)P application resulted in NO increase and resveratrol also increased this level of NO. Resveratrol increased the TAS levels decrased by B(a)P, and TOS levels were also increased by resveratrol interestingly. OSI levels determined with TAS and TOS levels, has no significant change between groups. GSH levels were decreased by B(a)P while resveratrol increased its' level to control level. mRNA expression levels of beta cell functions related genes INS-1, INS-2 and SIRT-1 were increased by resveratrol treatment. Insulin levels in cell and in medium were increased after resveratrol treatment. mRNA expression level of FOXO-1 gene was increased while PDX-1 was decreased by resveratrol treatmeant. B(a)P suppressed the mRNA expression of p53 gene, but resveratrol increased. The effects of B(a)P on pancreatic beta cells and the protective effects of resveratrol on this cells were investigated in vitro with this research firstly.

The results obtained from this research showed that oxidative changes, functional impairment and carcinogenetic effects of B(a)P in pancreatic beta cells could be blocked by resveratrol.

P-09.04.4-034
The protective effect of vitamin E (alpha-tocopherol) on ischemia-reperfusion injury in rat liver

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Ischemia-reperfusion (I/R) process is usually used during transplantation and resection of the liver but liver dysfunction and cellular death due to lack of oxygen in tissues, changes in the balance of oxidant/antioxidant in favor of oxidants, and inflammatory response are inevitable during this process. In the present study, it was aimed to investigate whether vitamin E(alpha-tocopherol), an antioxidant agent, has a protective effect against liver ischemia reperfusion injury in rats by using morphometric methods.

For this purpose, 21 adult Sprague-Dawley male rats were divided into 3 groups as; control, ischemia / reperfusion (I/R), and vitamin E+ischemia/reperfusion (Evit +I/R). In experimental groups, the total hepatic ischemia was applied for 45 minutes followed by a 24 hour of reperfusion. In the treatment group, 7 days before ischemia 40 mg / kg dose of vitamin E was administered intraperitoneally once a day. After the termination of the reperfusion, the rats were perfused by cardiac way and liver tissues were dissected. Following volume and weight calculations, the livers were subjected to the standard histological preparation methods and embedded in paraffin. Serial sections at 5 μm thickness were obtained from these blocks, stained with hematoxylin-eosin, and analyzed with morphometric methods.

In light microscopic examinations of the I/R group, irregularity in lobules, dilatation in central veins and sinusoids, extensive areas of necrosis and pycnotic nuclei were seen in hepatocytes. The volume density of sinusoids to liver parenchyma was estimated as 16% in the control group, whereas it was 36% in the I/R group. This value was decreased to 32% in the Evit + I/R group. However, no significant difference was found among the groups in the lobule area calculated by the point counting method.

These results show that intraperitoneal vitamin E administration for 7 days prior to ischemia partially inhibits damage caused by ischemia-reperfusion injury in the liver.

P-09.04.4-035
Biological effects of soursop are mediated through reactive nitrogen species

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The leaves, fruit and bark of Annona muricata, a member of the Annonaceae family, are commonly used in the traditional medicine of tropical and subtropical regions. In recent years, many studies have shown their anti-cancer, anti-convulsant, anti-arthritic, anti-parasitic, anti-malarial, and anti-diabetic activities. It should be noted that these characteristics have been described using different types of extracts from different parts of the plant. Our studies have focused on the systematic characterization of activities most easily accessible from an aqueous extract of leaves, with hitherto documented antihypertensive and hepatoprotective effects. We found that the extract shows almost 54% efficiency against hydroxyl radicals. With increasing concentrations, the effectiveness weakened, reaching a second peak (40%) at a concentration of 50 mg/l. The scavenging activity against NO degradation products maintained a continuously increasing trend with a maximum at a concentration of 100 mg/l. Surprisingly, the extract initiated peroxynitrite production in a similar trend, except at 50 mg/l, where it scavenged peroxynitrites with relatively high efficiency, up to 34%. These findings are consistent with the elevated levels of reduced glutathione detected after incubation of liver mitochondria with extract to a maximum concentration of 50 mg/l, with subsequent sharp decline. The activity of glutathione S-transferase was decreased, although not significantly. This indicates a reduction of metabolic processes of compounds, allowing their action over a longer period of time. In a live system, even antihypertensive effects can be observed. However, a significant outflow of GSH to create the GSH adducts of active substances, and particularly S-nitrosoglutathione from increased production of peroxynitrites, can cause liver toxicity. The study was supported by grant VEGA 1/0782/15 and 1/0018/16.

P-09.04.4-036
The role of polyamine metabolism in curcumin induced apoptosis via reactive oxygene species (ROS) generation in growth hormone (GH) overexpressed T47D breast cancer cells

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Autocrine Growth hormone (GH) signaling triggers cell proliferation, growth, metastasis and drug resistance in cancer cells. Polyamines (PAs) play an essential role in cell cycle, proliferation, growth and carcinogenesis of various cancer types such as prostate, colon and breast cancer. ODC (ornithine decarboxylase) is the key enzyme in the PA biosynthetic pathway that is under control of Antizyme (AZ) and Antizyme Inhibitor (AZI) activity. PA catabolic enzymes polyamine oxidase (PAO) and spermine/spermidine acetyle transferase (SSAT) by-products triggers reactive oxygene species (ROS) generation and apoptotic cell death. Curcumin decreased cell viability in dose and time dependent manner in T47D wt and GH+ cells. Although forced GH expression induced cell proliferation, 20 μM curcumin inhibited cell invasion. Curcumin (20 μM) induced apoptosis by acting on intrinsic and extrinsic pathway in both cell lines. Moreover, curcumin supressed ODC, AZI expression in wt and GH+ T47D cancer cells. Although curcumin decreased AZ expression in T47D wt cancer cell, increased AZ expression was determined in T47D GH cancer cell. PAO and SSAT expressions were upregulated in T47D GH+ cells. Concomitantly, putrescine levels were increased in T47D GH+ cancer cell compared to wt cells and curcumin depleted spermidine and spermine levels in wt and GH + T47D cells. Curcumin induced-apoptotic cell death via ROS generation and co-treatment of N-acetyl cysteine (NAC) overcame curcumin effect. Conclusion, forced GH expression triggers cell proliferation and growth via acting on polyamine metabolism and curcumin-triggered ROS generation was prevented by NAC treatment in T47D wt and GH+ breast cancer cells.

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P-09.04.4-037
The radio-protective effects of propolis and *Nigella sativa* oil on oxidative/nitrosative stress in liver tissue of rats exposed to total head irradiation

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Our purpose is to investigate Propolis and Nigella sativa oil (NSO) for their antioxidant effects on the liver tissue of rats exposed to ionizing radiation.

A total of 32 Sprague–Dawley rats were divided into five groups to test the radioprotective effectiveness of of Propolis and NSO administered by orogastric tube. Appropriate control group was also studied. Biochemical parameters in liver tissue of rats were determined by spectrophotometer.

Xanthine oxidase (XO), nitric oxide synthase (NOS), superoxide dismutase (SOD) activities, nitric oxide (NO•) and malondialdehyde (MDA) levels were higher in IR group while glutathione-S-transferase (GST), glutathione peroxidase (GSH-Px) level in the IR group were lower in this group when compared to the other groups. GST activity in IR plus Propolis group was statistically higher than in all other groups.

Propolis and NSO clearly protect liver tissue from radiation-induced oxidative stress.

P-09.04.4-039
The effects of royal jelly on the antioxidant parameters in the breast tissues of the rats with breast carcinoma treated with paclitaxel or not

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Royal jelly is a bee product which has health protective effects by antioxidant, antitumor, anti-inflammatory and immunomodulatory activities. The aim of this study of was to determine the

effects of royal jelly on the breast tissue antioxidant parameters in rats with breast carcinoma treated with paclitaxel or not.

8–12 weeks old female Sprague-Dawley rats (n = 37) included in current study were divided into 5 groups: control group (n = 8) with healthy rats; breast cancer group (n = 8); breast cancer group (n = 7) treated with 15 mg/kg paclitaxel injection (once a week for 3 weeks); breast cancer group (n = 7) treated with 100 mg/kg royal Jelly (by oral gavage for 30 days); and finally breast cancer group (n = 7) treated 100 mg/kg royal Jelly in addition to 15 mg/kg paclitaxel injection. Rats with breast carcinoma was obtained at 150th days after a single dose injection of 50 mg/kg N-methyl-N-nitrosourea (MNU). All cancer groups were followed by 30 days with treatment of paclitaxel and/or royal jelly. The antioxidant parameters in rat breast tissues, superoxide dismutase (SOD) and catalase (CAT) activities were determined by spectrophotometric colorimetric methods and glutathione (GSH) by High Performance Liquid Chromatography (HPLC).

All the antioxidant parameters decreased in breast cancer group without any treatment ($p < 0.05$). But, statistically non significant increases were observed by paclitaxel and royal jelly treatment ($p > 0.05$).

This study indicated that royal jelly supplementation can not be sufficient to increase the antioxidant parameters in breast cancer. We are going to continue to identify the effects of royal jelly on breast cancer detail.

P-09.04.4-040
The effects of N-acetylcysteine on microsomal and serum paraoxonase 1 activities at high fat diet induced obese rats

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Obesity is a chronic disease that develops from the interaction between genotype and environmental factors and increase in the accumulation of body fat. It is related with glucose and lipid metabolism disorders, cardiovascular diseases and increased oxidative stress. Paraoxanase 1 (PON1) is an enzyme which plays a protective role in oxidative stress, inflammation and liver diseases. It has been suggested that PON1 has protective effects against high fat diet induced obesity and obesity related disorders. N-asetylcysteine (NAC) is a potent antioxidant due to its ability to stimulate glutathione synthesis. The aim of this study was to evaluate the microsomal and serum PON1 enzyme activities (paraoxonase, arylesterase and lactonase) at high fat diet induced obese rats in the presence of NAC.

This study consisted of control, obese and NAC-supplemented obese (2 g/l NAC) groups. Eighteen Sprague-Dawley rats were randomized into three groups (n = 6). Control rats were fed by standart food and obese and NAC groups were fed by high fat diet. The microsomal and serum paraoxonase, arylesterase and lactonase activities were determined by colorimetric methods.

Serum paraoxonase, arylesterase and lactonase activities decreased in obese and NAC groups when compared to control groups. On the other hand microsomal paraoxonase, arylesterase and lactonase activities increased in NAC group when compared to control and obese groups. However there was no statistically significant difference between the groups.

It has been concluded that the microsomal and serum paraoxonase 1 enzyme activities did not change at high fat diet induced obese rats in the presence of N-asetylcysteine.

P-09.04.4-042
The effects of gallic acid on the acute necrotizing pancreatitis in rats

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Reactive oxygen species, playing an active role in the early and late course of acute pancreatitis, lead to dysfunction of cell membrane and releasing of lysosomal enzymes, and thereby to the injury in pancreatic cells. Gallic acid, found in vegetables such as green tea, is an active component which has antioxidant, anti-inflammatory, antiviral, anticancer activities. The aim of this study was to investigate the effects of gallic acid in experimental acute necrotizing pancreatitis (ANP) model in rats.

Eighteen male Sprague-Dawley rats were divided into three groups (6 rats in each group). Group 1: sham + saline; group 2: ANP induced by intraductal glycodeoxycholic acid and intravenous cerulein; and group 3: ANP + gallic acid (100 mg/kg/day, intraperitoneal). At the end of 18th hours, pancreas histopathology was examined. The levels of serum amylase as a diagnostic marker of pancreatitis, interleukin-6 (IL-6), total antioxidant status (TAS), nitrite + nitrate, total thiols as antioxidant marker and thiobarbituric acid reactive substances (TBARS) to measure malondialdehyde (lipid peroxidation product) were determined by spectrophotometric methods.

Serum amylase, IL-6, plasma TBARS levels were significantly higher but total thiols levels were lower than sham group in ANP group without treatment ($p < 0.05$). However; TAS and nitrite + nitrate levels did not show any significant difference ($p > 0.05$). On the other hand, while serum amylase, IL-6 and TBARS levels were lower, total thiols levels higher in gallic acid treatment group than in the untreated ANP group, but statistically insignificant ($p > 0.05$).

In conclusion, gallic acid treatment is beneficial but not sufficient to treat the acute necrotizing pancreatitis in rats.

P-09.04.4-043
Evaluation of oxidant/antioxidant system parameters, IL-6 and IL-10 levels in amniotic fluid of pregnancies with Down sydrome

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Introduction and aim: Down sydrome (DS) can be diagnosed at high-risk of down syndrome pregnancies by invasive prenatal testing. In this study we aimed to demonstrate antioxidant/oxidant system markers, IL6 and IL10 levels in amniotic fluid samples of pregnancies affected by DS.

Materials and methods: For this purpose we collected amniotic fluid samples from 18 pregnancies affected by down sydrome and 36 normal healthy pregnancies who applied to Zekai Tahir Burak Research and Training Hospital Genetic Center and were proceeded with amniocenthesis. In the amniotic fluid samples; malondialdehyde (MDA), superoxide dismutase (SOD), glutathion peroksidase (GSH-Px) xhantine oksidase (XO), catalase

(CAT), adenozine deaminase (ADA), nitric oxide (NO), nitric oxide senthase (NOS) enzymatic activities were evaluated by spectrophotometric methods, IL6 and IL10 levels are evaluated by ELISA. For statistical analysis Student's t–test and Spearman corralation analusis are used.

Results: It was found that SOD levels are significantly elevated in study group compared to control group ($p < 0.05$). Besides this, in study group, CAT and IL-6 levels are found singnificantly lower than control group ($p < 0.05$). We couldn't find any significant difference between two groups in terms of MDA, GSH-Px, XO, NO, NOS, ADA ve IL-10 levels ($p > 0.05$).

Discussion and conclusion: There is an important decrease in inflammation compared to normal pregnancie in the amniotic fluid of pregnancies having DS. Based on these results, SOD enzyme may be used as a marker for prenatal diagnosis of DS. For this purpose these experiments should be tried on larger sample groups.

P-09.04.4-044
Prooxidant and antioxidant properties of oxygenated monoterpene linalool in Hep G2 cells

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The aim of our work was to compare prooxidant and antioxidant properties of linalool, which is the oxygenated monoterpene compound reported to be a major volatile component of the essential oil of several aromatic species, in Hep G2 cells.

Cytotoxicity of linalool was assayed by CellTiter-Blue® Cell Viability Assay. Malondialdehyde levels result in membrane damage in Hep G2 cells were assayed by using fluorometric method.

Hep G2 cells were incubated with linalool at 24, 48 and 72 hours. The viability of Hep G2 cells decreased in a manner dependent upon concentration and incubation time. The IC_{50} values were calculated 81.5 μg/ml (24 hours), 72.7 μg/ml (48 hours) and 64.7 μg/ml (72 hours). But, cell viability of Hep G2 cells increased when the cells preincubated with linalool at lower concentrations ($<IC_{50}$) against H_2O_2 cytotoxicity. Membrane-damaging effects of linalool were increased with accelerating concentrations. On the other hand, membrane damaging effect of H_2O_2 was decreased when the cells preincubated with linalool before H_2O_2 incubation.

Oxygenated monoterpene linalool had both prooxidant and antioxidant properties showing membrane damaging and protective effects on Hep G2 cells depend on concentration.

P-09.04.4-045
Determination of oxidant-antioxidant status at postprandial lipemia

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Postprandial lipemia is primarily characterized by increasing triglyceride levels after the lipid rich meal. Postprandial lipemia may cause oxidative stress by increasing free radical production and increasing oxidative stress could be responsible for the development of many diseases.

Plasma oxidant-antioxidant status was evaluated in healthy individuals with postprandial hypertriglyceridemia generated by performing oral triglyceride tolerence test (OTTT). The study group included 86 subjects (38 female and 48 male). Ferric reducing ability of plasma (FRAP), total thiol and thiobarbituric acid reactive substances (TBARS) levels were determined by colorimetric methods at fasting and 2nd, 4th and 6th hours following OTTT.

The levels of FRAP and thiol were significantly higher in males than females ($p = 0.0001$ and 0.042, respectively). Thiol levels decreased significantly in both gender at postprandial 2nd, 4th and 6th hours as compared with fasting condition ($p = 0.0001$). While TBARS levels increased at postprandial 2nd hour, that was only significant for male individuals ($p = 0.0001$).

It has been concluded that postprandial lipemia may change oxidant-antioxidant balance in favor of oxidants and gender is an important criteria while assessing the oxidant-antioxidant status in postprandial lipemia

P-09.04.4-046
Ischemia modified albumin and C-reactive protein levels in prediabetes

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Prediabetes is a state of abnormal glucose homeostasis characterized by the presence of impaired fasting glucose, impaired glucose tolerance, or both. The aim of this study was assess serum ischemia modified albumin (IMA) in prediabetes and determine its correlation with other risk factors for chronic complications such as inflammation and hyperglycemia.

Glucose, insulin, total cholesterol, HDL cholesterol, triglycerides, albumin, C-reactive protein (CRP) and IMA were measured in 30 patients with prediabetes and 30 controls.

Prediabetes patients had higher levels of IMA and CRP in comparison with control subjects but there was no significant difference between groups for IMA. Significant positive correlation was observed between CRP and fasting glucose, insulin. There was no significant correlation between IMA levels and the parameters tested.

