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SPEED TALKS

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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 Late-breaking abstracts and abstracts withdrawn after Congress cancellation are not included in this issue.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Abstracts submitted to the Congress are **not peer-reviewed**.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.

How to cite these abstracts

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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.

** The Abstract number begins with the letters ST and can be found atop each abstract's title in the PDF file.

SPEED TALKS

Sunday 4 September
17:30–19:30, Hall A

Developments in biomaterials and tissue engineering

ST-07.01.3-001
In vitro biomineralization of hydroxyapatite crystals controlled by recombinant bone extra cellular matrix proteins

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Biomineralization is deposition of hydroxyapatite, the crystallized mineral form of calcium and phosphate, by cells to their extracellular matrix (ECM), and it is an essential mechanism of bone and teeth formation in humans. Biomineralization is especially important in adults for tissue regeneration in bone defects. ECM molecules regulate mineral formation and provide crystal growth and nucleation. One of the most important ECM molecules, Alkaline Phosphatase (ALP), is the key enzyme in biomineralization process by the activity of converting organophosphate into inorganic phosphate. Moreover, osteocalcin and osteopontin are small soluble noncollagenous proteins of ECM and they regulate biomineralization by binding to calcium atoms available at crystal surfaces due to their highly negative charged amino acid residues. In this study, ALP, osteocalcin and osteopontin are expressed in bacterial systems and purified to assess *in vitro* biomineralization. Optimization of *in vitro* biomineralization activities with osteocalcin and osteopontin proteins provided the understanding of the effect of protein concentrations in crystal structure of calcium crystals. Understanding of the effect of protein concentrations will provide control over biomineralization in different cell types by designing synthetic genetic circuits. Programming non-biomineral formation cells for biomineral formation will enable differentiation free bone mineral formation. Reprogramming of non-biomineral formation cells will help to treat bone defects and bone-impairing diseases, such as osteoporosis. Consequently, it is an outstanding approach to understand the activity of bone ECM proteins and construct synthetic genetic systems that can reprogram non-mineral formation cells for biomineralization within the scope of bone tissue engineering.

ST-07.01.3-002
Cytotoxicity of folic acid conjugated PEGMA nanoparticles toward Caco-2 cells with and without encapsulated irinotecan

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The development of external stimulus responsive smart nanoparticle systems has received significant attention in recent years in biomedical sciences and medicine especially with controllable drug delivery system being used in cancer therapy. In the past decade, nanomaterial especially anorganic nanomaterials are frequently used as therapotic agent in the field of photothermal therapy. But, because anorganic nanomaterials are raising concerns regarding their potential long-term toxic effects, their application are limited in biomedical and medicine fields. Therefore, nontoxic and more biocompatible or biodegredable PTT agent, such as organic ones, are urgently needed to be developed. So, limited literature exist on the succesful utilization of biocompatible polymer stabilizer added PEGMA nanoparticles in vitro and in vivo photothermal cancer therapy applications.

Caco-2 cells were cultured in RPMI-1640 medium with fetal bovine serum (20%), 2 mM L-glutamine, 1 $mg{\cdot}mL^{-1}$ gentamycin. The cells were cultured in a humified incubator (at 37°C, 5% CO_2). At 90% confluence, the cells were harvested using 0.25% trypsin and were subcultured into 96-well plates with each well 10 000 cells. The next day, at increasing doses PEGMA (4–500 $\mu g{\cdot}mL^{-1}$) were added on to the cells. Then, XTT were added to each well. After incubation (at 37°C, 5 h) optic density determined in ELISA Reader (460 nm). IC_{50} value was calculated for PEGMA nanoparticles.

The selective cytotoxicity of PEGMA nanoparticles was further enhanced by conjugation of folic acid and incorporation of irinotecan: at 24 h and an equivalent irinotecan concentration of 0.35 $\mu g{\cdot}mL^{-1}$, viable Caco-2 cells were reduced to 45%. Folic acid conjugation served to enhance the viability of Caco-2 cells in this work. Careful optimization of the folate content should further improve the cell specificity of the PEGMA nanoparticles, thus providing a viable targeting platform for cancer therapy.