We have shown higher level of CRP in prediabetes. These results support the hypothesis that chronic inflammation may be involved in development of hyperglycaemia.

P-09.04.4-048
The effects of $S\dot{I}O_2$ nanoparticles of rat uterine smooth muscle

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Usage of nanoparticles in daily life is increasing with technology. Like a lot of chemicals, nanoparticles has toxic effects. Uterus smooth muscle are important many different reproductive functions including sperm and embryo transport, implantation, menstruation, gestation and parturition. In this study, effects of

specially used in textile field SiO_2 nanoparticles on uterus smooth muscle was aimed to be researched.

In this study 64 Wistar albino female rats were used. Rats were separated in 4 groups as control, dose 1 (250 μg/ml), dose 2 (500 μg/ml) and dose 3 (1000 μg/ml). Nanoparticle's size was chosen as 20 nm. Preparations of four groups were evaluated for biochemical and histological examinations. All isolated uterine smooth muscle stripts except the controls were treated with SiO_2 for two hours. In biochemical examinations in order to evaluate oxidative stress level of malondialdehit (MDA), activity of superoxide dysmutase (SOD) and glutathione peroxidase (GSH-Px) were measured. In histological examinations via electron microscope ultrastructure of uterus was examined as well as apoptotic cells detected with immunofluorescent labeling method. Intergroups differences were defined by statistical analysis.

While MDA level increased depending on the dosage, SOD level was decreased depending on the dosage. GSH-Px rate was decreased for each dosage with respect to control. However, no significant difference is detected between groups. In electron microscopic examination no changes were observed in uterus ultrastructure with compare to control. However, in immunofluorescent labeling it was detected that apoptosis increased in dosage groups with compare to control group.

As a result, it was thought that application of SiO_2 nanoparticles, in 20 nm size and in 250, 500 and 1000 μg/ml dosages caused of oxidative stress and apoptosis. This results suggested that SiO_2 has toxic effects on uterine smooth muscle.

P-09.04.4-049
Evaluation of oxidant and antioxidant status in patients with uterine myoma

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Uterine myomas are the most common benign pelvic tumors arising from myometrium. They are rarely associated with mortality but responsible for significant morbidity and have adverse effects on quality of life especially in reproductive age women. Reactive oxygen species and superoxide dismutases, as well as sex steroids play important roles in the reproductive physiology processes. In addition, oxidative stress and impaired antioxidant defense system have been linked to pathophysiology of various diseases including malignant gynecological disorders. Clinical investigations indicate that women with myoma may have increased risk of developing malignant tumors particularly sarcomas.

The present study aimed to investigate the possible role of oxidant and antioxidant status in myomas. Blood and urine samples of 21 myoma patients were collected. Activities of erythrocyte antioxidant enzymes [copper-zinc superoxide dismutase (CuZn-SOD), catalase (CAT), glutathione peroxidase (GPx1)] and levels of lipid peroxidation biomarkers [plasma malondialdehyde (MDA) and urine 8-epi-prostaglandin $F_{2\alpha}$ (8-epi-PGF2α)] were determined. The results were compared with those of 21 controls. The groups were matched in terms of age, body mass index, smoking habit, coexisting chronic diseases, menopausal status and sex steroid hormone levels.

All antioxidant enzyme activities were higher (37% for CuZn-SOD, $p = 0.003$; 52% for CAT, p0.05) and the levels of lipid peroxidation biomarkers were lower (%59 for MDA, $p = 0.011$ and 43% for 8-epi-PGF2α, $p > 0.05$) in myoma patients compared to controls. Correlation analyses showed a significant negative correlation between erythrocyte CuZnSOD activity and plasma MDA levels ($r = -0.431$, $p = 0.005$).

The decreased lipid peroxidation may be the consequence of elevated antioxidant enzyme activities and the data suggests a protective role of activated antioxidants especially CuZnSOD and CAT in patients with myoma.

P-09.04.4-050
Investigation of ischemia-modified albumin levels in patient with acute limb ischemia

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Introduction: Acute limb ischemia commonly occurs as a result of embolus caused by cardiac origin and which may end up with limb loss or even death if left untreated. Thrombosis are usually seen where the arteries give branches and tendency to atherosclerosis is more serious at these sites. Involvement of several arteries in either embolus or in situ thrombosis limits the adequacy of collateral circulation. Restriction of blood flow due to arterial stenosis or occlusion often leads patients to complain of muscle pain on walking. Any further reduction in blood flow causes ischemic pain at rest, which affects the foot. Early recognition may prevent limb loss or death. Ischemia can alter the capacity of the amino terminus of the albumin to bind free metal atoms such as cobalt, copper and nickel. This new, chemically changed albumin is called ischemia modified albumin (IMA). IMA is a sensitive marker of myocardial ischemia, skeletal muscle ischemia, pulmonary embolism, mesenteric ischemia and stroke. Therefore, in this study it was aimed to investigate the IMA level in acute limb ischemia.

Materials and methods: In this study, 24 patients with acute limb ischemia (LI group; mean age 62 years) and 34 healthy individuals (control group; mean age 42 years) were included. IMA levels were detected in control and LI group by ELISA (Organo Teknika, Avusturya) using IMA ELİSA kit.

Results: IMA values were compared with nonparametric methods Mann Whitney U test, and significantly decreased IMA level was statistically significantly different between LI group and control group ($p < 0.001$).

Conclusion: There is a significant increase in serum IMA in limb ischemia, so that alterations also might be clinically useful in the diagnosis of limb ischemia, but should be supported with further studies.

P-09.04.4-051
Antioxidant status in women with polycystic ovary syndrome

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Object: Polycystic ovary syndrome (PCOS) is a multifaceted disorder with a pathogenetic pathway that is not fully understood yet. Apart from hormonal derangements, insulin signaling defects and adipose tissue dysfunction, oxidative stress, defined as an imbalance derived from excessive formation of oxidants in the presence of limited antioxidants defenses, has been actively

implicated in the etiology of the syndrome. The aim of this study was to determine of serum myeloperoxidase activity (MPO), paraoxonase 1 activity (PON1) and arylesterase activity (ARE) in patients with PCOS.

Material and methods: The study was carried out on 150 women consisted of 103 patients with PCOS and 47 healthy ones as control. Serum PON1 activities were measured spectrophotometrically using diethyl-p-nitrophenylphosphate as substrate. Phenylacetate was used as substrate for ARE measurement, and ARE activity was determined by measuring absorbance of the resulting phenol at 270 nm. Molar absorptivity coefficients were used in the calculation of PON and ARE activities as 1 nmol phenol/ml serum/min.

Result: The MPO and ARE activities were significantly lower in the patient groups when compared with the control group (72.09 ± 60.66 - 119.74 ± 90.81 U/ml $p < 0.001$, 47.43 ± 12.21 - 83.93 ± 26.38 U/ml $p < 0.001$, recpectively). The PON1 activities are higher in the patient group (145.55 ± 98.23 U/ml) compared to the control group (153.33 ± 101.90 U/ml) are found, but are not statistically significant.

Conclusion: Lower serum MPO and ARE activities might contribute to the increased susceptibility for the development of diseases risk in women with PCOS. Because free oxygen radicals are thought to contribute to the complication of many chronic diseases, the PCOS may be related to oxidative stress.

P-09.04.4-052
The association between subclinical hypothyroidism and levels of ischemia-modified albumin

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Subclinical hypothyroidism, defined as an elevated serum thyroid stimulating hormone level associated with serum thyroid hormone concentrations within the reference range. Free radical-mediated oxidative stress has been implicated in the pathogenesis of thyroid disorders. The ischemia-modified albumin (IMA) has been proposed as a marker of protein oxidative damage, which has been found to reflect hypoxic stress. This study aimed to investigate the influence of subclinical hypothyroidism on serum IMA levels.

Thirty-one subclinical hypothyroidism patients and 27 control subjects were enrolled in the study. Albumin, IMA were measured and IMA/albumin ratio was calculated. To determine the IMA levels the measurement method based on albumin cobalt binding assay was used.

Serum IMA levels of patients with subclinical hypothyroidism were 0.58 ± 0.08 ABSU, IMA levels of control subjects were 0.49 ± 0.08 ABSU. IMA levels were significantly higher in patients with subclinical hypothyroidism patients than in control subjects ($p < 0.0001$). When IMA values were normalized for albumin concentrations, the IMA/albumin ratio was also significantly elevated in patient group compared to control group ($p < 0.01$).

IMA levels are increased in patients with thyroid dysfunction. Elevated levels of IMA can be a clinically useful marker of protein oxidative damage in subclinical hypothyroidism.

P-09.04.4-054
The effects on endothelial dysfunction of quercetin in streptozocin-induced diabetic rats

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Background/Aims: The activation of lectin-like oxidized low density lipoprotein receptor-1 (LOX-1) on endothelial cells leads to intracellular oxidative stress and inflammation and a feed-forward cycle of injury in diabetes, since both oxidized low density lipoprotein (oxLDLs) and glucose increase LOX-1 expression. Quercetin (QR) is part of a subclass of flavonoids called flavonols. Polyphenolic compounds affect the development of atherosclerosis not only through antioxidant properties but also by modulation of serum lipids, thereby influencing the immune and inflammatory processes associated with the development of atherogenic diseases. We investigated the effects of dietary QR on endothelial dysfunction mediated by oxidized low density lipoprotein (oxLDL)/lectin-like oxidized low density lipoprotein receptor-1 (LOX-1) in animal model of type 2 diabetes mellitus.

Methods: We compared 4 groups of male adult Wistar albino rats: a control group, an untreated diabetic group, diabetic groups treated with QR, and QR group. Diabetes was induced by a single injection of STZ (50 mg/kg). Animals were kept in standard condition. In 30 day after inducing diabetic, serum was collected for biochemical parameters. Glucose, lipid profiles, microalbuminuria, oxLDL and LOX-1 levels were determined.

Results: Serum triglyceride, LDL, VLDL levels in diabetic control group (without treatment) was significantly higher than control group (normoglycemic untreated group). Supplementation with quercetin decreased serum total cholesterol and increased HDL-cholesterol compared with the control group. Serum oxLDL and LOX-1 levels in diabetic control group (without treatment) were significantly higher than control group (normoglycemic untreated group).

Conclusions: Consumption of quercetin reduced oxLDL and LOX-1 levels. Thus, quercetin could be effective in improving hyperglycemia, dyslipidemia, and endothelial damage in type 2 diabetes.

P-09.04.4-055
Investigation of some oxidative stress parameters in Bdnf heterozygous mice liver tissue

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Brain-derived neurotrophic factor (BDNF) is a neurotrophin which is responsible for protection, development and plasticity of central and peripheral nervous system. BDNF is primarily synthesis in neurons, therefore BDNF and its receptor TrkB are synthesize in skeletal muscle, hearth, liver and adipose tissue. Recently, researchers were focused on BDNF in order to investigate its energy metabolism associated dysfunctions. Reactive oxygen species (ROS) physiologic concentrations are mandatory for normal redox situation, cell function and intracellular signalization. However, ROS are

The FEBS Journal **283** (Suppl. 1) (2016) 129–417 DOI: 10.1111/febs.13808

excessively produced in pathologic conditions. Ultimately, imbalance between oxidants and antioxidants results with oxidative stress (OS). In this study, we investigated some OS parameters in standard (20% protein, 70% (7% sucrose) carbohydrate, 10% lipid) and sucrose (20% protein, 70% (35% sucrose) carbohydrate, 10% lipid) diet fed BDNF heterozygous mice liver tissues.

The male C57BL6 strain wild type (+/+) and BDNF heterozygous (+/−) mice (5 weeks) were obtained. The animals were fed ad libitum by special standard and sucrose diets. Twenty four mice were divided into four groups and each group consist six mice. All mice were fed for 16 weeks. First group involved in C57BL6 wild type mice and fed by standard diet. Second group contained C57BL6 BDNF heterozygous mice and fed by standard diet. Third group consisted C57BL6 wild type mice and fed by sucrose diet. Fourth group involved in C57BL6 heterozygous mice and fed by sucrose diet.

In first group, MDA levels, SOD and CAT activities were higher than other groups. In second group, CAT activities were lower than other groups. But, we could not find any statistically significant differences between all groups about MDA, SOD, CAT levels in BDNF heterozygous mice liver tissues.

In conclusion, standard and sucrose diet feeding may not affect MDA, SOD and CAT levels in BDNF heterozygous mice liver tissues.

P-09.04.4-056
Effect of high fat diet on liver oxidative stress parameters in BDNF heterozygous mice

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Brain-derived neurotrophic factor (BDNF) is member of neurotrophin family which plays critical roles in the development, differention, survival, maintenance of the central and peripheral nervous systems. BDNF also contributes to food intake and body weight control. BDNF heterozygous mice display increased body weight and mild hyperphagia. Expression of BDNF is not limited to the brain, it also express some peripheral tissues like adipose tissue, liver, kidney, skeletal muscle, heart. Even though roles of BDNF are well known relatively in central nervous systems, effects of this protein is not clear in peripheral tissues. As mentioned before, it is expressed in organs involved in energy, lipid and glucose homeostasis, including the liver, adipose and muscle tissues, but its role there remains unclear.

In this study, we aimed to investigate role of BDNF on liver oxidative stress parameters in heterozygous mice model fed fat diet induced obese mice. In this study, we used C57BL/6 mice inbred strain wild type and BDNF heterozygous (+/−) mice. Animals were divided to two groups: wild type (n = 5) and BDNF heterozygote mice (n = 5). The animals were fed ad libitum by high-fat diet during 4 month. Weight gain was recorded every 15th days. In liver tissues were measured malondialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT) by spectrophotometric methods.

Liver MDA levels decreased in obese BDNF heterozygous mice compared to obese wild type group and statistically significant difference between groups. BDNF heterozygous mice CAT activities were higher than the other group and this difference was statistically significant. There was no statistically significant difference between the groups in terms of SOD activities.

It has been concluded that the MDA levels and SOD enzymes activities changed at high-fat diet induced obese BDNF heterozygous mice compared to wild type mice liver tissues.

P-09.04.4-060
Disturbances of microelements profile in serum of overweight/obese adult females with acute and persistent pro-inflammatory *Chlamydia pneumoniae* infection

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In some adult and elderly populations the acute and/or persistent infection with the common intracellular respiratory pathogen *Chlamydia pneumoniae* (CHL) may be associated with increased risk of developing obesity or cardiovascular disorders. Thus, 19 microelements modifying oxidative stress status were determined by ICP-MS/MS in the HNO_3 diluted serum samples collected from CHL-positive adult females (n = 39) living in urbanized area in Poland. CHL infection was confirmed by IgG+ antibody ELISA and real-time PCR assay. All females were classified under their body-mass index values to the normal-weight (NW), over-weight (OW) and obese group (OB). Control groups comprising the age-matched CHL-negative adult female subjects (NCH, n = 64). Prevalence of CHL infection increased with age of studied females and was significantly higher among OW/OB subjects in comparison with NW females (44.78 versus 20.9%; $p < 0.03$). The significantly ($p < 0.05$) increased concentration of Mn, Fe, Cu, Zn, Sb, Ba, Hg and decreased concentration of B, Al, V, Co, Ni, As, Cd, Pb were observed in serum of the NW, OW and OB females indicating CHL infection. In each female subgroup the serum concentration ratio Cu/Zn and Cu/Fe significantly decreased after CHL infection. Compare to the NCH females the strongest decreased serum Cu/Zn and Cu/Fe ratio (1.5 and 3.0 fold, respectively) was observed in the OB-CHL group. Similarly, the highest increase in the serum Fe concentrations was noted in the OB-CHL (106%) females followed by OW-CHL (53%) and NW-CHL (19%) group. Contrary, reversed increase of serum Cu concentrations after CHLA infection was observed in following order: NW-CHL(21%), OW-CHL(9%), OB-CHL(4%). In addition, infection of CHLA in females was accompanied by no significant change in serum concentrations of Mg, Cr and Se in the NW-CHL/NCH and OW-CHL/NCH group. However, in the OB-CHL group a significant increase (8%; $p < 0.05$) of serum Se was noted which could attenuate CHL related systemic inflammation.

P-09.04.4-061
Determination of reactive oxygen species induced dna damage using modified cupric reducing antioxidant capacity (CUPRAC) colorimetric method

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Reactive oxygen species (ROS) term is a common name of a group of species. Hydroxyl radical and singlet oxygen can be taken into account as ROS samples. ROS may be formed as a result of endogenous or exogenous reasons. Although ROS have some beneficial functions, they should be balanced by antioxidants (AOx). Excessive amounts of ROS can attack biological

macromolecules including DNA. DNA damage is usually related with mutagenic and carcinogenic changes. That's why determination of DNA damage is so important and there are a great many studies in literature comprising different techniques. One of the most common of them is the 'comet assay'. But application of the method and interpretation of the results is not easy. Investigation of certain DNA damage products is also very common. These methods usually need expensive instrumentation such as using tandem mass spectrometry. On the other hand, depicting total DNA damage on a certain product may cause misinterpretations.

In the presented study, DNA was decomposed by hydroxyl radicals produced by Fenton method. In the study while DNA is not CUPRAC reactive the oxidation products can react with the CUPRAC reagent. The effect of AOx was also investigated. For this purpose, selected AOx compounds were added to the reaction medium. Because of their radical scavenging effect, the CUPRAC absorbance decreased in the presence of AOx. In the presence and absence of AOx, absorbance differences were calculated. The calibration graphs between final concentration and absorbance differences were drawn for each AOx. Gallic acid was determined as the most effective one among the tested AOx samples. For statistical comparison with the presented study, TBARS was used as reference method.

Direct use of DNA as a probe material to determine oxidative damage may be an advantage to understand DNA hazard in biological systems. The proposed method can be applied in all laboratories having a spectrometer as a cost-effective and simple procedure.

P-09.04.4-062
Effects of alpha-1 antagonists on oxidative system of rat heart tissue

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Benign prostate hyperplasia is a progressive process occurring in the stromal and epithelial components of the prostate. Alpha-1 receptor blocking agents are used for relaxation of the smooth muscles in the prostatic stroma. Our aim was to investigate the effects of alpha-1 antagonists on oxidative system of rat heart tissue.

33 male wistar albino rats were divided into 5 groups randomly. 1) tamsulosin (1 mg/kd/day), 2) terazosin (5 mg/kg/day), 3) doksazosin (25 mg/kg/day), 4) alfuzosin (10 mg/kg/day), 5) control. All drugs were administered every other day single doze via oral. Rats were sacrificed after 30 days. Heart tissue was taken for biochemical analysis. Malondialdehyde (MDA), nitric oxide (NO), protein carbonyl (PC) levels and superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) enzyme activities were determined in supernatant samples.

There was not an significant difference between terazosin, doxazosin, alfuzosin, tamsulosin groups in means of SOD, MDA and GSH-Px levels. NO levels were significantly different between tamsulosin group and the control group ($p = 0.004$). In addition, tamsulosin group and terazosin group were also significantly different ($p = 0.032$). According to these results we can say that tamsulosin group had higher NO levels than control and terazosin group.

Tamsulosin's enhancer effect on NO levels leads to relaxation of the heart muscle and vascular relaxation, and so fewer side effects than other alpha antagonists.

P-09.04.4-063
The effect of rat liver tissue radical metabolism and the protective role of *Hippophae rhamnoides* L. on cold and immobilization stress model

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Cold and immobilization stress is a widely used model for study the changes that occur on oxidant-antioxidant balance. *Hippophae rhamnoides* L. (Seabuckthorn; SBT) a unique and valuable plant has recently gained worldwide attention, mainly for its medicinal and nutritional potential. This study was aimed to investigate the protective role of SBT which is a natural herbal product with high antioxidant content on oxidative and nitrosative stress induced by cold and immobilization stress in rats.

32 Wistar albino rats were divided into 4 groups randomly. Control (i.p. physiological saline), SBT (i.p. 200 mg/kg/48 hours SBT), Stress (i.p. physiological saline; 6 hours cold + immobilization stress) and SBT + Stress (i.p. 200 mg/kg/48 hours SBT; 6 hours cold + immobilization stress) groups were formed. 3-nitrotyrosine levels were determined by ELISA whereas total antioxidant capacity, total thiol, total glutathione, nitrite-nitrate levels and superoxide dismutase and glutathione peroxidase activities were measured by colorimetric methods.

SBT + Stress group nitrite-nitrat ($p = 0.0001$), total glutathione ($p = 0.0001$) levels and glutathione peroxidase activities ($p = 0.0001$) were found to be significantly higher whereas superoxide dismutase activity was found to be lower ($p = 0.007$) when compared to Stress group. There was no significant differences between Stress group total thiol and total antioxidant capacity levels compared with Stress + SBT group. Stress + SBT group oxidative and nitrosative stress marker 3-nitrotyrosine level was found to be significantly higher when compared with Control group ($p = 0.002$) whereas there was no significant differences between Stress and Stress + SBT groups.

All this data show that SBT has antioxidant properties on cold and immobilization induced oxidative and nitrosative stress in rat liver tissue.

P-09.04.4-065
Investigation of the antiulcerogenic effects of ether extract of *Hippophae rhamnoides* L. leaf on indomethacine-induced stomach ulcer in rats

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Introduction: *Hippophae rhamnoides* L. (wild spindle) has been extensively used in traditional medicine as antiulcerogenic in Eastern Turkey. Peptic ulcer is one of the major gastrointestinal

disorders with an incidence of approximately 10% of the world population. Although there are many drugs currently used in the treatment of peptic ulcer, such a drug providing radical treatment without side effects is not available. Since oxidative stress is involved in peptic ulceration, this study was designed to investigate antiulcerogenic and antioxidant effects of *Hippophae rhamnoides* L. ether extract on indomethacine-induced stomach ulcer in rats.

Materials and methods: Thirty-five *Sprague Dawley* male rats (weights ranging 180–220 g) were randomly divided into 7 groups, as each composed of 5 rats. After *Hippophae rhamnoides* L. leaf ether extracts of 100 mg/kg, 250 mg/kg and 500 mg/kg doses and 20 mg/kg doses of famotidin orally administered, 25 mg/kg doses of indomethacine were orally applied to rats in order to make ulcer. On the sixth hour of indomethacin administration all rats were sacrificed using thiopental (50 mg/kg). The stomachs were removed, and ulcer areas were evaluated macroscopically. Superoxide dismutase activity (SOD), glutathione (GSH) and malondialdehyde (MDA) levels in stomach tissues of rats were determined by ELISA method with respective kits.

Results: İt is determined that three doses of *Hippophae rhamnoides* L. significantly recover the ulcer disease in rats. *Hippophae rhamnoides* L. administration provided significance increment in superoxide dismutase activity and glutathione level, and significant reduction in malondialdehyde levels compared to control group ($p < 0.05$, at all parameters).

Conclusions: We can conclude that the ether extract of *Hippophae rhamnoides* L. leaves reduces free radical formation and has antiulcerogenic effects on stomach tissue. This study was supported by Ataturk University (project number: 2013/58).

P-09.04.4-066
Effect of oxidative stress on cognitive functioning in children and adolescents with obesity

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Obesity is a major health problem with growing incidence and accompanying complications. Its relation with diminished cognitive functions was reported. This study aims to evaluate the effects of obesity induced oxidative stress and metabolic alterations on the cognitive functions of children and adults.

33 children and adolescents with obesity (age: 8–18); and 33 age and gender matched healthy subjects were enrolled. All subjects completed the battery tests of CNSVS via computer. The scores were compared by using commercial software (IBM SPSS Statistics 19). Biochemical parameters, malondialdehyde (MDA) and protein carbonyl (PC) levels were estimated.

MDA and PC levels were significantly higher in subjects with obesity (0.78 ± 0.16 μmol/l;198.30 ± 84.45 nmol/ml) than the controls (0.5 ± 0.10 μmol/l; 125.35 ± 43.52 nmol/ml) (<0.001). There was statistically significant difference between study and control groups on all cognitive performance domains. Significant correlation was detected between MDA, PC levels and the cognition indexes.

Children with obesity should be evaluated for the cognitive functions, together with the metabolic follow-up. Obesity induced oxidative stress may be the reason of the diminished cognition in children as well as the changes in the lipid profile and inflammation, but we need larger study groups to lighten these complex process.

P-09.04.4-067
Relative contribution of nitric oxide synthase (NOS) isoforms to oxidative/nitrosative stress in the cerebral cortex of rat with acute liver failure (ALF)

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Acute liver failure (ALF) is associated with deregulation of NMDA/cGMP/NO signaling and oxidative/nitrosative stress in the brain. However, the relative roles of the different NOS isoforms and the mechanisms underlying alterations in their activities during ALF are not fully clear. Here we investigated gene and protein expression of NOS isoforms, NOS activity, eNOS uncoupling and total NO production in cerebral cortex of rats with thioacetamide (TAA)- induced ALF. Sprague Dawley rats (250–280 g) received three i.p. injections of TAA (300 mg/kg) at 24 hours intervals. The brain cortex expression NOS isoforms (eNOS/iNOS/nNOS) was measured by Real-time PCR and Western Blot, NOS activity was tested by monitoring the conversion of radiolabeled arginine to citrulline. Reactive oxygen species (ROS) were quantified in the presence of NOS substrate L-arginine, using the carboxy-H_2DCFDA probe. NO was measured with the Griess procedure. The eNOS expression was decreased, whereas the eNOS dimmer/monomer ratio and nNOS/iNOS expression were elevated in TAA treated rats. While the total NOS activity was decreased, the iNOS activity was elevated and NO concentration tended to increase. ROS production was elevated by TAA. Unspecific NOS inhibitors L-NAME and L-NNA attenuated ROS production in both control and TAA rats, but with higher efficiency in the latter case. Ca^{2+} chelation had almost the same effect as pharmacological NOS inhibition suggesting that Ca^{2+} -independent iNOS activity is not the main source of ROS. Incubation with high dose of tetrahydrobiopterin (BH4) with which is critical for eNOS dimerization and subsequent NO production also reduced ROS production indicating the eNOS uncoupling phenomenon in TAA cortex. The study points to eNOS downregulation due to lowered protein expression and uncoupling as a novel mechanism contributing to enhanced superoxide O_2^- anion formation, and confirms the role of iNOS/nNOS in enhancing NO synthesis in ALF-affected brain.

P-09.04.4-068
Antioxidant and antidiabetic activity of ethanol extract of *Myrtus communis* L. berries on streptozotocin-induced diabetic rats

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Introduction: Diabetes mellitus (DM) is an endocrine disorder of world which is characterized by altered blood glucose levels and related complications including hepatic injury. *Myrtus communis* L. (MC) is widely used by diabetic patients in the folk medicine of Turkey as well as they are used worlwide. It is known that of leaves, oil and fruit of *Myrtus communis* L. (MC) have therapeutic effects on diabetes mellitus (DM). This study was aimed to analyze the possible antidiabetic and

hepatoprotective effects of MC berries in streptozotocin (STZ) induced diabetic rats.

Materials and methods: A total of thirty rats composed of six groups as each included five rats were used. 40 mg/kg STZ was injected once to animals to induce DM. After STZ injection, rats were exposed to three different ethanol extracts of MC berries (250, 500 and 1000 mg/kg) by oral gavage during 14 days.

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were determined in serum and glutathione (GSH), malondialdehyde (MDA) levels and superoxide dismutase (SOD) activity were determined in liver tissue.

Results: MC administration provided significant reducement in the altered serum glucose, AST and ALT levels in all diabetic groups. MC extract showed significant antioxidant activity by altering SOD activity and GSH level and reducing MDA levels in diabetic rats compared to controls ($p < 0.05$). Serum AST and ALT levels were reduced by MC administration in all diabetic groups. MC administration provided significance increment in SOD activity and GSH level, and significant reduction in MDA levels compared to controls ($p < 0.05$). The maximum hypoglycemic and antioxidant effects were observed at 1000 mg/kg dose of MC.

Discussion and conclusion: Results demonstrated that ethanol extract of Myrtus communis L. has antidiabetic and antioxidant activity. Our study confirms the traditional usage of this plant as an antidiabetic herb.

P-09.04.4-069
The effect of calcium on the antioxidant activities of PON1Q192R isoenzymes

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Background: Human serum paraoxonase 1 (PON1) is a calcium dependent esterase that hydrolyzes organophosphates and also arylesters such as phenyl acetate. PON1 prevents LDL oxidation by hydrolyzing lipid peroxides. PON1 is inhibited by various chelating agents, heavy metal ions, and sulfhydryl reagents. In our study we investigated the effect of calcium on LDL oxidation of purified PON1 Q192R isoenzymes.

Methods: PON1 Q192R isoenzymes were partially purified from human serum. Both allozymic forms were treated by preincubation with 1 mM EDTA for 15 minutes. LDL oxidation was induced by copper ions. Formation of thiobarbituric acid-reacting substances (TBARS) was used as a measure of lipid peroxidation. Homocysteine thiolactonase (HTLase activity) and arylesterase activities were measured spectrophotometrically by using homocysteine thiolactone and phenylacetate as the substrates.

Results: Addition of 1 mM EDTA to partially purified HDL-PON1 Q192R isoenzymes inhibited 100% of HTLase and arylesterase activities. Inactivation of PON1 for arylesterase/HTLase activity by the addition of EDTA did not reduce the abilities of both allozymic forms in protecting LDL from oxidation.

Conclusion: Ca^{+2}-dependent inhibition of PON1 Q192R arylesterase/HTLase by using the metal chelator EDTA, did not alter PON1's ability to inhibit LDL oxidation. PON1's ability to protect LDL from oxidation may not require calcium.

P-09.04.4-070
Evaluation of cholinesterase inhibitory effect, anti-radical and anti-lipid peroxidation activities of *Mentha pulegium*

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Introduction: Many studies indicated that intake of dietary and medicinal plants is effective in preventing or suppressing many diseases, therefore, there is a growing interest in plant'sbioactive compounds. *Mentha pulegium*, is widely used in gulf countries in herbal teas or as additives in commercial spice mixtures for many foods to offer aroma and flavor.

The aim of this study is to investigate the in vitro radical activity, the total phenol and flavonoid content, anti-lipid peroxidation and the cholinesterase inhibitory effects of *Mentha pulegium* methanol extract.

Methods: The acetylcholinesterase and butyrylcholinesterase inhibitory potentials of extracts, were evaluated by colorimetric assay. The in vitro antioxidant activity was measured by DPPH assay, the total phenols content was measured by folin–ciocalteau assay, the flavonoids content by the $AlCl_3$ colorimetric method, and the protective effect of menthe *Mentha pulegium* extracts against lipid peroxidation was evaluated using a liposome oxidation system.

Results: The methanol extract showed a scavenging activity nearly equivalent to Vitamin C which is attributed to its high phenolic and flavonoid contents. The extract possessed protective effect against lipid peroxidation in a dose dependent manner. The methanol extract shows very little anticholinesterase activity as compared to the standard compound, physostigmine.

Conclusion: Results presented here indicate that *Mentha pulegium*possess strong antioxidant activity and protective effects and they can therefore be used as a natural additive in food, cosmetic and pharmaceutical industries.

P-09.04.4-071
Evaluation of micronuclei in the buccal epithelial cells of type 2 diabetic patients

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Type 2 Diabetes Mellitus is a long term metabolic disorder that is characterized by hyperglycemia and insulin resistance. Because of the hyperglycemia and free radicals, diabetes can cause cellular instability. Micronuclei is a sensitive indicator of genetic damage and a marker of DNA damage. Micronuclei is also a morphological marker of chromosomal instability. In this study, we aimed to evaluate the frequencies of micronuclei in Papanicolaou stained buccal cells of type 2 diabetic patients.

A total of 30 type 2 diabetic patients and 30 healthy individuals were involved into our study. Buccal smear samples that belong to these individuals were stained by using Papanicolaou method for cytologic examination and the stained slides were evaluated by light microscopy (Olympus BX-51).

Cells with micronuclei in each Papanicolaou stained buccal smear sample were counted under light microscopy. The frequency of micronucleated epithelial cells were seen as significantly higher in type 2 diabetic patients than the control group ($p < 0.05$).

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The frequency of micronuclei may reflect the DNA damage in the buccal epithelial cells. Micronucleus scoring may help to screen DNA damage of epithelial cells in type 2 diabetic patients. Further studies are needed to investigate the potential role of type 2 diabetes on DNA damage.

P-09.04.4-073
The effect of sodium perborate tetrahydrate some antioxidant enzyme activities

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One of the boron compounds is sodium perborate tetrahydrate ($NaBO_3.4H_2O$), which the most widely used solid peroxygen compounds. It is used in safety bleach formulations, detergents and tooth powders. As known these products are commonly used in daily life. However, the actions on blood antioxidant defenses of sodium tetraborate against reactive oxygen species are not identified yet. It is reported that oxidative stress caused by ROS damages.

In this study, we searched enzyme activities of superoxide dismutase (SOD), catalase (CAT), glutathione-*S*-transferase (GST), glutathione reductase (GR), glutathione peroxidase (GSH-Px) and glucose-6-phosphate dehydrogenase (G6PD) also the effect sodium perborate tetra hydrate on activities of these enzymes from human erythrocyte under in vitro conditions. According to our findings sodium perborate tetrahydrate caused significant ($p < 0.05$) increasing in the CAT activity from red blood cell. The other antioxidant enzyme activities (SOD, GST, GR, GSH-Px and G6PD) did not show any changing by influence of sodium perborate tetrahydrate.

P-09.04.4-074
Inflammatory markers and oxidative stress

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Metabolism of obese individuals could be exposed to risk of chronic low-grade pro-inflammatory effect and oxidative stress. Some inflammatory and oxidative markers have been studied recently. Plasma total antioxidant status (TAS) and total oxidant status (TOS) parameters can be non-invasive markers of diseases such as fatty liver disease, laparoscopic procedures (pneumoperitoneum), accompanying inflammatory condition like urinary tract infection, diabetic neuropathy, chronic hepatitis.

The study groups have been comprised of two groups with normal to over-weight children. TAS and TOS levels were detected and the oxidative stress index (OSI) was computed as a marker of the grade of oxidative stress. The over-weight group displayed higher levels of fasting glucose, insulin resistance, the body mass index. Also, we know that insulin resistance leads to increased lipolysis and free fatty acid output. Higher TOS as well as CRP is related to the group, also lower TAS than other group is shown. CRP levels in plasma were positively correlated with insulin and glucose levels. In addition, there was a significant relationship between OSI and insulin resistance in the over-weight group.