Stem cells and cancer

ST-05.03.3-001
Epidermal β-catenin activation remodels the dermis via sequential signalling to distinct fibroblast lineages

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Sustained epidermal Wnt/β-catenin signalling expands the stem cell compartment and drives cells of the interfollicular epidermis and sebaceous gland into hair follicle lineages, thereby inducing ectopic hair follicles (EF) in adult skin. This is accompanied by extensive fibroblast proliferation and extracellular matrix (ECM) remodelling in the underlying dermis to a neonatal state. We unravelled that epidermal Hedgehog (Hh) and Transforming Growth Factor beta (TGFβ) signalling mediate the dermal changes. Pharmacological inhibition or genetic deletion of these pathways prevents β-catenin-induced dermal reprogramming and EF formation. Epidermal Shh stimulates proliferation of the papillary fibroblast lineage, while TGFβ2 controls proliferation, differentiation and ECM production by reticular fibroblasts. Hh inhibitors do not affect TGFβ target gene expression in reticular fibroblasts, and TGFβ inhibition does not prevent Hh target gene induction in papillary fibroblasts. However, when Hh signalling is inhibited, the reticular dermis does not respond to epidermal β-catenin activation. We conclude that the dermal response to epidermal Wnt/β-catenin signalling depends on distinct fibroblast lineages responding to different paracrine

signals. These findings are of particular interest, given the many different epithelial tumours in which there is inappropriate activation of Wnt signalling accompanied by changes in the underlying connective tissue.

ST-05.03.3-002
Targeting ALDH+, CD44+, CD24− breast cancer cell population by silencing with MDR1 siRNA

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Introduction/Aim: Cancer stem cells generate the basis of tumorigenicity and metastasis by means of their extraordinary characteristics. According to cancer stem cell theory, cancer stem cells increase in number and make the chemotherapy impossible during chemotherapy. Cancer stem cells are immortal, tumor initiating and self renewing pluripotent cells. Breast cancer stem cells have some phenotypic markers profoundly which are CD44+, CD24−, connexin 43, CK18, GATA3, MUC, vimentin ve ostenectin. Generally most of the cancer stem cells are resistant to chemotherapy due to overexpression of drug resistance genes (*MDR1, BCRP* i.e.). Cancer stem cell markers should be clarified to target stem cells and especially this will play very important role to reverse drug resistance.
Materials and Methods: Breast cancer cell population bearing the cancer stem cell markers were sorted by FACS cell sorter. CD44, CD24low and ALDH markers were used during sorting procedure. Expression levels of *MDR1, ALDH* and *EGFR* genes were analyzed by real-time PCR using cDNA as templates. The cells were transfected by *MDR1* and mock siRNA and tranfection efficiency was determined by fluorescent siRNA. Effects of *MDR1* gene silencing were determined by molecular and toxicological analyses.
Results: The cells with ALDH+, CD44+, CD24− phenotype were found to over-express cancer stem cell marker *ALDH, EGFR* and *MDR1* genes profoundly when compared to MCF-7 parent cell line. Inhibiting MDR1 gene expression, by siRNA silencing technology, reversed drug resistance. siRNA silencing also caused the alterations on the expression levels of the stem cell marker genes to some extent.
Conclusion: These results will lead to explain the complicated behaviors of breast cancer stem cells and will provide background for the investigation to target cancer stem cells.

ST-05.03.3-004
STAMP2 is required for human adipose-derived stem cell differentiation and adipocyte-facilitated prostate cancer growth *in vivo*

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Six Transmembrane Protein of Prostate 2 (STAMP2) has previously been implicated in both prostate cancer (PCa) and metabolic disease. STAMP2 has unique anti-inflammatory and pro-metabolic properties in mouse adipose tissue, but there is limited information on its role in human metabolic tissues. Using human adipose-derived stem cells (ASCs) we report that STAMP2 expression is dramatically upregulated during adipogenesis. shRNA-mediated STAMP2 knockdown in ASCs significantly suppressed adipogenesis and interfered with optimal expression of adipogenic genes and adipocyte metabolic function. Furthermore, ASC-derived adipocyte-mediated stimulation of prostate tumor growth in nude mice was significantly reduced upon STAMP2 knockdown in ASC adipocytes. These results suggest that STAMP2 is crucial for normal ASC conversion into adipocytes and their metabolic function, as well as their ability to facilitate PCa growth in vivo.