TAS and TOS are together more accurate sings of oxidative and antioxidative status of people. As well as a raise over weight-related subclinical inflammation and a fall antioxidant capacity is significant even in children. This condition may eventually develop the risk of long-term vascular damage.

P-09.04.4-075
The effects of hydrogen peroxide pre-treatment on antioxidant enzyme activities in calli tissues of two eggplant genotypes under salinity

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The effects of hydrogen peroxide (H_2O_2) pre-treatment on catalase (CAT) and superoxide dismutase (SOD) were investigated and lipid peroxidation measured as malondialdehyde (MDA) content of the calli from salt-sensitive (Artvin) and salt-tolerant (Mardin) eggplant genotypes under salinity stress.

The seeds from each genotypes were germinated on MS medium for 4 weeks and hypocotyl tissues from these plantlets were used as explants for calli induction on MS medium including 1 mg/l 2,4-D and 0.1 mg/l kinetin. As for the pre-treatment, the subcultured calli tissues were transplanted on the mediums containing 50 and 100 μM H_2O_2 for 48 hours and then transplanted on the mediums including 150 mM NaCl for 24 hours. Antioxidant enzyme analysis and MDA measurement was carried out for the Control, NaCl-only, H_2O_2-only and H_2O_2 pre-treated tissues.

Pre-treatment with H_2O_2 decreased the deleterious effects of salt stress on MDA contents. In comparison with salt stressed groups, H_2O_2 pre-treatment with or without NaCl reduced MDA content especially in Artvin. Comparing two genotypes, a decrease was observed on SOD activity in Artvin genotype and an increase in Mardin genotype by comparison of salt stressed groups. Higher increase on SOD activity was observed in 100 μM H_2O_2 + NaCl groups on each genotypes. Comparing two genotypes, a decrease was observed on SOD activity in Artvin genotype and an increase in Mardin genotype by comparison of salt stressed groups. Higher increase on SOD activity was observed in 100 μM H_2O_2 + NaCl groups on each genotypes.

The result showed pre-treatment of 100 μM H_2O_2 induced acclimation of the plants to salinity. In addition, 100 μM H_2O_2 pre-treatment, as a stress signal, could trigger the activation of antioxidant enzymes in calli and in this way alleviated the oxidative damage in calli growth under salinity.

P-09.04.4-076
The investigation of effects of ghrelin and cannabinoid CB1 receptor agonist and antagonist on oxidant and antioxidant mechanisms on brain tissues of penicillin-induced epileptic rats

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The aim of this study is to investigate the individual effects of ghrelin and cannabinoid type1 (CB1) receptor agonist ACEA,

the antagonist AM-251 and the interaction of these two different systems on oxidant and antioxidant systems in the brain, cerebellum and brain stem tissues of penicillin-induced epileptic rats.

In this study 56 male Wistar albino rats were used weighing 20–250 g. Each group was consisted of 8 rats. Study groups: 1: Control, 2:Penicilin(500 IU), 3:Penicillin(500 IU) + Ghrelin(1 μg), 4:Penicillin(500 IU) + AM-251(0.25 μg), 5:Penicilin (500 IU) + ACEA(7.5 μg), 6:Penicillin(500 IU) + AM-251(0.25 μg) + Ghrelin (1 μg), 7:Penicillin(500 IU) + ACEA(7.5 μg) + Ghrelin(1 μg). Than the levels of MDA, GPx and SOD are measured in plasma and tissue samples of these rats.

Penicillin was found to be induced lipid peroxidation in the brain, cerebellum and brain stem tissues in our study. Ghrelin and ACEA, which both have anticonvulsant effects, were shown to be effective in reversing the oxidative damage caused by penicillin and proconvulsant AM251 was found to further increase the oxidative stress caused by penicillin in these tissues. Ghrelin also was found to suppress the oxidative stress caused by AM251 in the cerebellum tissue but it did not contribute to antioxidant effects produced by ACEA.

Since the role of oxidative stress in epilepsy has been established, it may be suggested that ghrelin and ACEA may have anticonvulsant effects via their antioxidant features. The discovery of inhibitors for enzymes that metabolize endogenous ghrelin and cannabinoids through new studies may contribute to the improvement of seizure resistance in epilepsy.

P-09.04.4-078
Serum endocan levels in patients with ankylosing spondylitis

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Accelerated atherosclerosis in patients with Ankylosing Spondylitis (AS) give rise to increased cardiovascular morbidity and mortality. Endothelial dysfunction could be the initial process in the development of atherosclerosis. Human endothelial cell-specific molecule-1 (endocan) is a novel human endothelial cell-specific molecule. Therefore, we assessed serum endocan levels and carotid intimamedia thickness (CIMT) as a surrogate marker of atherosclerosis in patients with AS.

A total of 57 patients with a diagnosis of AS according to Newyork ctriteria and 50 control subjects were included in our study. Serum endocan, interleukin-6 (IL-6), tumor necrozis factor-α (TNF-α), C reactive protein (CRP) and CIMT were measured in all participants. Serum endocan, IL-6, TNF-α levels were measured with ELISA. The other parameters were done by routine biochemical methods.

AS patients exhibited increased serum endocan levels and CIMT compared to matched controls ($p < 0.05$). Whereas, serum IL-6, TNF-α were similar between grous. In patient with AS, there were no significant differences between active and inactive patients by means of IL-6, TNF-α, endocan and CIMT. In AS group, CIMT correlated with disease duration and age ($r = 0.597$, $p = 0.000$; $r = 0.721$, $p = 0.000$). We could not find any significant correlation between serum endocan levels and parameters studied.

Our study shows increased CIMT in AS patients without traditional risk factors such as increased BMI, lipid profile compared to controls. Although we found increased circulating endocan levels in patients with AS, the other factors could affect increased atherosclerosis in this population because of lack of correlation between endocan and CIMT. Probable biomarkers could be related to increased CIMT in patients with AS should be investigated in larger study groups.

Keywords: Ankylosing spondylitis, atherosclerosis, carotid intima media thickness, endocan

P-09.04.4-079
Investigation of pentose phosphate pathway and oxidative stress in erthrocyte infected Babesia ovis

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Introduction: Babesia infections occur in cattle, sheep, goat, horse, dog, cat pig and rodents. In this study, the effects of Babesia ovis living and present in the erythrocytes to glucose metabolism was researched. At the same time, biochemical parameters were also associated with parasitemia.

Materials and methods: Babesia ovis (Israel) cell culture was provided from Dr. Abel Martin González Oliva (Portugal). Culture passaged 48 or 72 hours according to parasitemia state (8–10%). Biochemical analyses were performed in erythrocyte culture in which parasitemia between 1% and 16%. Cell counts and hemoglobin concentration of erythrocytes culture suspension were measured at cell counter instrument and than it was washed 3 times with physiological saline, erythrocyte suspensions were stored at-80oC analysis. GSSG (oxidize glutathione), GSH (reducte glutathione), NADPH, Glukoz 6 P dehydrogenaz, GSHpx (Glutathione peroxidase), GSHRx (Glutathione reductase) were determined by commercial kits. All experiments were done in duplicate, the results were calculated by the number of erythrocytes.

Results: Parasitemia was positively correlated with GSH, NADPH and GSHRx ($p < 0.05$). A correlation between other biochemical parameters was not observed.

Discussion: The pentose phosphate pathway in erythrocytes has an important role such as to provide pentose sugar required for the synthesis of nucleic acid, to reduce glutathione, to produce NADPH and to protect from methemoglobin accumulation. In studie sthat naturally infected erythrocytes with Babesia parasites, it was seen to be caused to oxidative stress, however GSH results in these investigation were obtained differently .

Conclusion: According to the results of this study that performed in vitro, it can be suggest that their glutathione metabolism and pentose phosphate pathway of parasites may active.Key words: Babesiosis, GSH, GSSG, NADPH, G6PDH, GSHPx, GSHRx

P-09.04.4-080
In vitro protective effect of betaine on peroxidative injury caused by ethanol and aspirin exposure on rat brain synaptosomes

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Aspirin intake of specific daily doses are advised by doctors to postmenapausal women and men above 40 years of age to prevent heart attack and even cancer in recent times. In this study, the aim is to investigate the in vitro cytototoxic effects of different doses of ethanol (50 mM, 100 mM ve 200 mM) alone and

together with 100 μg/ml aspirin, and possible protective role of 1 mM betaine on rat brain synaptosomes. 15 male Sprague Dawley rat forebrains were divided into equal pieces and pooled to form 10 study groups. Synaptosomal fractions extracted from pooled rat brains were incubated with different doses of ethanol, aspirin and betaine, and malondialdehyde (MDA) levels, an important indicator of cellular damage, were measured. A significant increase ($p < 0.05$) was observed in MDA level of 200 mM ethanol group compared to control group. Different doses of ethanol (50 mM, 100 mM ve 200 mM) + aspirin exposure significantly increased ($p < 0.001$) MDA levels compared to controls, whereas betaine administration significantly decreased ($p < 0.001$) lipid peroxidation caused by ethanol + aspirin treatment. We conclude that ethanol and ethanol + aspirin administration increases lipid peroxidation in rat brain synaptosomes while betaine helps prevent this peroxidative membrane injury.Keywords: Aspirin, betaine, ethanol, malondialdehyde (MDA)

P-09.04.4-081
Analyses of mitochondrial biogenesis in hepatocellular carcinoma treated with berberine

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Objective: Berberine (BBR) has been demonstrated to have anticancer activities against various cancer types, particularly hepatoma. In this project, we aimed to reveal the effect of BBR treatment on mitochondrial biogenesis through sirtuins and HIF-1α in hepatocellular carcinoma cell line, HEP3B under hypoxia.
Method: HEP3B cells were subjected to normoxia (21% O_2) and hypoxia (1% O_2) in the presence or absence of BBR treatment. The amount of BBR was optimized via cell viability (MTS) assay under normoxia. Then, immunoblotting experiments were performed to identify the effect of BBR on HIF-1α, PGC-1α, and sirtuins involved in mitochondrial stress. The variation in the OXPHOS complexes and the level of reactive oxygen species (ROS) were also measured to investigate the effect of BBR on mitochondrial energy stress state.
Results: Here, we present that cell viability was significantly decreased at 25 μM. BBR treatment has shown significant reduction in HIF-1α and SIRT6 which responsible for up-regulation of glycolysis. Also, succinate dehydrogenase (CII) and cytochrome *c* oxidoreductase (CIII) of the OXPHOS complexes were downregulated without any change in NADH dehydrogenase (CI) or ATP synthase (CV). BBR significantly abolished to oxidative stress under hypoxia, which was demonstrated as a reduction in the level of reactive oxygen species by decreasing on SIRT3 expression. BBR induces the overexpression of SIRT1 and its deacetylated-PGC-1α, which might be an indicator of being a potent protective agent against hypoxia by normalizing mitochondrial function and inducing mitophagy in impaired mitochondria caused by deficiency of glycolysis and OXPHOS.
Conclusion: Detailed information about the communication between HIF-1α and sirtuins and their relation to mitochondrial energy production was provided with the alteration of their activity by BBR treatment. It is highly expected that BBR and its derivatives might become important during the development of supplemental therapies.

P-09.04.4-083
The effects of *Ziziphus jujuba* on human glioblastoma cells

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Introduction: Reactive oxygen species are involved in a variety of biological phenomena, such as carcinogenesis, inflammation and aging. Among the targets of ROS, DNA appears most important in tumor biology since it is firmly established that cancer is a genetic disease. ROS induce several kinds of DNA damage, including strand breakage and DNA-protein cross-linkage. Fruit of *Zizyphus jujuba*, a traditional Chinese herb widely consumed in Asian countries, has been reported to possess several vital biological activities. This study intends to evaluate their antioxidant activity on glioblastoma cells.
Materials methods: Cell survival was quantified by colorimetric MTT assay. Human gliblastoma cells were pretreated with 100 μM H_2O_2 after 30 minutes 100 μM *Ziziphus jujuba* essential oil was added to the cells for three hours. Then, the cell homogenates were taken and glutathione, Total oxidant and total antioxidant capacity and nitric oxide levels were estimated using spectrophotometric methods.
Results: *Ziziphus jujuba* treated cells could prevent intracellular glutathione from being depleted following an exposure of H_2O_2. Also our data suggest that *Ziziphus jujuba* is effective in preventing H_2O_2 induced oxidative stress and nitric oxide levels.

Discussion: Some research showed that H_2O_2 was over produced in the pathological process of acute and chronic neuronal toxicity, the toxicity effect of β-amyloid on the cultured neuron and neuronal cell line was mediated by H_2O_2. The traditional medicine recommend several medicinal plants for providing relief from various inflammatory diseases. Many research has been reported that the essential oil from seeds of helping to prevent the oxidative stress and neuronal diseases in brain.
Conclusion: The antioxidant potential of *Ziziphus jujuba* may be attributed to the presence of flavonoids and the other constituents present therein. The findings substantiate the therapeutic applications of the plant in the indigenous system of medicine.

P-09.04.4-084
A new biosensor for rapid determining of oxidant agent hydrogen peroxide

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Introduction: Toxicity by oxygen radicals has been recommended as a major cause of cancer, heart disease, and aging. Oxygen radicals and other oxidants appear to be toxic in large part because they start the chain reaction of lipid peroxidation. Most of the analytical techniques for peroxide determination are generally time consuming and not very suitable for routine or on line analysis. We aimed to design a new biosensor for rapid determining of oxidant agent hydrogen peroxide.
Materials and methods: All reagents were of analytical grade unless stated otherwise, and were purchased from Sigma Aldrich. Firstly, the 2-Hidroxymetacrilate Metacriloamidoscystein nanoploymers were immobilized by binding covalently with sulphur atoms on the gold electrod's surface. Free NH_2 groups of catalase enzyme make Schiff bases between nanopolymer's carbonyl groups, then immobilization was actualysed with cross linking

reagent glutaraldehyde. We developed a biosensor system preparing ferrociyanide, selected as a mediator, in the buffer solution.

Results: PolyHemaMac nanopolymer and catalase complex were immobilized by glutaraldehite to construct a hydrogen peroxide biosensor. The responses of the biosensor are therefore proportional to the oxidation peaks of the complex at +0.019 V potential. The cyclic voltammograms obtained from the experiments showed that, pottasiumferrociyanide mediator complex positively affected the biosensor responses for hydrogen peroxide determination.

Discussion and conclusion: As a result of this study, the method developed by the catalase enzyme electrode was found to be more advantageous in comparison to other methods reported in the literature so far; it was determined that the method is sensitive, economic, practical and less time-consuming. Since biosensor technology provides economical, practical, specific and sensitive results for the determination of hydrogen peroxide, it was improved very efficiently.

P-09.04.4-085
Impact of amoxicillin, gentamicin and cefazolin sodium antibiotics on antioxidant gene expression and enzymatic activities in mouse liver

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Reactive oxygen species (ROS) are highly reactive molecules, which are produced by living organisms as a natural byproduct of the normal metabolism and environmental factors. Living organisms have the antioxidant defence systems to block harmful effects of ROS. The imbalance between oxidants and antioxidants is termed oxidative stress. The antioxidant defence mechanisms are divided into two groups as enzymatic and non-enzymatic defences. Enzymatic defence mechanisms consist of enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glucose-6-phosphate dehydrogenase (G6PD) and glutathione S-transferase (GST).

The present study was designed to determine the effects of gentamicin, amoxicillin and cefazolin sodium antibiotics on the hepatic antioxidant system and to determine any possible correlation between enzymatic and molecular levels. For this reason, effects of these antibiotics on the transcription of the antioxidant system has been investigated by real time PCR, and then the enzyme activity of these enzymes have been measured in whole liver homogenate obtained from control group and the drug administered groups mice.

Our results demonstrate that administering antibiotics led to crucial inhibition of all antioxidant enzyme activity. While significant transcriptional activation for Sod and Cat was seen in the gentamicin treated group, the transcription of Gst and G6pd was decreased. However transcriptional activation was seen for Sod and Cat in amoxicillin administered group, the transcription of Gst was decreased as compared with the control group. In the cefazolin sodium treated group, while Cat and Gst transcription were elevated significantly, the expression of Sod and G6pd were decreased.

In conclusion, gentamicin, amoxicillin and cefazolin sodium affect the hepatic antioxidant system at the molecular and protein level. This work was supported by Scientific Research Project of Ataturk University of Turkey (Grant no: 2013/296).

P-09.04.4-086
Protective effects of curcumin supplementation on oxidant/antioxidant system changes created by organic phosphorus pesticide poisoning

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Organic phosphorus pesticides (OPP), widely used in agriculture or as insecticides in home, cause adverse health effects. Chlorpyrifos is one of the most commonly used OPP. We aimed to investigate the possible protective effects of curcumin (CUR) supplementation, the principal curcuminoid of turmeric, on poisoning symptoms and oxidant/antioxidant system changes caused by chlorpyrifos.

Adult Sprague-Dawley rats were used. CUR (30, 100 and 300 mg/kg) were administered orally for 5 days. On the sixth day, chlorpyrifos (279 mg/kg, s.c.) was administered. Twenty four hours after chlorpyrifos administration, body weights, locomotor activities and body temperatures of rats were measured. Following the measurements, rats were decapitated and the blood, brain and liver tissue samples were taken and prepared for the biochemical and histopathological measurements.