ST-05.03.3-005
Investigating the activity of *Thymus capitatus* essential oil on cancer by using human telomerase reverse transcriptase immortalized mesenchymal stem cells

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Plant derived natural products can provide promising anti-cancer activity and can support the development of novel anti-cancer treatment strategies as cytotoxic activity towards cancer cells was observed in the recent studies in which different human cancer cell lines were exposed to plant essential oils.

In our study, thyme (*Thymus capitatus*) essential oil was tested for its anti-cancer activity by using human telomerase reverse transcriptase immortalized mesenchymal stem cells (hMSC-TERT) and a tumorigenic cell line; human telomerase reverse transcriptase immortalized mesenchymal stem cells which are irradiated with 2.5 Gy of γ-rays. The hMSC-TERT and irradiated hMSC-TERT cells were exposed to increasing concentrations of thyme essential oil in order to investigate the effects of different concentrations of thyme essential oil on the hMSC-TERT and irradiated hMSC-TERT cells and to identify any cytotoxic activity of thyme essential oil towards these cell lines. The hMSC-TERT and irradiated hMSC-TERT cells were treated with 0.5% (v/v), 1% (v/v) and 2% (v/v) thyme essential oil. Cell morphologies were investigated under the microscope and a cytotoxic activity of thyme essential oil was observed for all three concentrations at different levels. Images of the cells were obtained at 0 h, 2 h, 4 h, 12 h, 24 h, 72 h and 120 h and at these time points dead cells were pelleted. Cells were followed for 20 days and at the end of 20 days the highest level of hMSC-TERT and irradiated hMSC-TERT cell death was observed at the concentration of 0.5% (v/v) thyme essential oil. These will be regarded as preliminary results and as promising results were obtained in this study, future experiments will be performed to further investigate the anti-cancer activity of thyme essential oil. Cell viability, senescence and apoptosis will be investigated in the further experiments by using 0.5% (v/v) thyme essential oil.

ST-05.03.3-006
PBX/Knotted 1 Homeobox 2 is an activator and a downstream target of transforming growth factor beta-1

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Introduction: PBX/Knotted 1 Homeobox 2 (PKNOX2) is a member of 3-amino acid loop extension (TALE) superfamily and is involved in sequence-specific DNA binding and actin monomer binding. Recently Pknox2 was reported as a supressor of Bone morphogenetic protein (Bmp)/Smad signaling as its overexpression inhibited the signaling. Studies showed that transforming growth factor-beta (TGF-β)/Smad signaling might be activated upon Bmp/smad pathway inhibition. We then analyzed results of

GEO dataset (GSE46019) and found that TGF-β1 stimulation upregulated *PKNOX2* expression. We hypothesized that PKNOX2 might be an activator and a downstream target of TGF-β/Smad signaling pathway.

Materials and Methods: Bone marrow mesenchymal stem cells (MSCs) were transiently transfected with PKNOX2-pCMV6 or empty vector. PKNOX2 protein level was determined following 48 hours of transfection. *PKNOX2* and *TGF-β1* gene expression were also determined using qPCR. Additionally, MSCs were cultured with two different doses (0.1 and 5 $ng{\cdot}mL^{-1}$) of recombinant human (r)TGF-β1 protein for 24, 48 and 72 h. *PKNOX2* gene expression level was determined by qPCR.

Results: MSCs transfected with PKNOX2 had higher levels of PKNOX2 protein and gene expression as well as *TGF-β1* expression. 24 hours after rTGF-β1 induction, *PKNOX2* expression increased as concentration increased. 48 hours after induction, there was a decrease in the gene expression with both doses of rTGF-β1. 72 hour after treatment, *PKNOX2* expression again increased in MSCs induced with high dose recombinant protein but there was no change in gene expression with low dose.

Discussion and Conclusion: Since TGF-β1 is known to induce epithelial-mesenchymal transition (EMT) and PKNOX2 is shown to localize at mesenchymal-epithelial transition (MET) sites, TGF-β1 induced PKNOX2 may be involved in EMT or MET transition. Morever, disregulation in PKNOX2 expression in disease states may change tissue identity.

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ST-05.03.3-007
Cytotoxic dose assessment of cell stress inducers on human bone marrow mesenchymal stem cells by real-time xCELLigence impedance analysis

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Cellular therapies with human mesenchymal stem cells (hMSCs) have shown great promise in medicine. Information on the effects of different cell stress inducers on hMSCs that may contribute to establishment of in-vitro injury models to investigate disease states is of great importance. H_2O_2, Rotenon (Rot) and EtBr are cell stress inducers affecting different cellular pathways but their role on hMSCs are still unknown. The aim of this study was to investigate the proliferative potential and cytological features of hMSCs subjected to acute or prolonged cell stress with these stress inducers. hMSCs isolated from bone marrow of 3 healthy individuals and brought to passage 3 were exposed to gradually increased concentrations ranging from 10 μM to 1.5 mM of H_2O_2, 250 nM to 500 μM EtBr, 5 nM to 5 mM Rot in 4 different experiments each as triplicate where non treated hMSCs were used as control. Cytotoxic effects of the inducers were monitored by real-time xCELLigence system for 3 days. Time-dependent xCELLigence cell survival curves revealed that hMSCs exposed to H_2O_2 between doses of 10–350 μM expanded exponentially same as control, they remained at steady state between 350–450 μM but cell viability started to decline sharply and sudden cell deaths were observed above 550 μM up until 1.5 mM. Growth of hMSCs remained exponential comparable to control at Rot concentrations ranging from 5 nM to as high as 1 mM; 5 mM was highly toxic and sudden cell deaths were observed. hMSCs cell index increased exponentially in EtBr doses between 250–500 nM similar to control; cells remained at steady state phase around 1 μM; slight increase followed by sharp decrease was observed at 3 μM till 500 μM. These results revealed that xCELLigence system is a powerful platform for continuous quantitative measurement of hMSCs viability and death in the experiments conducted for robust high-throughput dose screening of cell stress inducers.

Acknowledgement: Supported by Hacettepe University Research Fund #THD-2015-8178.

ST-05.03.3-009
Adenosine generated by ectonucleotidases of glioblastoma-initiating cells controls the migration/invasion of glioma cells enhancing EMT through the activation of low-affinity adenosine receptors

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Glioblastoma multiforme (GBM) is a highly invasive neoplasm, comprised of a cell subpopulation with tumorigenic capacity called Glioblastoma-initiating Cells (GICs). Inside the tumor has been described a hypoxic niche that promotes Epithelial-Mesenchymal Transition (EMT), migration/invasion and increased extracellular Adenosine levels, which activates the Low-Affinity Adenosine Receptors (L-AARs) A2BAR and A3AR. We postulate that this high concentration of Adenosine under hypoxia is mediated by an increased activity of ectonucleatidases (CD73 and/or PAP) in GICs, which controls the migration/invasion of GBM cells through the activation of L-AARs.

GICs of a GBM cell line were cultured under normoxia and hypoxia. The expression of ectonucleotidases, L-AARs and EMT markers were evaluated by RNA-seq/RT-qPCR, western blot and flow cytometry. HPLC fractionation was utilised to determine extracellular adenosine levels. Cell migration was measured using wound healing and neurosphere attachment assays; invasion was measured via agarose spot and transwell matrigel assays. To determine the ectonucleotidase activity and its effect on cell migration/invasion, CD73 and/or PAP knockdown cell lines were generated. The contribution of L-AARs on cell migration/invasion and expression of EMT markers were evaluated using selective antagonists of A2BAR and A3AR.

Ectonucleotidase activity was higher in GICs cultured under hypoxia than normoxia. CD73 and PAP were responsible for adenosine production under normoxia and hypoxia respectively. Knockdown of CD73 and PAP decreased cell migration/invasion under normoxia and hypoxia respectively. Inhibition of L-AARs decreased cell migration/invasion and EMT markers; A2BAR seems to be responsible for these processes under hypoxia.

High extracellular Adenosine concentration generated by increased activity of ectonucleotidases enhances migration/invasion in GBM cells, increasing the expression of EMT markers through the activation of L-AARs.