Chlorpyrifos administration increased the malondialdehyde (MDA) levels but decreased catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GRX) concentrations and reduced/oxidized glutathione (GSH/GSSG) ratio in the blood samples, brain and liver tissues compared with the control group ($p < 0.05$–0.001). The concentration of advanced oxidation protein products (AOPP) were increased only in the brain tissue after chlorpyrifos administration ($p < 0.001$). CUR administration reduced all of these changes ($p < 0.05$–0.001). Similarly, CUR at the doses of 300 mg/kg reduced the decreases in body weight, body temperature and locomotor activity with chlorpyrifos ($p < 0.001$). Additionally, the histopathological damage scores induced by chlorpyrifos ($p < 0.05$–0.01) were decreased by the administration of CUR ($p < 0.05$–0.01).

Our findings suggest that CUR supplementation can ameliorate the poisoning effects of chlorpyrifos via supporting the antioxidant mechanisms and CUR could be used for protective purposes against oxidative

stress and tissue damage caused by chlorpyrifos.

P-09.04.4-087
The effect of OGTT applied for screening in pregnancy on adenosine deaminase and xanthine oxidase activity in normal and prediabetic pregnant women

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Objective: It was reported that the activities of adenosine deaminase (ADA) were different in normal pregnant women and pregnant women with gestational diabetes mellitus (GDM). It was

also stated that the activity of xanthine oxidase (XO) was increased in pregnant women with GDM. The objective of this study was to evaluate if glucose have effects on oxidative stress in prediabetic women by affecting ADA and OX after 50 g OGTT which was applied in pregnant women for screening.

Methods: The serum specimens of 39 pregnant women who applied to the outpatient clinic of the obstetrics and gynecology department and had 50 g OGTT, were used in this study. ADA and XO activities were analyzed in the serum specimens taken from the normal (n = 20) and prediabetic pregnant women (n = 19) in the 0th and 60th minutes of OGTT. ADA and XO activities were measured with the spectrophotometric method and the U/l enzyme activity was calculated.

Findings: There was no significant difference between the 0th and 60th minutes regarding the ADA activities in the normal and prediabetic pregnant woman groups. However, we observed a significant difference between 0th and 60th minutes regarding the XO enzyme activity in normal pregnant women ($p = 0.001$). In normal pregnant women, the median XO enzyme activity in the 0th minute was 0.29 (0.11–1.33) U/l and it was 0.8 (0.25–2.26) U/l in the 60th minute. Nevertheless, there was no correlation between the XO activity and glucose level. As to the prediabetic pregnant women, there was no significant difference between the XO enzyme activities in 0th and 60th minutes.

Conclusion: The results of our study showed that the XO activity increased as a response to OGTT in the normal pregnant women compared with the prediabetic pregnant women. This finding made us think that the oxidative stress caused by OGTT did not affect the XO response in prediabetic pregnant women and that there would be some adaptive mechanisms against the chronic exposure to high level glucose.

P-09.04.4-088
Assessment of antioxidant enzymes and acetylcholine esterase enzyme activities in liver, gill and brain tissues of rainbow trout grown under different light wavelengths

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Rainbow trout (*Oncorhynchus mykiss*) aquaculture continuously increases in Turkey. The objective of the present study is to increase the productivity in fish farming of rainbow trout just via intervention in physical cases without the effects of any chemicals and investigate whether this conditions cause oxidative stress.

In this experiment eight tanks were used and 44 rainbow trout larvae were placed in each tank and these tanks were illuminated with light in different wavelengths; natural sunlight, and incandescent long-wave (red light), medium-wave (green light) and short-wave (blue light) LED lights. The experiment took 64 days. Biochemical changes in rainbow trout exposed to light in different wavelengths (red, green, blue) were analysed via the variations in GR, GST, G6PD, GPx, SOD and CAT enzyme activities, which are significant for enzymatic antioxidant defence system and in AChE activity, which plays an important role on central nervous system.

Maximum activity change in liver tissue was observed for GST and G6PD enzymes in fish grown under green light and for SOD enzyme in fish grown under blue light. In gill tissue, SOD and G6PD activities were affected the most, and in brain tissue, these were GST and SOD activities. It was observed that the average weight of the fish increased 1.2 times under red and blue lights and 1.3 times under green light. The highest weight increase was observed under green light, however, antioxidant enzyme activities increased in the liver and gills and decreased in the brain tissue under this light condition.

In conclusion, it was observed that productivity was 1.2 times under red light when compared with control group and it was determined that the fish grown under red light can tolerate oxidative stress more than other wavelength.

P-09.04.4-089
Effect of *Nigella sativa* on biliary obstruction-induced oxidative stress and apoptosis in rats

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Objectives: The aim of this study was to evaluate the possible protective effects of Nigella sativa (NS) against cholestatic oxidative stress and liver damage in the common bile duct ligated rats.

Methods: A total of 24 male Wistar albino rats were divided into three groups:sham control, bile duct ligation (BDL) and BDL+received NS; each group contain 8 animals. The rats in NS treated groups were given NS (0.2 ml/kg) once a day orally for 4 weeks starting 3 days prior to BDL operation.

Results: The changes demonstrating the bile duct proliferation and fibrosis in expanded portal tracts include the extension of proliferated bile ducts into lobules, mononuclear cells, and neutrophil infiltration into the widened portal areas were observed in BDL group. Treatment of BDL with NS attenuated alterations in liver histology. The alpha smooth muscle actin (α-SMA), transforming growth factor beta (TGF-β1) and the activity of TUNEL in the BDL were observed to be reduced with the NS treatment. BDL significantly increased the tissue hydroxyproline (HP) content, malondialdehyde (MDA) levels, and decreased the antioxidant enzyme (superoxide dismutase (SOD) and glutathione peroxidase (GPx)) activities. NS treatment significantly decreased the elevated tissue HP content and MDA levels and prevented the inhibition of SOD and GPx enzymes in the tissues.

Conclusion: The data indicate that NS attenuates BDL-induced cholestatic liver injury, bile duct proliferation, fibrosis, apoptosis and oxidative stress. The hepatoprotective effect of NS is associated with antioxidative potential.

P-09.04.4-090
Molecular studies of organophosphate toxicity in cultured neural cells

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Organophosphorous compounds (OPs) are used widely as pesticides for agricultural purposes, as oil additives or as flame retardants. However, due to their widespread use, there are major

human safety concerns, since that these agents may not only cause acute toxicity via inhibition of acetylcholinesterase but they can also induce delayed toxicity in the nervous system. A key interest to the current work is the potential correlation between gene expression and cytoskeletal protein changes in differentiating neural cells exposed to sub-lethal neurite outgrowth inhibitory concentrations of specific OPs, which was addressed by analysing the underlying changes in the levels of cytoskeletal gene expression and protein levels. To assess the molecular effects of OP exposure, phenyl saligenin phosphate (PSP), chlorpyrifos (CPF) and its metabolite chlorpyrifos oxon (CPFO) were applied at the point of induction of differentiation of rat C6 glioma and mouse N2a neuroblastoma cells and incubated for 24 hours. At sub-lethal concentrations (1, 3, 10 μM) all three OPs used in this study were able to inhibit the development of neurites with no significant effect on cell viability, as determined by neurite outgrowth and MTT reduction assays. To understand the possible effects of OPs on cytoskeletal gene expression, primers for genes encoding glial fibrillary acidic protein (GFAP), βIII tubulin, growth associated protein 43 (GAP43) and neurofilament heavy chain (NEFH) were optimized for qPCR analysis and the levels of the corresponding proteins were detected by western blot analysis. Exposure to OPs caused in most cases a reduction in the levels of cytoskeletal proteins, and the results from qRT-PCR analysis also indicated reductions in the gene expression of GFAP in C6 cells, and of NEFH and βIII tubulin in N2a cells, in a dose dependent manner. Thus, the observed changes in protein levels are at least partly due to altered gene expression.

P-09.04.4-091
The effects of curcumin on human platelet activation, apoptosis and aggregation

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Curcumin is extracted from a perennial herbaceous plant known as *Curcuma longa*. In recent years, considerable interest has been focused on curcumin due to its use to treat a wide variety of disorders without any side effects. Earlier studies have shown that curcumin has anti-apoptotic, anti-inflammatory, antiproliferative, antiangiogenic, anticancer and antiplatelet activities. The goal of the present study was to investigate the effects of curcumin on peroxy radical-induced oxidative changes in human platelets.

15 healthy volunteers were enrolled in the study. None of the study participants were on anticoagulation therapy. Citrated venous blood samples were centrifuged at 200 g for 10 minute to obtain platelet-rich plasma (PRP). The platelet pellet was washed and suspended with Tris-NaCl buffer. Then, platelets were incubated with H_2O_2 absence and presence of curcumin (50–500 μg/ml) for 1 hours at 37 °C. To determine the preventive effects of curcumin on the oxidative stress and apoptosis induced by peroxy radicals in human platelets were determined by measuring levels of of lipid peroxidation, total antioxidant capacity, caspase 3, 8 and 9 activities, and mitochondrial membrane potential. Additionally, we also studied the effects curcumin on platelet aggregation induced by ADP.

Pre-treatment of platelets with curcumin caused a marked reduction in oxidative stress, activation and apoptotic markers in a dose-dependent manner. On the other hand, pre-treatment of platelets with increasing doses of curcumin resulted in inhibition of platelet aggregation induced by ADP.

In the light of our findings, we suggest that curcumin may have a therapeutic potential to prevent platelet activation related disorders.

P-09.04.4-092
Cytostatic and hepatoprotective effect of edible mushroom *Tricholoma anatolicum* extracts

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People have been using mushrooms in the treatment of diseases as well as food, for centuries. Most of the edible and inedible mushroom species were used in important medical studies and their effects were begun to be used in the treatment of diseases. This study focuses on the hepatoprotective effects of *Tricholoma anatolicum*, which is endemic specie in Turkey, against oxidative stress based on hydrogen peroxide (H_2O_2) on HEPG2 liver cancer cell line.

T. anatolicum used in this study was extracted with the help of ultrasonication and fraction methods. Then the cytostatic effects of extracts on HEPG2 cells were explored and their hepatoprotective effects were determined. Moreover, various concentrations of aqueous extract (EHTA) of *T. anatolicum* were determined by HEPG2 cells's 24–48–72 hours effect analysis on their cellular morphology, XTT and real-time cell analysis in of Xcelligence device. EHTA extract's cell pathway (apoptosis and necrosis) effects on HEPG2 cells were determined with flow cytometry method with the help of Annexin V-APC and 7AAD fluorescent dye. Finally, the phenolic compounds found in EHTA extracts were determined with the help of HPLC methods.

According to XTT cytotoxicity analysis, the EHTA extract values were determined as follows: 24 hours $IC_{50} > 2000$ μg/ml, 72 hours IC_{50}. Furthermore, according to the real-time cell analysis made with Xcelligence, EHTA extracts were found to be; 24 hours $IC_{50} = 241.539$ μg/ml, 48 hours $IC_{50} = 418.135$ μg/ml, 72 hours $IC_{50} = 285.694$ μg/ml. Increasing concentrations of EHTA extracts were determined to direct HEPG2 cells to apoptosis. Moreover, considering the HPLC analysis –according to the reference point of 100 mg in 100 g sample– within EHTA extracts, catechins and vanillic acid peaked.

The final results revealed that *T. anatolicum*'s effect on HEPG2 was cytostatic at low doses, and cytotoxic at high doses.

P-09.04.4-093
Relationship between serum ceruloplasmin levels and coronary blood flow

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Background: There is growing evidence that oxidative stres plays an important role in the development of the slow coronary flow (SCF) phenomenon. Ceruloplasmin (Cp) is a copper containing metalloenyzme which has antioxidant functionthrough its ferroxidese 1 activity and is associated with cardiovascular diseases. We aimed to investigate the relationship between SCF and serum Cp level.
Methods: Patients who underwent elective coronary angiography and had no significant epicardial coronary disease were included in the study. Patients who had Thrombolysis in Myocardial Infarction frame counts (TFCs) above the normal cutoffs were considered to have SCF and those within normal limits were considered to have normal coronary flow (NCF). A total of 90 patients (55 subject as SCF and 35 subjects as NCF)

The FEBS Journal **283** (Suppl. 1) (2016) 129–417 DOI: 10.1111/febs.13808

were analyzed. 5 ml blood samples were taken from the groups to study ceruloplasmin activity. Serum ceruloplasmin levels were determined spectrophotometrically.

Results: The serum Cp levels were statistically lower in SCF group than in the NCF group (414 ± 79 versus 469 ± 99 ng/ml, p = 0.009). Also there was a significant correlation between serum Cp levels and TFCs ($r = -0.378$, p = 0.013).

Conclusion: The findings of this study suggests that patients with SCF had lower serum Cp levels correlated with TFCs. We concluded that reduced serum Cp levels might represent a biochemical marker of SCF.

P-09.04.4-094
Effects of thyroid hormone supplementation on oxidative stress after sleeve gastrectomy

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Introduction: Sleeve gastrectomy (SG) has been used for the surgical treatment of morbid obesity, as a first step or definitive treatment. Alterations of thyroid hormones in gastrointestinal surgery were previously studied. The aim of the present study was to determine the effects of triiodothyronine (T3) supplementation on oxidative stress parameters in anastomotic tissue level.

Materials and methods: Twenty-four male Wistar albino rats were divided into control (n 12), and experimental (n 12) groups. Rats were underwent a SG, with a hand-sewn suture. Experimental group rats received a single dose of T3 (400 mg/100 g) in postoperative day. Rats were sacrificed on postoperative day 7. Serum thyroid stimulating hormone (TSH), free T3 (FT3), and free thyroxine (FT4) were analysed using ELISA. Each tissue was homogenized in ice-cold PBS (pH: 7.4) and centrifuged at 2000 rpm for 20 minutes (4 °C) to avoid contamination with cellular debris. The supernatants were used to measure total oxidant status (TOS), total antioxidant status (TAS), nitric oxide (NO) and malondialdehyde (MDA) levels. All tissue parameters were analysed by spectrophotometric methods. Oxidative stress index (OSI) values were calculated.

Results: Rats given T3 hormone had not decline in FT3 levels compared with the control groups. A significant decrease in FT4 levels was found in T3 given rats on postoperative day 7. Whereas tissue TOS levels did not alter by thyroid hormone treatment, TAS levels significantly decreased. OSI values were not statistically different in tissues. Tissue NO levels were also similar in both groups. MDA levels increased in T3 given rats compared with the control group.

Discussion and conclusion: This study showed that anastomosis after sleeve gastrectomy is associated with decreased FT4 level. Although TOS levels and OSI values were similar in both groups, T3 supplementation induced lipid peroxidation by increasing tissue MDA levels that might deplete tissue antioxidant level.

P-09.04.4-095
The impact of Tip60 gene on antioxidant system

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Reactive oxygen species (ROS) are reactive chemical molecules, which are produced by living organisms as a natural byproduct of the normal metabolism and environmental factors. Although intracellular ROS level is essential molecules for the signal transduction pathways, elevated intracellular level of ROS leads to oxidative stress that causes damage to DNA, proteins and lipids. Therefore, excessive ROS levels have to be eliminated by antioxidant defence systems. TIP60 (Tat interacting protein, 60 kDa) is a histone acetyltransferases (HATs) that catalyses multiple functions in metabolism such as DNA repair, apoptosis, etc. We thought that if TIP60 has a role in signal transduction and apoptosis, it might have direct or indirect relationship with the antioxidant system.

The present study was designed to determine the impact of Tip60 gene on the hepatic antioxidant enzymes including Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPX), and glutathione reductase (GR) both gene and protein level. For this reason, quantitative gene expression analysis on the antioxidant system has been investigated by real time PCR, quantitative protein expression has been investigated by western blot analysis, and then the activity of these enzymes have been measured in whole liver homogenate collected from control and liver-specific Tip60 conditional knockout mice. Additionally, since any change of reduced glutathione (GSH), oxidized glutathione (GSSG), malondialdehid (MDA), and hydrogen peroxide (H_2O_2) level in the cell might be an indication for the accumulation of ROS, the relative levels of them were also studied.

Our data showed that the absence of Tip60 affects the antioxidan system both gene and protein level. In conclusion, our initial data suggest that Tip60 may be essential for the (*ROS*) *homeostasis* and redox regulation. This work was supported by Scientific Research Project of Ataturk University of Turkey (Grant no: 2013/293).

P-09.04.4-098
Antiapoptotic effects of curcumin against ischemia-induced injury in rat uterus

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Curcumin is a major chemical component produced from the rhizome of the plant *Curcuma longa*. lt has been demonstrated that curcumin has an antioxidant, anti-inflammatory, and antiproliferative effects and, protects tissues against ischemia/reperfusion (I/R) injury. I/R has detrimental effects on transplanted organs including uteri. The major consequence of l/R injury is oxidative stress leading to the generation of ROS. Uterine transplantation (UT) has been gaining popularity around the worId in the past few years. The aim of our study was to examine the antiapoptotic effects of curcumin on uterine l/R injury.

The rats were randomized into three groups of seven rats each, Group I consisted of rats that did not receive any treatment, group ll exposed to 0.5 hour of lschemia and 1 hour of reperfusion, group III of rats that received intraperitonealy curcumin (200 mg/kg) 0.5 hour before the induction of I/R. Then, the rat uterine tissue levels of MDA, TAC, and activities of caspase 3, 8 and 9 were measured. Furthermore, the apoptotic index was determined immunohistochemically by the TUNEL method using light microscopy.

Biochemical analysis results showed that curcumin decreased the MDA and caspase-3 and 9 IeveIs, and increased the uterine tissue levels of TAC but, caspase 8 activity did not changed by curcumin suggesting that curcumin induces apoptosis via intrinsic apoptotic pathway. On the other hand, an high apoptotic index was observed in I/R group (27.37 ± 11.56%) and decreased after treatment with curcumin (8.69 ± 7.49%).

In conclusion, we demonstrated the protective effect of curcumin on apoptosis immediately after reperfusion induction in uteri and we can say that curcumin could improve IR injury and decrease apoptotic index. We propose that curcumin may be a novel approach for improvement of uteri I/R injury.

P-09.04.4-099
Effect of chlorophylline as an antioxidant molecule on glutathione and related enzymes in breast cancer model

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Glutathione and the related enzymes belong to the defence system of the tissues against chemical and oxidative stress. These enzymes especially Glutathione S-transferase are often overexpressed in tumor cells and are regarded as a contributor to their drug resistance and are thought to play an important role in cancer progression.

The purpose of this study is to evaluate the protective effects of chlorophylline as an antioxidant molecule which has inhibitory effects on GST P1-1 on chemically-induced breast cancer model. In our previous work, we had observed that this molecule led to proliferation in breast cancer cells.

In this study, N-methyl-N-nitrosourea (MNU) used for inducing carcinogenesis in eighteen, 21-day-old female Sprague-Dawley rats. Chlorophylline and MNU solutions were injected intraperitoneally when the rats were 21, 28, 35 and 42 days old. Their weight and tumor diameters were measured throughout the 5 months study period. At the end of the study, all animals were sacrificed and determined both glutathione levels and related enzymes activities (Gluathione S transferase, Glutathione reductase and Glutathione peroxidase) in tumor and tissues such as liver, kidney, heart and spleen were studied and analyzed.

As a result, in breast cancer model, glutathione and related enzyme activities were protected by chlorophylline treatment whereas MNU made them decreased compared to the control group. In conclusion, chlorophylline with antioxidant features decreased the toxic effect of MNU by regeneration of glutathione and enhancement of its related enzyme activities. The use of antioxidant molecules, because of proliferative effects and defence-oriented behaviours, should be discussed in cancer therapy.

P-09.04.4-100
Effect of overexpression of *Bacillus* catalase on *Lactococcus lactis* nisin production

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Nisin, has been used commercially (E234) in food preservation for approximately 40 years. It's the only bacteriocin which is approved by World Health Organization as a food additive. The fundamental problem that limits nisin usage in food preservation is low product yield by producer strains. Because of high commercial potential of nisin, studies about increasing the production efficiency of nisin is kept in the forefront in recent years. Since nisin biosynthesis and bacterial growth are occuring in parallel to each other, conditions that promote growth are also expected to encourage nisin production. It is known that, when supplied with exogenous heme, *Lactococcus lactis* cells can respire under aerobic conditions and produce higher energy which in turn cause higher biomass. However, aerobic conditions also cause oxidative stress since catalase enzyme, which detoxify hydrogen peroxide, is absent in *L. lactis*.

In this study, to complete the missing component of the defence mechanism of *L. lactis*, catalase (*katE*) gene of aerobic bacterium *Bacillus subtilis* was overexpressed in facultative anaerobe *L. lactis* cells. For this, *katE* gene of *B. subtilis* was amplified by polymerase chain reaction (PCR). Plasmid constructions were established in *E. coli* by using an *E. coli-L. lactis* shuttle vector and then the recombinant plasmid was transferred to *L. lactis* cells by electroporation.

The presence of catalase activity in the recombinant strain grown on the solid medium was first detected by dropping hydrogen peroxide directly on the cells, then with enzyme assays. Fermentation studies are going on to determine nisin production of the recombinant strain.

To the best of our knowledge, this study presents the first preliminary results that shows the effect of overexpression of catalase gene on nisin production.

P-09.04.4-101
Anticancer activity of humic acid *in vitro*

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Cancer is among the leading causes of morbidity and mortality worldwide. Chemotherapy is one of the major cancer treatment strategies. Remarkably, natural products have garnered increased attention in the chemotherapy drug discovery field because they are biologically friendly and have high therapeutic effects. Humic acid (HA) is a natural product which is forming during decomposition of organic matter in humus. In recent years, there are some resarches on the medical use of humic acid. The present study investigated anticancer effects of HA in several human cancer cell lines.

HA was purchased from Sigma-Aldrich. In this study, we used several human cancer cell lines: The human breast cancer cell line, MCF-7, colon cancer cell line, HT-29, lung adenocarcinoma cell line, A549, and servical cancer cell line, HeLa. The cells were maintained in DMEM medium supplemented with 10% heat-inactivated FBS and 1% penicillin/streptomycin. Cells were grown in petri dishes in a humidified atmosphere containing at 37oC. Five different concentrations (100 ug/ml, 50 ug/ml, 25 ug/ml, 10 ug/ml, 5 ug/ml) were prepared using a stock solution of HA. The cell proliferation and migration was measured. On the

The FEBS Journal **283** (Suppl. 1) (2016) 129–417 DOI: 10.1111/febs.13808

other hand, the apoptotic mechanisms induced by HA in cancer cells were investigated using "Apoptosis antibody array kit".

The effects of HA on cancer cell lines were evaluated over 72 hours. According to our results, HA induced a decrease in HT-29, A549 and HeLa cell numbers in a dose-dependent and time-dependent manner. Contrary to this, HA induced proliferation of MCF-7 cells in dose dependent manner. HA inhibited cell migration in a dose dependent manner except MCF-7 cell line. It was also determined apoptotic pathways in cancer cells induced by HA.

It was concluded that HA has an inhibitory effect on certain some cancers. Since the effect of HA on tumor progression is unknown, further studies are needed to clarify the rol of HA on cancer activity.

P-09.04.4-102
Chronic immobilization stress in rats: fluoxetine and amisulpride protects against chronic immobilization stress-induced biochemical alterations

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In the present study, the effects of Amisulpride and Fluoxetine on serum total sialic acid (TSA) and lipid bound sialic acid (LSA) levels was investigated in the rats exposed to chronic immobilization stress.

The study was administered using 40 male Wistar albino rats weighing 200–250 g. Rats were divided into five groups (n = 8/group). Group I comprised the control group, group II was exposed with saline + immobilization stress (30 minutes daily immobilization stress for 15 days and 0.5 ml saline was administered perorally 30 minutes before immobilization), group III was exposed amisulpride (10 mg/kg/day) + immobilization stress, group IV was exposed fluoxetine (10 mg/kg/day) + immobilization stress and V. group was exposed amisulpride (10 mg/kg/day) + fluoxetine (10 mg/kg/day) + immobilization stress.

Statistical analysis showed that the saline + stress, amisulpride + stress and amisulpride + fluoxetine + stress groups was significantly higher than the control group with regards to TSA levels ($p < 0.05$). Whereas, the fluoxetine group was significantly lower than the group regarding TSA levels ($p < 0.05$). On the other hand, saline group was significantly higher than the control group with regards to LSA level ($p < 0.05$). Whereas, no significant differences in LSA levels were observed in the amisulpride, fluoxetine and amisulpride + fluoxetine groups, as compared to the control group ($p > 0.05$).

The present study demonstrated beneficial effect of fluoxetine and amisulpride on the concentration levels of LSA and TSA in stress.

P-09.04.4-104
Protective effect of borax and boric acid on total sialic acid and lipid-bound sialic acid levels against 3-methylcholanthrene and benzo(a)pyrene induced oxidative stress in rats

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The present study was performed to investigate total sialic acid (TSA) and lipid bound sialic acid (LSA) levels as possible in vivo chemoprotective effect of borax (BX) and boric acid (BA) against3-Methylcholanthrene (3-MC) and benzo(a)pyrene (B(a)P) induced oxidative stress in rats. The rats were divided into nine groups of six rats each. Group I: Control, untreated animals were given % 0.9 NaCl, Group II: The B(a)P were administered 25 mg/kg via ip. four times. Group III: The 3-MC-treated animals were administered 25 mg/kg via ip. four times, Group IV: BA was given 300 mg/l/day with water. Group V: BX was given 300 mg/l/day with water. Group VI: B(a)P 25 mg/kg via ip four times + BA 300 mg/l/day dosage with water. Group VII: 3-MC 25 mg/kg via ip four times + BA 300 mg/l/day with water. Group VIII: B(a)P 25 mg/kg via ip four times + BX 300 mg/l/day dosage with water. Group IX: 3-MC 25 mg/kg via ip four times + BX 300 mg/l/day with water. The experimental period was continued for 150 days. Statistical analysis showed that the 3-MC + BA group was significantly higher than the control group with regards to TSA and LSA levels $p < 0.001$, $p < 0.001$,p

P-09.04.4-105
Effects of aluminum exposure on trace elements in rat tissues

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Aluminum (Al) is the most abundant metal and the third most abundant element in the earth's crust. People are constantly exposed to Al which is found in most rocks, soils, waters, air and foods, due to a result of an increase in industrialization and improving technology practices. The study was designed to examine the possible effects of aluminum exposure in different durations on trace elements in rat tissues.

Twenty-four healthy male Wistar rats weighed 180–200 g were randomly divided into three groups: control group (GC) received only drinking water, short-term group (GS) and long-term group (GL). The study groups were orally exposed to 40 mg/kg body weight $AlCl_3$ in drinking water for 8 and 16 weeks, respectively. At the end of the treatment period, rats were sacrificed and the kidney, liver, brain and cerebellum tissues were removed to analyse the levels of Al, Ar, B, Ni, Si, Cr, Cu, Fe, Mg, Mn, Se, Cu and Zn by ICP-OES.

The statistically significant increase were determined in cerebellum Al, Cu, As, B and Cr levels in GL according to the GC. While As levels were statistically increased, Ni levels were decreased in GL in the kidney and liver. While Cu, Mg and Cr levels were higher, Se and B levels were lower in the GS than GC in the brain. There were no significant difference in Si and Mn levels. As a result of our study, it may be concluded that Al accumulation may lead to changes in tissue trace element levels.

P-09.04.4-106

The gastroprotective effect of water extract of *Cinnamomum aromaticum* in experimental rat model: This effect is related to its *in vivo* and *in vitro* antioxidant parameters

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Introduction: In this study, the antiulcerogenic effect of a water extract (CAWE) obtained from a spices sample, *Cinnamomum aromaticum*, was investigated using indomethacin-induced ulcer models in rats.
Materials and methods: Experimental groups consisted of six rats. Antiulcerogenic activities of 50, 100, 200 and 400 mg/kg body wt. doses of the CAWE were determined by comparing the negative (treated only with indomethacin) and positive (famotidine) control groups.
Results and discussion: Although all doses of the CAWE showed significant antiulcerogenic activity as compared to negative control groups, the highest activity was observed with 400 mg/kg body wt. doses (45%). The CAWE showed similarly antioxidant activity when compared with trolox and ascorbic acids used as positive antioxidants. In addition, the activities of catalase (CAT) and myeloperoxidase (MPO) enzymes were determined in the stomach tissues of rats and compared with those of the negative and positive control groups to expose the effects of these enzymes on antiulcerogenic activity. The enzymatic activities of CAT and MPO and lipid peroxidation (LPO) level in indomethacin-administrated tissues were increased significantly by indomethacin in comparison to control groups. These enzymes and LPO level were decreased, however, by the CAWE. In contrast to LPO level, CAT and MPO activities, glutathione (GSH) level was decreased by indomethacin and increased by all doses of CAWE and famotidine. The present results indicate that the CAWE has a protective effect in indomethacin-induced ulcers, which can be attributed to its antioxidant potential.

P-09.04.4-107

Plasma thiol levels in patients with prostate cancer

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Introduction: Thiol groups (-SH) are important anti-oxidants and essential molecules protecting organism against the harmful effects of oxidative stress. The aim of our study is to evalute thiol-disulphide homeostasis with a novel and automated method in patients with prostate cancer (PC) before and after radical prostatectomy (RP).
Material and methods: 18 patients with prostate cancer and 17 healty control subjects were enrolled into the study. Plasma samples were collected from patients before RP and 6 months after the RP operation. Thiol-disulphide homeostasis was determined with a recently developed novel method. Prostate specific antigen, albumin, total thiol, native thiol, disulphide and total antioxidant status (TAS) were evaulated and compared between the groups.
Results: Native thiol levels were 419.8 ± 54.87 μmol/l in the control group, 350.7 ± 46.35 μmol/l in the patients before RP, and 364.3 ± 46.88 μmol/l in patients after RP. Native thiol, total thiol and TAS levels were significantly higher in the control group than the patients before RP (p values < 0.001). Native thiol, total thiol and TAS levels were higher 6 months after RP compared to before RP in patients, but these changes were not significant statictically (p values 0.3, 0.3 and 0.09 respectively).
Discussion and conclusion: Our study demonstrated that antioxidant defense mechanism was weakened as indicated by the decreased thiol levels in the patients with PC. Increased oxidative stress in prostate cancer patients may cause metabolic disturbance and have a role in the pathogenesis of prostate cancer.

P-09.04.4-108

Is there any relation between 50 g oral glucose challenge test and serum total oxidant-antioxidant status in pregnant woman?

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The purpose of this study was to test the hypothesis that any degree of antepartum screening for gestational diabetes mellitus with oral 50 g glucose challenge test (GCT) should be associated with oxidant-antioxidant status.

In this prospectif study, oral glucose challenge test was applied to 25 pregnant women aged 25–40 years and at 24–28 weeks of gestation. Plasma glucose concentrations were measured initial, 1 hour and in addition to test 2 hours after ingestion of 50 g glucose. At the same time serum insulin, cortisol, total antioxidant status (TAS), total oxidant status (TOS) levels were measured and the oxidative stress index (OSI) was calculated.

Ten pregnant women (forty percent) had a positive glucose challenge test (GCT). A positive moderate relation with initial and 1 hour serum total antioxidant status (TAS) levels (r = 0.68) and the oxidative stress index (OSI) (r = 0.67) was found. There was a positive weak correlation with initial and 2 hours total oxidant status (TOS) levels (r = 0.22) but statistically significance difference was not found (p > 0.05).

In this study after ingestion of 50 g glucose serum total antioxidant status (TAS), serum oxidant status levels (TOS) and serum oxidative stress index (OSI) levels were higher than the initial levels.

The results of this study suggest that antepartum screening for gestational diabetes mellitus with 50 g oral glucose challenge test (GCT) weakly associated with oxidant-antioxidant status and to confirm this results the longer follow-up studies with more participants are necessary.

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Phytochemical contents and antioxidant activities of some bread (*Triticum aestivum* L.), durum (*Triticum tugidum* ssp. durum Desf.), and hulled einkorn (*Triticum monococcum* ssp. monococcum) wheats

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Wheat (*Triticum* ssp.), cultivated for centuries in the Middle-East, Central Asia, Europe, and North-Africa, is one leading staple crops around the World, and its marginally grown ancestor einkorn (*Triticum monococcum* ssp. *monococcum*), possesses rich gene resources for wheat improvement and have bioactive compounds reducing and preventing chronic diseases such as diabetes, cancer, alzheimer, and cardio vascular diseases, beside their nutritional properties. However, as more attention has been given to wheat cultivars with strong gluten, protein content, starch composition, and resistance to biotic and abiotic stresses in bread wheat and yellow-colored pasta product in durum wheat health compounds such as fibers, phytochemicals, and bioactives have been underestimated so far. The aim of this study was, then, to examine the total phenolics and flavonoids, quantify their phenolic acids, α-tocopherol by high performance liquid chromatography (HPLC), and their 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity of bread (*Triticum aestivum* L.), durum (*Triticum turgidum* ssp. *durum* Desf.) wheat cultivars and einkorn (*Triticum monococcum* ssp. *monococcum*) wheat populations collected from different provinces (Bolu and Kastamonu) of Turkey. Ferulic acid (148.67–764.04-μg/g), p-coumaric (5.06–54.09-μg/g), and total phenolic content (ranged 2.06–8.11-μmol GAE/g) of einkorn populations were significantly higher than bread and durum wheat cultivars. Results suggested the possibility of production of einkorn wheat populations, and hopefully cultivars rich in particular health beneficial component(s) may provide benefit to the consumers. In addition, higher phenolic content of einkorn may offer novel wheat genetic resources for the improvement of new wheat cultivars and the development of wheat-based functional foods.

P-09.04.4-110
The relationship between NGAL and oxidative stress parameters in patients who have undergone on-pump coronary artery grafting

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Oxidative damage due to ischemia and acute kidney injury (AKI) after coronary artery bypass graft (CABG) surgery are the leading complication during this process. In the kidney, ischemia/reperfusion injury contributes to AKI that is a clinical syndrome with rapid kidney dysfunction and high mortality rates. Some animal and clinical studies have demonstrated an increase in serum and urinary neutrophil gelatinase-associated lipocalin (NGAL) expression after renal ischemic injury.

In this study, our aim was to investigate the relationship betwwen NGAL and oxidative stress parameters due to ischemia caused by total perfusion time (TPT) in patients who have undergone on-pump CABG.