ST-05.03.3-010
Cell state transitions during early differentiation of mouse embryonic stem cells

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Cell state transitions during development or in vitro differentiation result from the cell's current transcriptional and epigenetic state, as well as the processing of external signals, both mechanical and biochemical driving changes in gene expression. Discerning the relative effect of internal state and external signals in early differentiation is essential for understanding differentiation dynamics during development and for future control of in-vitro tissue composition. Live imaging provides an opportunity to study cell decisions at single cell resolution. By using fluorescent markers it is possible to follow the dynamics of gene expression and morphological changes over time. Cell lineage, correlated with the epigenetic state memory, and external signals (molecular and mechanical) correlated with cell location, may play different roles in fate decisions.

We combine live imaging, spatial analysis and statistical approaches to quantify these effects on cell state transitions. We developed a high throughput method to characterize the decision point of mouse embryonic stem cells in colonies (2D) and in Embryoid bodies (3D) in mesoderm differentiation.

Comparing these systems provides insights on the signals affecting the decision as they differ in cell to cell contact and cells exposure to medium. We look for tissue scaling effects on signal and fate patterns by decoupling the differentiation time from the tissue size.

Our preliminary results show that mesoderm formation in EB is largely depends on a mechanical signal from the contact point with the surface regardless the starting EB size. Moreover, differentiation in an ECM-like environment abolishes this effect and changes the timing and dynamics of the mesoderm formation. Using a localized BMP4 signaling inside an EB precedes the mesoderm onset and affects its localization. We expect these results will enhance our understanding on how internal state and external signals are integrated in differentiation decisions.

ST-05.03.3-011
IGF-1 and IGFBP-3 levels and their correlations with CEA in colorectal cancer patients

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Colorectal cancer is one of the most frequently seen cancers worldwide. Currently, CEA is the most commonly used tumor marker in colorectal cancer. The changes in IGF/IGFBP equilibrium is known to cause carcinogenesis. In this study, we aimed to monitor IGF-I/IGFBP-3 levels, the changes in IGF-I/IGFBP-3 ratio and correlations of the levels of these peptides with the common marker CEA. 55 colorectal cancer patients and 35 control group patients admitted to Ankara Numune Training and Research Hospital in six month period are included in these study. Serum CEA, IGF-I and IGFBP-3 levels of all specimens are measured with chemiluminescence method.In colorectal cancer patients, IGF-I levels found to be increased, IGFBP-3 levels decreased and IGF-I/IGFBP-3 ratio was increased; when compared to control group ($P < 0.05$). A moderately significant correlation was found between the conventional tumor marker CEA and IGF-I and IGF-BP3 ($r = 0.533$ and $r = -0.573$ respectively). IGF-I/IGFBP-3 ratio among with CEA can be a useful marker in follow-up of colorectal cancer patients.

Miscellaneous

ST-Mis-001
USP29 is a new regulator of HIF signalling

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Hypoxia Inducible Factor (HIF) is the central transcription factor that regulates a variety of cellular processes that allow cells to adapt to and survive low oxygen conditions (hypoxia). In healthy cells, HIF signalling is tightly controlled via the availability of its α-subunit. HIF-α is degraded by the ubiquitin-proteasome system (UPS) in well oxygenated cells but the protein is stable in hypoxia. HIF-α is also stabilised in a variety of cancer cells of different origin due to genetic alterations independently of the present oxygen level and sustained expression of HIF-α has been associated with aggressiveness, migratory and metastasis-initiating potential, and therefore worse prognosis.

In order to better understand which proteins might be responsible for pathological activation of HIF signalling, the family of deubiquitinating enzymes (DUBs) was silenced in an RNAi screen and the impact of the selected DUBs was further characterised using standard biochemical methods and confocal fluorescence live cell imaging techniques.

We identified a new non-canonical positive regulator of HIF-α stability. USP29 belongs to the family of ubiquitin-specific proteases (USPs) and efficiently stabilised HIF-α in a O_2/PHD/pVHL-independent and a proteasome-dependent way. Furthermore, USP29 expression correlated with disease progression in prostate cancer.

Our data present a novel hypoxia-independent HIF-α regulation and suggest that the maternally imprinted USP29 gene might act as an oncogene. In the light of DUBs being a class of highly druggable proteins, our results might open up possibilities for new therapeutic approaches.