Materials and methods: The study was conducted in 30 patients who received on-pump CABG at University of Istanbul, Cerrahpasa Medical Faculty, Department of Cardiovascular Surgery. Blood samples were collected prior to surgery and after 2 hours following the termination of cardiac pulmonary bypass (CPB) . Following centrifugation, serum samples were separeted and stored at -80 C until analysis. Serum NGAL, IMA (Ischemia modified albümin), PCO (Protein carbonyl), NT (Nitrotyrosine), LPD (Lipid peroxide) levels were determined by ELISA procedure.

Results and discussion: Serum NGAL, PCO, NT levels in after 2 hours following CPB were significantly higher than the before surgery ($p < 0.001$, $p < 0.001$, p

Serum NGAL levels in after 2 hours following CPB was found to have positive correlation with IMA, PCO, NT, and LPO levels ($r = 0.59$, $p < 0.01$; $r = 0.77$, $p < 0.001$; $r = 0.68$, $p < 0.001$; $r = 0.77$, $p < 0.001$ respectively). NGAL levels were positively correlated with total perfusion time after CBP ($r = 0.37$, $p < 0.05$).

The results of our study show that, increased NGAL levels 2 hours after CPB were positively correlated with oxidative stress parameters and total perfusion time.

P-09.04.4-111
Investigation of the effects of thymoquinone against indomethacine induced gastric damage in rats

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Introduction: Incidence and high cost of acute stomach mucosa damages make this issue a very interesting issue for study. For this reason, it is aimed to investigate the effects of different dosage of thymoquinone (TQ) against indomethacine induced gastric damage in rats.

Material and method: In our study, six groups of 36 Wistrar male rats were used. Groups are named as healthy, IND control, Famotidine (40 mg/kg) and three different doses of TQ (0.5, 1 and 2 mg/kg). While any treatment or drug administration will done on healthy group, model was generated to other groups by giving FAM or TQ with tap water via oral gavage. 5 min later, 25 mg/kg IND administrated to each rat. Animals were sacrified about 6 hour later and stomach samples of each groups were collected for macroscopic study and GSH levels measurements.

Results: Lower doses of TQ is more effective and all TQ groups exhibit reduced ulcer region with respect to the IND control group. GSH level of IND control group is lower than healthy group. The GSH level of TQ, especially in lower doses, and FAM groups statistically exhibit an increase in GSH level.

Conclusion: It is observed that IND induced gastric damage cause ulcer and increase in free radical. It is determined that lower doses of TQ (0.5, 1 and 2 mg/kg) is also exhibit a protective effect on IND induced model. It is tought that quinone in TQ structure have a strong redox feature and this feature clean up the free radicals caused by IND, it reduces the oxidative stress and protect the stomach from ulcer.

P-09.04.4-112
Investigation of some biological active properties of Anzer bee-pollen

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Anzer honey is the most famous honey in Turkey with many endemic species flowers. The Anzer Plateau is located Rize province of Eastern Black Sea Region. In this study, antioxidant and anti-hyaluronidase and anti-urease activities were investigated of the plateau bee pollen. The antioxidant capacity was determined by total phenolic content (TPC), total flavonoids contents (TFC), ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. The antioxidant results of TPC, TFC, FRAP, DPPH activities were 753.52 ± 15.20 mg GAE/100 g, 245 ± 10.56 and 390.65 ± 2.29 μmol Trolox/100 g, 1.47 ± 0.05 mg/ml, respectively. Inhibition effects of Anzer bee pollen on urease and hyaluronidase enzymes were calculated as the IC_{50} values. The results of hyaluronidase IC_{50} were 0.07 ± 0.01 g/ml and 1.32 ± 0.01 mg/ml. In additional, there are positive correlations between enzyme inhibitions and antioxidant capacity. In conclusion, Anzer bee pollen is an important blossomed honey and have antioxidant, anti-inflammatory and gastroprotective effects and regularly consumed may be improve human health.Keywords: Anzer bee pollen, antioxidant, anti-inflammatory, gastroprotective

P-09.04.4-113
Antioxidative nanosystem: an advanced strategy combating atherosclerosis

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Atherosclerosis is the leading cause of mortality worldwide, and as a chronic inflammatory disease, caused by a complex interplay between inflammatory and oxidative events.

Quercetin, a plant derived flavonoid and a well- known antioxidant, has shown great promises with regards to its protective effects against oxidative stress.

However due to its physicochemical properties, the optimum pharmacokinetic behavior is a challenging issue.

Herein, we aimed to fabricate quercetin loaded solid lipid nanoparticle (Quer-SLN) to improve the bioavailability and therapeutics efficiency. Furthermore the in-vitro capacity of Quer-SLN for ameliorating TNF-α induced oxidative stress in human endothelial vein cell (HUVEC) was evaluated. Quer-SLNs were prepared by simple hot homogenizing method and characterized by means of drug loading (DL), encapsulation efficiency (EE), cytotoxicity, size, zeta potential and morphology. Antioxidant activity of plain quercetin and Quer-SLNs were then investigated using intracellular reactive oxygen species (ROS) detection method (DCFH-DA assay) by FACS flowcytometry

In conclusion, the results here showed superior control of oxidative stress by quercetin nanosystem as compared to plain quercetin. Precirol based SLNs as a biocompatible/biodegradable lipid, may provide a novel drug delivery system for quercetin with improved beneficiary impact in atherosclerosis.

P-09.04.4-115
The effects of zinc on oxidant/antioxidant systems in the absence of oxidative stress

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Objective: Zinc is known as an antioxidant essential trace element. We aimed to evaluate the dose-dependent effects of zinc on the oxidant-antioxidant system in liver, kidney and brain tissues of rats and the histological alterations in the absence of oxidative stress (OS).
Material and methods: Thirty-nine female weighing about 150 gr Wistar albino rats were divided into four experimental groups as ad libitum (AL) diet (control), AL diet + 3 mg/kg Zn sulfate (low dose; group 1), AL diet + 12 mg/kg Zn sulfate (middle dose; group 2) and AL diet + 25 mg/kg Zn sulfate (high dose; group 3). Zn sulfate solutions were administered 0.2 ml/day orally for 13 days and in day 14 rats were sacrificed and tissues were excised for detecting malondialdehyde (MDA), advanced oxidation protein products (AOPP), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione reductase (GR), and glutathione-S-transferase (GST) activities. Histological evaluation was also performed to confirm the effects of zinc.
Results: In liver tissues AOPP levels decreased in all groups receiving zinc as compared to the control group. Liver MDA levels were increased in group 1 and 3; SOD and GSH-Px levels were both increased while GST levels were decreased in all groups compared to control. GR levels were increased only in Group 2. In kidney; AOPP level was decreased only in Group 1 and SOD level was only decreased in Group 2 as compared to control while GR levels were increased in all doses of zinc. In brain; AOPP, GSH-Px and GR levels were decreased in all groups receiving zinc as compared to control group. SOD activity in brain tissues was increased by the administration of middle dose of zinc (Group 2). GST level was decreased in only group 1
Conclusions: The biochemical and histological findings of this study suggest that zinc has various effects on liver, kidney and brain tissues in the absence of OS.Key Words: Zinc, liver, kidney, brain

P-09.04.4-116
The effect of diabetes mellitus on oxidative stress and antioxidant capacity in humour aqueous and serum

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Introduction: This study aimed to investigate the effect of diabetes mellitus (DM) on oxidative stress and antioxidant capacity in humour aqueous (HA) and venous serum using total antioxidant capacity (TAC) and total oxidative stress (TOS) levels in serum and HA in cataract patients.
Materials and methods: In this study patients were divided into two groups. Group 1 was composed of patients with type 2

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DM and cataract and group 2 was composed of patients with cataracts who are not accompanied by DM and cataract patients who are not accompanied by systemic diseases. Each group consisted of 20 patients, totally 40 patients were included in the study. The HA which was collected from the eyes at the beginning of the cataract surgery and venous blood serum collected from the same patients were analyzed. In both groups, HA and serum TAC and TOS levels were measured with ELISA.

Results: : Serum TAC levels in the DM group were significantly lower than in the control group ($p < 0.05$). TOS serum levels in DM group was statistically higher than the control group ($p < 0.05$). Differences between TAC and TOS levels were not statistically significant when compared the two groups' HA results ($p > 0.05$). Group 1, divided into two subgroups according to their HbA1c levels, there was no statistically significant difference between the subgroups when HbA1c levels were compared with the relationship between serum and HA's TAC and TOS levels ($p > 0.05$). There was not an association between the gender, age and the levels of TAC-TOS in both groups ($p > 0.05$).

Discussion and conclusion: Presences of DM is the only risk factor for increase of oxidative stress and decrease of anti-oxidant capacity in patients without a systemic complication of DM and diabetic retinopathy. In our study, diabetic patients without retinopathy showed similar HA TOS and TAC levels to healthy individuals, this finding indicates that blood-aqueous barrier is protected in these patients.

P-09.04.4-117
The effect of ferulic acid against testicular ischemia/reperfusion injury in rats

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Testis torsion is one of the urologic emergencies occurring frequently in neonatal and adolescent period. Testis is sensitive to ischemia/reperfusion (I/R) injury and, therefore, ischemia and consecutive reperfusion cause an enhanced formation of reactive oxygen species (ROS) that result in testicular cell damage and apoptosis. Ferulic acid, known as an antioxsidant, is a phenolic acid found in seeds and leaves of the plants. We aimed to investigate potential protective effect of ferulic acid against testis I/R injury.

Thirty five Wistar rats were randomly divided into 5 groups; control, ethyl alcohol, ischemia, I/R, I/R-ferulic acid groups. Animals were exposed to 2 hours of ischemia followed by 2 hours of reperfusion. Ferulic acid was administered (100 mg/kg) before reperfusion intravenously. Testicular cell damage was examined by H-E staining and PAS. TUNNEL, active caspase-3, iNOS and MPO were evaluated by immunostaining. Malondialdehyde (MDA), glutathione (GSH) levels, glutathione peroxidase (Gpx) and superoxide dismutase (SOD) activities were assessed by biochemical methods.

Histological evaluation showed that ferulic acid pretreatment reduced significantly testicular cell damage and decreased TUNNEL, caspase 3 positive cells; iNOS and also MPO expression. In addition, ferulic acid administration decreased significantly the MDA levels increased by I/R. Morever, ferulic acid increased significantly the SOD activity levels, which was decreased by I/R. There were no statistically significant differences in the levels of GSH and GPx activity in all groups.

The present results suggest that ferulic acid is a potentially beneficial agent in protecting testicular I/R.

P-09.04.4-118
The drugs used in heart failure treatment reduces oxidative stress in adults

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Background: In heart failure (HF), angiotensin antagonists (AA), beta-blockers (BB), spironolactone, diuretics and acetylsalicylic acid are often used. Top 3 pharmaceutical groups reduce mortality. On the increased oxidative stress (OS) in patients with HF, it is known to have beneficial effects of certain groups of drugs. However, the net effect of these drugs in OS is unknown. The aim of this study was to investigate the effects of drugs used in HF on OS.

Materials and methods: 133 patients were included in the study. All of the patients had systolic heart failure and all of them were under treatment. Drugs used by the patients were recorded. The levels of total antioxidant status (TOS), total oxidant status (TAS), the enzymatic activity of ceruloplasmin, paraoxonase-1 and arylestherase were measured according to Erel's method. Serum total thiol levels were measured with SH Modified HU method and the lipid hydroperoxide levels were measured with the ferrous ion oxidation xylenol orange assay. The percentage TOS / TAS was determined as OSI.

Results: In patients treated with acetylsalicylic acid (ASA), spironolactone, beta blocker and furosemide, there were increased TOS, decreased TAS and OSI ($p < 0.05$). In patients treated with angiotensin blockers, increased TAS and LOOH, and decreased SH were found ($p < 0.05$). In patients treated with nitrates and CCB, TOS and OSI were found decreased. Correlation analysis showed that increased TAS correlated with the use of angiotensin blockers, ASA, furosemide and beta blocker positively; and with the TOS and OSI level correlated with the use of spironolactone, ASA spironolactone and furosemide ($p < 0.05$).

Conclusion: Current medical agents that are being used in HF are effective in reducing OS in HF patients. One of the effective mechanisms to reduce the mortality of some of these drugs may decrease OS.Key Words: Heart Failure, drug use, oxidative stress

P-09.04.4-119
Across adjacent ring formed titanium phthalocyanine-mediated photodynamic therapy alters and degrades filamentous actin cytoskeleton and internal membranes

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Photodynamic therapy (PDT) is widely accepted as a promising and minimally invasive treatment strategy due to its applicability on a wide range of cancer diseases. This clinically approved treatment method relies on the dramatic production of singlet oxygen and reactive oxygen species (ROS) in target tissue to evoke apoptotic cell death [1]. We, therefore, focused on the intracellular ROS accumulation, internal membrane degradation, filamentous actin cytoskeleton alteration and nucleus morphology changes induced by PDT-mediated across adjacent ring formed titanium phthalocyanine which was previously synthesized Bis(ethane-1,1-p-phenol-2,2-p-phenoxy) phthalocyaninatotitanium (IV).

Characterization of the synthesized metallophthalocyanine was accomplished by using UV-vis, IR, ^{1}H-NMR and MALDI-TOF-mass spectroscopies. The dark and PDT-mediated activities of bare and phosphonolipids (max. 5%) charged titanium phthalocyanine (0.625, 1.25, 2.5, 5 and 10 μM) were determined on A549 human lung carcinoma and HaCaT human keratinocyte cell lines by using intracellular ROS assay, DIOC(6), TRITC-phalloidin and DAPI staining protocols. Waltmann PDT 1200 l was used as the non-toxic light source at 100 J/cm^2 fluence and 150 mW/cm^2 fluence rate. The experiments showed that PDT-mediated titanium phthalocyanine leads to significant and concentration dependent reactive oxygen species accumulation. Moreover, internal membrane degradation, apoptotic bodies on nucleus and filamentous actin cytoskeleton alteration were observed. Consequently, the activity mechanism of PDT-mediated titanium phthalocyanine seems to be in a tight relationship with ROS accumulation-mediated internal membrane degradation, filamentous actin cytoskeleton alteration and apoptotic pathways activation.

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P-09.04.4-121
Determination of serum thiol levels in retinal vein occlusion by a novel method

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Introduction: Retinal vein occlusion (RVO) is a common retinal vascular disorder that can affect visual acuity and cause blindness in elder population. Sulphur containing aminoacids such as cysteine (Cys), cysteinylglycine, glutathione, homocysteine and γ-glutamylcysteine are reported to be associated with the pathogenesis of RVO. Thiols are organosulfur compounds that are formed of a carbon-bonded sulfhydryl group. Sulphur containing aminoacids slightly contribute the composition of plasma thiol pool. Thiols can undergo oxidation reaction via oxidants and form disulphide bonds Our purpose is to research the relationship between a novel oxidative stress marker serum dynamic thiol–disulphide homeostasis and retinal vein occlusion.
Materials and methods: 22 RVO patients and 22 controls were included in the study. Native thiol, total thiol, disulphide levels are measured in the serum samples of RVO and control group by using an automated method described by Erel et al. Also disulphide/native thiol and disulphide/total thiol ratios were calculated.
Results: There were no significant difference between the RVO and control group in native thiol, total thiol, disulphide disulphide/native thiol and disulphide/total thiol ratios.($p > 0.05$ for all)
Conclusion: Our study is the first report evaluating the dynamic thiol–disulphide homeostasis in RVO patients by a newly developed method by Erel et al. Further large sample sized studies investigating the levels of sulphur containing aminoacids may additionally be planned to verify this study.

P-09.04.4-122
Serum oxidative and antioxidative parameters in obstructive sleep apnea syndrome patients

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Purpose: The purpose of this study was to evaluate markers of systemic oxidative stress and antioxidant capacity in subjects with severity of OSAS.
Methods: A total of 106 OSA patients were included in the study (18 controls, 14 with mild, 14 with moderate, and 60 with severe OSA). Patients were grouped according to apnea-hypopnea index (AHI) as mild, moderate and severe OSA. Patients with AHI<5 served as control group. Known risk factors for oxidative stress, such as age, sex, obesity, smoking, hypelipidemia, and hypertension, were investigated as possible confounding factors. Plasma arylesterase, total oxidative stress (TOS), total antioxidant capacity (TAC), total thiol, catalase (CAT) levels were measured for all patients.
Results: The mean age was 52.49 ± 12.9 years and 40.6% (43/106) of the study population was female. Plasma arylesterase, TOS, TAC, total thiol, and CAT plasma values were not different between mild, moderate, severe OSA groups and controls ($p > 0.05$). Catalase levels were significantly lower in women patients with severe OSA compared to healthy women controls ($p < 0.05$). There was a negative correlation between AHI and serum total thiol levels ($r = -0.289$, $p < 0.05$) in severe OSA groups. ConclusionThe present prospective study provides evidence that OSA might be associated with decreased antioxidant burden possibly via catalase way.