MicroRNAs and noncoding RNAs

ST-01.03.3-001
Aldosterone induction alters microRNAs profile in rat kidney

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Naturally occurring, small non-coding RNA molecules; miRNAs are complementary to one or more mRNA molecules, and by downregulating gene expression regulate expression of many genes including cancer and cardiovascular diseases. Aldosterone, steroid hormone is produced in the cortex of the adrenal glands, has a crucial importance on the regulation of blood pressure and like many hormones its expression is also regulated by miRNAs. The aim of this study to clarify which miRNAs have been altered after treatment with aldosterone in rats. In order to investigate the relation between miRNAs and aldosterone regulation, aldosterone induced rat models were generated. Quantitative amount of aldosterone was measured by Aldosterone ELISA Kit. Systolic blood pressure of rat was measured by non-invasive "Tail-cuff" method. RNA samples from heart tissue were isolated using

TRIZOL, quality and quantity of RNA samples were calculated using Bioanalyzer using Agilent RNA 6000 Nano Kit. Alteration in miRNA expression has been detected via customized miRNA array and selected aldosterone related gene expressions subsequently confirmed by qRT-PCR. Serum samples of rat were analyzed for aldosterone concentration and aldosterone concentration increased from 288.1 $pg{\cdot}mL^{-1}$ to 623 $pg{\cdot}mL^{-1}$ when compared with control group ($P = 0.0008$). Blood pressure measurement studies showed that aldosterone induction causes formation of hypertension in rats (control group is 118 ± 9 mmHg while ALDO group has blood pressure as 164 ± 2 mmHg ($P < 0.0001$). miRNA array studies showed that 723 miRNAs have been elevated (2-fold, $P < 0.05$) and 29 of which showed 5-fold regulated and 14 of them altered 10-fold after treatment with 75 $\mu g{\cdot}kg^{-1}$ aldosterone in heart tissues. These results indicate that elevation in miRNA expression may modulate aldosterone level and causes formation of hypertension.
Acknowledgement: This project was supported by TUBITAK (114Z734).

ST-01.03.3-003
miR-21 is elevated in cumulus cells of women with poor ovarian response to stimulation

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Micro RNAs (miRNAs) are a large family of short (~21-nucleotide) non-coding mRNAs that repress gene expression through degradation of target mRNAs and/or inhibition of their translation. miR-21 has been impicated in ovarian function and follicular growth. In this study, we asked whether miR-21 expression is associated with the number of oocytes retrieved in women undergoing in vitro fertilization (IVF) and aimed to assess its regulation in ovarian cells using in vitro culture and down-regulation. Pooled cumulus cells were collected from women undergoing in vitro fertilization-intracytoplasmic sperm injection (IVF-ICSI). Expression of miR-21-5p (active strand of miR-21) and miR-21-3p was tested in poor responders ($n = 21$) and non-poor responders ($n = 29$) using quantitative real time polymerase chain reaction (qRT-PCR). Regulation of miR-21-5p and miR-21-3p in KGN cells by estradiol was tested *in vitro*. miR-21-5p and miR-21-3p levels were down-regulated in vitor by treatment with miRNA Inhibitors (chemically modified, single-stranded nucleic acids). qRT-PCR analysis showed that miR-21-5p expression iss significantly upregulated in poor responder patients ($P < 0.05$), while miR-21-3p expression was significantly lower ($P < 0.005$), suggesting that elevated miR-21-5p expression in cumulus cells is not regulated at the pre-miR-21 level in poor responders. Lastly, we found that both miR-21-5p and miR-21-3p are increased in KGN cells in response to higher doses of estradiol ($P < 0.05$), while their expression is not affected at lower estradiol concentrations. In this study, we found that poor response to IVF is associated with elevated expression of miR-21-5p, and that this elevated expression is independent of lower serum estradiol levels in poor responders. Whether miR-21 plays a role in human cumulus cell function and whether miRNA expression in cumulus cells may be used as a biomarker for oocyte or follicular viability remains to be investigated.

Developmental biology

ST-02.09.1-001
Mismatch repair activity in human preimplantation embryos

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Mismatch repair (MMR) plays an important role in repairing DNA replication errors and it is known to be effective in repairing both base-base mismatches and insertion/deletion of loops. MMR repair genes were shown to be expressed in the early stages of mammalian development and they were shown to be crucial for healthy development. Therefore developing a functional assessment for MMR is important. This study aims to develop a functional assay for MMR in human preimplantation embryos.

Homo/heteroduplexes were formed in the presence and absence of nicks using oligonucleotides. The constructs were exposed to commercially available nuclear/whole cell extracts and extracts obtained from mouse and human blastocysts. The efficiency of MMR was confirmed by minisequencing. Control studies were carried out in the absence of any nuclear/whole cell extracts.

Both nicked and non-nicked heteroduplexes were repaired in nuclear and whole cell extracts. Control studies in the absence of nuclear and whole cell extracts showed no repair. Synthetic oligonucleotides were used to form heteroduplex constructs and MMR efficiency was semi-quantitatively analysed after exposure to nuclear/whole cell extracts (as little as 2.5 μg) and extracts obtained from pooled mouse and human blastocysts.

In vitro plasmid-free assay was successfully developed to assess the functional activity of mismatch repair (MMR) in preimplantation embryos in which MMR was shown to be active in human blastocysts.

ST-02.09.1-002
Angiogenic effect and wound healing potential of *Enteromorpha linza* L. (Linnaeus) J.Agardh (Green algae) methanol extract

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Marine macroalga have rich secondary metabolites and they have entered in many areas such as medicine, pharmacy and food of our lives. In this study, we aimed to determine the angiogenic effect and wound healing potentials of methanol extract of *Enteromorpha linza* from the Aegean Sea.

CytoSelectTM 24-Well Wound Healing Assay Kit (Cell Biolabs, INC) was used for wound healing activity of the extract. Cells ($2–5 \times 10^4$) were seeded for migration in the plates and incubated for 24 and 48 h at 37 ± 1°C. After incubation, cells were stained with DAPI and Giemsa for 5 min. Also, cells were monitored for migration into the wound field until the wound closed. In vivo antiangiogenic activity is determined by the HET-CAM (Hen's Egg Test Chorio-Allantoic-Membrane) method. Fertilized eggs (day 0) were placed in an incubator at 37 ± 1°C and 58 ± 2% hum. for 5 days. On day 5, air cells of the eggs were cut, algae extracts (300, 600 and 900 $mg{\cdot}mL^{-1}$) were placed directly onto the CAM and observed at 0.5, 2 and 5 min. for sign of hemorrhage, coagulation or lysis reactions.

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Growth rates of the cells (respectively $22 \pm 3\%$ and $43 \pm 6\%$) in wound healing assay after treatment of the extract was significantly faster than that of untreated control cells. However, HET-CAM results show that while methanol extract has angiogenic effect in 300 and 600 $mg{\cdot}mL^{-1}$ treatment, it has a high antiangiogenic score as 10.84 led to severe vascular irritation, mainly through vessel lysis in 900 $mg{\cdot}mL^{-1}$.

Angiogenesis is a tightly regulated process playing an essential role in wound healing. The effects on wound healing are related to the stimulation of angiogenesis by the extract. Our study showed that *E. linza* extract is a potent angiogenic agent both *in vivo* and *in vitro*. *In conclusion,* these findings highlight the potential of application of *E. linza* for promoting cell regeneration and wound treatment in parallel with in vivo studies.

ST-02.09.1-003
Retinoic acid metabolite (acitretin) showed developmental stage-associated differences of ADAMTS4 in rat fetuses

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Retinoic acid shows teratogenic effects through strengthening programmed cell death. It is suggested that the increase in cell death causes various malformations. ADAMTS proteinases take place in many physiological and pathological processes. ADAMTS enzyme is found in many tissues. Furthermore, it is expressed that they are synthesized in the embryonic period though the light of recent studies. Herein, we aimed to investigate the effects of acitretin on ADAMTS enzymes in rat fetuses. It was been demonstrated that acitretin, taken during pregnancy, caused fetal malformations and induced apoptosis in cranial and anal regions.

This study was performed on 50 fetus obtained from 20 adult female rats. 11, 13, 15, 17 and 19 weeks fetuses. Immunohistochemical (IHC) analyses were performed with the aggrecan, versican and ADAMTS1, 4, 5, 8, 9 respectively. A serious expression with ADAMTS1, 4 and 5 were found in the cortex, liver and cartilage proportionally to the increase in the age of the fetus whereas there was no detectable staining with ADAMTS9. It has been observed that aggrecan and versican significantly synthesized in vertebral disk, sternum, costa and other structures showing cartilage feature. ADAMTS 1, 4 and 5 were found more in cranial and anal regions. Our results showing that acitretin induce ADAMTSs and this may cause any effect on developing fetus by ADAMTSs.