P-09.04.4-123
Oxidative stress and inflammation biomarkers change in type 2 diabetes mellitus

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Introduction: Oxidative stress and inflammation are main complications in patients with Diabetes Mellitus (DM). To compare oxidative and inflammatory damages between type 2 DM patients and normal people serum 8-hydroxydeoxyguanosine (8-OHdG), malondialdehyde (MDA) and interleukin-6 (IL-6) measured.
Methods: We collected serum samples of 50 Iranian type 2 DM and 50 healthy subjects from ages 27–70 years. The levels of 8-OhdG, IL-6 and MDA evaluated by ELISA and TBARS assays, respectively.
Results: The sera 8-OHdG, MDA and IL-6 were significantly higher in diabetic group than control group ($P < 0.05$). Although there was a notable positive correlation between MDA and 8-OHdG, there was no a relationship between 8-OHdG or MDA with IL-6.
Discussion: In agreement with previous studies our data illustrated that high levels of oxidative stress is associated with increased production of oxidized lipids and nucleobases in diabetic patients compared to control group. Also enhanced proinflammatory cytokine, IL-6, induced inflammmation in these patients.

Conclusion: Oxidative stress and inflammation play pivotal roles in the development of diabetes and can cause major complications in DM. So we suggest that early detection of these measurable indicatores can help to diagnosis the severity or presence of some complication in diabet.

P-09.04.4-125
The effect of quercetin on erythrocyte glucose-6-phosphate dehydrogenase enzyme activity in ethanol treated rats

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In this study, we aimed to evaluate the effects of ethanol on erythrocyte (G-6-P-D) enzyme activity and the effects of quercetin on erythrocyte G-6-P-D activity in the recovery of the effects of ethanol.

Rats were randomly divided into four groups. The control group (n = 6) received physiological saline. The quercetin group (n = 7) received quercetin (120 mg/kg/ day) via i.g. route The Alcohol Group (n = 7) received ethanol (80% v/v, 1 ml/day) via i.g. route. The Alcohol + Quercetin Group (n = 5) received 1 ml of Ethanol (80% v/v) 2 hours after quercetin treatment (120 mg/kg/day). Experimental procedures were peformed for 30 days. Erythrocyte G-6-P-D activity was found to be higher in the Quercetin Group than those in the Alcohol Group ($p < 0.001$). In the Alcohol Group, the erythrocyte G-6-P-D activity was found to be significantly decreased than those in the Control Group ($p < 0.001$). Statistically significant differences were observed in erythrocyte G-6-P-D activity between the Alcohol Group and the Alcohol + Quercetin Group ($p < 0.001$).

As a conclusion, our results demonstrate that ethanol decreased erythrocyte G6PD activity and quercetin was found to be beneficial in the prevention of toxic effect raised by ethanol.Key words: Erythrocyte, ethanol, G6PD, oxidative stress, quercetin

P-09.04.4-126
Effects of the sulphasalazine to the cerebral hypoxia reperfusion injury in rat

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Background: Cerebral ischemia/ reperfusion (I/R) injury is still a difficult process to treat and rehabilitate today. This study was designed to investigate beneficial effects of sulfasalazine in cerebral I/R injury in rat.

Methods: Except CONTROL group (n = 5), 20 Wistar albino rats were divided into four groups for acute and chronic stage investigation of I/R injury, and temporary aneurysm clips were attempted to both internal carotid arteries for duration of 30 minutes. Four hours later, except CONTROL, SHAM-A, SHAM-C groups, 40 mg/kg once a day sulfasalazine was administered to animals, orally. Animals were sacrified and then necrotic neuronal cells of hippocampal CA1, CA2, and CA3 region, and cortical necrotic neurons, perivascular edema, pyknotic neuronal cells, irregularities of intercellular organization (IIO) were counted and scaled histopathologically. Tissue IL-1β, IL-6, malonyldialdehyde (MDA), myeloperoxidation (MPO), NO, and TNF-α levels were measured by using ELISA, too.

Results: Sulfasalazine could reduce perivascular edema, IIO, cortical and hippocampal neuronal cell death in both stages. It could decreased MDA in acute stage, but not reduce IL-1β, IL-6, MPO, NO, and TNFα levels. It could increased IL-1β levels in chronic stage but not affect to IL-6, MPO, MDA, NO, TNF-α levels.

Conclusion: Sulfasalazine could improve histopathological architecture of hypoxic tissue in both stages of I/R injury. It could inhibit lipid peroxidation cascades in acute stage but not affect to tissue MPO, NO, IL-6, and TNF-α levels in any stage in rat. These results suggested that therapeutic mechanisms of sulfasalazine should be investigated by using more specific laboratory methods in future studies.

Key words: antiinflamatory, cerebral hypoxia reperfusion injury, sulfasalazine, stroke.

P-09.04.4-127
Study on nitrate and nitrite reducing activity of xanthine oxidase in camel and horse milk

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Camel and horse milk xanthine oxidase (XO) was found to catalyze the reduction of nitrate and nitrite to nitric oxide (NO) under aerobic condition. To date, mammalian nitrate reductase (NaR) and nitrite reductase (NiR) have not been identified. NO, a gas, is found to control a seemingly, limitless range of functions in animals.

One assay was used to determine NaR and NiR activities of milk XO: (1) nitrite formation from nitrate by NaR, and (2) nitrite utilization by NiR. Nitrite concentrations were determined by using sulfanylamide and N-(1-naphtyl)-ethylenediamine, which form red color measured at 485 nm.

These activities of the milk XO require NADH as a physiological electron donor. High XO, NaR and NiR activities are detected only after heat treatment (80 °C, 5 min) of the fresh milk in the presence of molybdate. In both camel and horse milk NaR activity of XO was almost two times higher than its NiR activity. It is well known that XO can be reversibly converted from the dehydrogenase form to the oxidase through the oxidation of sulfhydryl groups. Cysteine and, to a lesser extent, glutathione increased NiR activity of milk XO but not its NaR activity. The mechanism of this increase of NiR activity remains unclear and is currently under study. Substitution of tungsten for molybdenum under above conditions gave no detectable NaR and NiR activity of milk XO. The molybdenum site-directed inhibitor, tungsten inhibited in a dose-dependent manner. Therefore, nitrate and nitrite are clear to interact with Mo center of XO.

Camel and horse milk are traditional drinks in Central Asia and Kazakhstan. Therefore, it is very important that XO provide a mechanism for generation of NO in camel and horse milk where nitric oxide synthase, NO producing enzyme, does not exist.

P-09.04.4-128
The influence of phytomedicine on metabolic processes of white rats undergone to ionized radiation

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The study of Peroxide lipids oxidation (PLO) process is used as one of stability parameters of organism's changes and as a key mechanism for understanding of adaptation reactions and of pathogenesis of different diseases. It's determined by high biological activity of products which are formed in the PLO reactions, in this relation lipids with high contents of fat acids play important role. To investigate the influence of phytomedicine Eminium Regelii on the metabolic processes (Peroxide lipids oxidation) of white rats' organism in conditions of ionized radiation.

The animals were exposed to ionizing radiation (gamma-radiation 60 Co) on the radiotherapeutic equipment Teragam in a dose of 6 Gy and received phytomedicine Eminium Regelii in a dose of 2.5 mg/kg orally within 14 days following the ionizing radiation exposure. Gamma-rays caused the increase of lipid peroxidation (LPO) primary (DC) and secondary products' (MDA) concentrations in spleen, liver, thymus and adrenal glands.

Treatment by phytomedicine resulted in contents of DC decreased in 3 times in spleen, in 7 times in thymus, in 5 times in adrenal glands, in liver in 4 times, in lymph nodes of small intestine it in 3 times. MDA decreased in liver up to 6 and 3 times, in spleen in 3.2 times, in thymus in 9 times, in liver in 6 times, in adrenal glands in 12 time, no changes in lymph nodes of small intestine.

The effect of phytomedicine treatment of organisms exposed to sublethal dozes of gamma-radiation results in the LPO primary and secondary products concentrations decrease in spleen, liver, thymus and adrenal glands.

P-09.04.4-131
Evaluation of serum levels of ischemia modified albumin (IMA) in bipolar disorder patients

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Introduction: Bipolar disorder is one of the most debilitating psychiatric disorders characterized by disruptive episodes of mania/hypomania and depression. Considering the complex role of biological and environmental factors in the etiology of affective disorders; recent studies have focused on oxidative stress, which may damage nerve cell components and take part in pathophysiology. Aim of our study is to contribute these data about oxidative stress in bipolar disorder, by detecting Ischemia Modified Albumin (IMA) levels of bipolar disorder patients in remission and also by comparing these results with healthy controls.
Methods: Study population consisted of 35 patients meeting the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) criteria for bipolar disorder I. 36 healthy subjects were included as control group (HC). Serum Ischemia Modified Albumin (IMA) levels of all participants were determined.
Results: Statistical analysis on serum Ischemia Modified Albumin (IMA) levels did not show any significant difference between bipolar disorder patients in remission and healthy controls.
Conclusion: Studies on oxidative stress in bipolar disorder have reached controversial results up till now. In this study, no statistically significant difference was detected between oxidative parameters of bipolar disorder patients in remission and healthy controls. In order to evaluate oxidative stress in bipolar disorder comprehensively, further studies are needed. Keywords: Bipolar disorder, Ischemia Modified Albumin (IMA), oxidative stress

P-09.04.4-133
Xanthine oxidase and adenosine deaminase activity in patients with familial mediterranean fever (FMF)

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Objective: FMF is an autosomal recessive dissease which is characterized by recurrent fever and inflammation of serous membranes. In this study we measured serum adenosine deaminase (ADA) and xanthine oxidase (XO) levels in FMF cases.
Method: Serum ADA levels were measured with a sensitive colorimetric method described by Giusti and XO levels were analysed by the method of Worthington in 30 FMF patients and 30 healthy controls.
Results: There was a significant difference in XO and ADA levels between controls and cases. ADA and XO levels were higher in patents with FMF.
Conclusion: ADA plays an important role in the function of immune cells. XO plays a role in nucleotide metabolism.

P-09.04.4-134
Paradoxical role of humic acid in some of cancer cell lines

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Humic acid (HA) is a natural product which is forming during decomposition of organic matter in humus. In recent years, there are some resarches on the medical use of HA. The present study was undertaken in order to evaluate the anticancer properties of the HA using a prostate cancer and osteosarcome cell lines PC-3, SJSA1 as an in vitro model system.

HA was purchased from Sigma-Aldrich. The cells were maintained in DMEM medium supplemented with 10% heat-inactivated FBS and 1% penicillin/streptomycin. Cells were grown in petri dishes in a humidified atmosphere containing at 37°C. Five different concentrations (100 ug/ml, 50 ug/ml, 25 ug/ml, 10 ug/ml, 5 ug/ml) were prepared using a stock solution of HA. We measured cell proliferation and migration to understand of progression effects of HA in PC-3 and SJSA1 cell lines in vitro.

According to our results, HA treatment caused cytotoxicity and induced cell death in vitro in PC-3 cells with an IC50 value of 67.9 μg/ml. Contrary to this, HA induced proliferation of SJSA1 cells in dose dependent manner. HA demonstrated the highest proliferatif activity against SJSA1 cells with an IC50 value of 100 > μg/ml. On the other hand, cell migration was reduced in PC-3 cell line and interestingly, migration was accelerated in SJSA1 cell line.

Our study may provide new insights into the regulatory effect of HA in cancer, but further studies are needed to clarify the role of HA in cancer pathogenesis.

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The effects of EGCG and CAPE through PI3K/Akt/mTOR pathway on ischemia-reperfusion damage in rat testicular tissue

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Testicular torsion (TT) is public urologic emergency among children. Main pathophysiology of testicular damage in TT may be due to ischemia/reperfusion injury of testis. The aim of this study was to investigate the effects of Epigallocatechin-3-gallate (EGCG) and Caffeic acid phenethyl ester (CAPE) on PI3K/Akt/mTOR pathway in experimental TT model. Rats were divided into five groups (n = 7). Control group; 2 hours torsion/4 hours detorsion group (T/D); all other groups were saturated for four days EGCG, CAPE and EGCG+CAPE (10μml/kg). Sections were taken from Bouine's-fixed and paraffin-embedded testicular tissue blocks and stained with H&E. Immunohistochemistry was applied for the detection of PI3K, Akt and mTORC. Intensities were evaluated as mild (1), moderate (2) or strong (3). Serum 8OHdG, plasma MDA levels were analyzed using ELISA method. Results were analyzed by ANOVA statistical test. Testis samples in control group exhibited normal histological morphology. Disorganization and separation of seminiferous tubule cells and accompanying interstitial edema and vessel dilation were observed in T/D group. Administration of EGCG before T/D reduced the histological detoriation in testicular tissue. Spermatogenic cells were seen to have 3/2/3 PI3K/ Akt/mTORC immunoreactivities in control group; 1/1/1 in T/D group, 2/2/3 in CAPE group, 2/3/2 in EGCG group, 2/1/2 in CAPE+EGCG group, respectively. Serum 8OHdG, plasma MDA levels from T/D group increased significantly when compared to control group. While MDA level decreased significantly in CAPE+EGCG group, 8OHdG level showed significant increase in CAPE group. In conclusion, CAPE and EGCG exerted protective effects on TT. Effects may be achieved through PI3K/Akt/mTORC pathway involved in cell proliferation, angiogenesis, apoptosis. Prophylactic use of EGCG prior to TT surgery improved testicular morphology, therefore could prevent destructive effects of TT and could be important treatment for patients with possible future infertility.

P-09.04.4-136
Evaluation of acylcarnitine levels measured in our laboratory

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Objectives: Deaths from inherited metabolic disorders may remain undiagnosed after postmortem examination and may be classified as sudden infant death syndrome (1). Plasma/serum and dried blood spot (DBS) acylcarnitine profiles (ACPs) are key to the diagnosis of mitochondrial fatty acid β-oxidation disorders (FAODs) (2). In this study admitted to our hospital between May 2015-May2016, 131 male, 100 female (mean age, 550.27 ± 620.979 day.) acylcarnitine results in 231 patients were evaluated.

Materials and methods: 20 acylcarnitine levels were evaluated in this study. Acylcarnitines were analysed in dried blood spots using LC-MS/MS in male and female patients.

Results: Acylcarnitine levels of patients were; mean ± SD; C0 (23.57 ± 10.693 μmol/l), C2 (19.03 ± 9.706 μmol/l), C3 (1.62 ± 1.258 μmol/l), C4 (0.26 ± 0.190 μmol/l), C4DC (0.32 ± 0.194 μmol/l), C5:1(0.02 ± 0.030 μmol/l), C5(0.21 ± 0.121 μmol/l), C5DC (0.10 ± 0.078 μmol/l), C5OH (0.29 ± 0.380 μmol/l), C6(0.19 ± 0.529 μmol/l), C8(0.07 ± 0.052 μmol/l), C10:1(0.10 ± 0.067 μmol/l), C10 (0.08 ± 0.068 μmol/l), C12(0.11 ± 0.072 μmol/l), C14:1(0.08 ± 0.062 μmol/l), C14(0.11 ± 0.064 μmol/l), C16 (1.38 ± 0.911 μmol/l), C18:1(0.56 ± 0.351 μmol/l), C18:1-OH (0.02 ± 0.017 μmol/l), C18 (0.58 ± 0.278 μmol/l)), respectively.

Conclusions: This study is important for Konya region, mitochondrial fatty acid β-oxidation disorders studies subject areas. This study is the first study to assess acylcarnitine levels of patients living in our region. We believe that our results will be useful for future studies. Key words: Acylcarnitine, Mass spectrometry, Dried blood spot

P-09.04.4-137
Binding of FAs and Cu(II) ions to HSA changes its Cys34 thiol group antioxidant capacity and carbonylation pattern with methylglyoxal

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Human serum albumin (HSA), the most abundant serum protein is transporter of free fatty acids (FAs), Cu and other metal ions. HSA represents major plasma antioxidant due to its Cys34 reduced/sulfhydryl form. FAs binding to HSA could lead to changes of Cys34 thiol group accessibility, reactivity and antioxidant property. The aim was to investigate the effects of selected FAs (stearic, miristic, oleic, fish oil extract-FO, FAs mixture-MixFAs), on Cu(ii) binding and synergistic influence on HSA-SH and HSA carbonylation by methylglyoxal (MG).

Changes of thiol group reactivity (and content) were followed by determination of pseudo first order rate constant (k') for thiol reaction with Ellman reagent. Changes of HSA were monitored by determination of guanidine group content, native PAGE and fluorescence spectroscopy. For FA/HSA molar ratios screening qTLC and GC were used.

Binding of FAs increases the HSACys34-SH reactivity, k' values increased in order: MixFAs, oleic, stearic, fish oil extract and myristic acid. Binding of Cu(ii) ion (0.1 mol/mol HSA) led to increase of k' value if fish oil extract was present, but for other FAs k' value decreased. The content of free HSACys34-SH decreased for 10% after Cu(ii) ion binding, and during 24 hours incubation at 37 °C, it was further decreased for 10% (stearic acid, MixFAs) and 20% (myristic, fish oil extract, oleic acid). Carbonylation of FA-HSA-Cu(ii) complexes with MG (20 mol/mol HSA), lead to decrease in Cys34-SH content depending on FA present: 30%-35% for myristic and stearic acid, 40% for oleic acid and MixFAs and 40% for fish oil extract.

Carbonylation of FA-HSA-Cu complexes could contribute to further enhancement of oxidative and carbonyl stress in diabetes as well as other diseases. Carbonylation level and reactivity of Cys34-SH depend on type of FAs bound to HSA which implies the possibility for modulation of SH reactivity by FAs as a supplement (Project No 172049 Ministry of Education and Science, Serbia).

Author Index

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