In conclusion, acitretin as a teratogenic agent may affecting ADAMTS proteinases and therefore these enzymes played an active role in the pathogenesis of developing disease and malformation in the womb.

Sunday 4 September
17:30–19:30, Hall B

Miscellaneous

ST-Mis-007
Paclitaxel applied mice's peripheral nervous system gene expression profiles detected with microarray

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As an anticancer agent, paclitaxel (PTX) has neurotoxic effects especially on peripheral nervous system (PNS). The aim of this study is to investigate the gene expression profiles in PNS of paclitaxel applied mice.

PTX was dissolved in vehicle (ethanol: Cremophor EL). Animals were divided into three groups as; paclitaxel (PTX, $n = 8$), vehicle (VEH, $n = 8$), saline (SLN, $n = 11$). Total of 27 male BALB/c mice were used during experiments under permission of ethical committee (No: 2015-059). Animals received treatment on 0th, 4th, 8th, and 9th days. On 9th day animals were sacrificed via cervical dislocation and sciatic nerves were removed. Total mRNA isolations were done from pooled down sciatic nerves of each group. SurePrint G3 Mouse Gene Expression 8X60K Microarray kit was used for gene expression detection. Genes with a p value less than 0.05 were determined as significant and fold change by 2 considered as differentially expressed.

Up regulated and down regulated genes were determined. Then, up regulated and down regulated genes were grouped within. The dramatic alteration was observed for the catabolic activity genes. Catabolic activity genes up regulated most when compared to other subgroups. Also for sciatic nerves PTX and VEH received animals had higher apoptotic gene levels when compared to SLN group.

The neurotoxic effects of PTX was known however it is important to reveal which group of genes were affected most from that agent. The side effects of PTX on PNS was shown at the behavioral and histological levels but first time with that study total gene expression profiles were reported. The expression levels of determined genes must be validated with real time PCR assays.

This study show the up regulation of catabolic activity genes in sciatic nerves of mice may depend on the PTX neurotoxicity.

Extracellular matrix and metalloproteinases

ST-02.07.5-002
Assessment of serum prolidase and lactate dehydrogenase activities and spirometric values in patients with silicosis

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Silicosis is a fibrotic lung disease caused by inhalation of free crystalline silicon dioxide. Occupational exposure to respirable crystalline silica dust particles may occur in industry. Impairment of lung function increases with disease progression, even after the patient is no longer exposed. Prolidase, a member of the matrix metalloproteinases family, is a cytosolic enzyme that participates in the

regulation of collagen biosynthesis. It was aimed to investigate serum prolidase activity in patients with silicosis and also possible correlations between serum LDH activity and pulmonary function tests.

In this study there were 40 males in each groups which contained patients with silicosis (first group), individuals having similar symptoms with silicosis from same occupational area (second group) and healthy subjects (third group). Subjects were compared for serum LDH, prolidase activities and spirometric values (FEV1, FEV1%, FEV1/FVC, FEV1/FVC%, FEF 25–75 and FEF 25–75%). Correlations between serum LDH, prolidase activities and spirometric values were also investigated.

The mean age of the first, second and third group was 40.6 ± 6.5, 38.4 ± 7.2 and 41.9 ± 10.5 years, respectively. The silicosis group had a statistically significantly higher serum prolidase activity than other groups ($P < 0.05$). There was a negative correlation between prolidase activity and LDH and spirometric values except FEV1/FVC%.

As prolidase activity plays an important role in the production of collagen and it is an indicator of increased collagen turnover, high serum prolidase activity might be considered as a marker of pulmonary parenchymal involvement in patients with silicosis. As yet, no curative treatment exists, but comprehensive management strategies help to improve quality of life and slow deterioration in silicosis. Further efforts are needed for recognition and control of silica hazards, especially in developing countries.

Chemical and biochemical aspects of oxidative stress

ST-09.04.4-001
Traits of tolerance to arsenic and aluminum stresses in *Tamrix gallica*

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Metal pollution is one of the most serious environmental problems, aggravated by human activities. The lack of knowledge of plant tolerance and differential response to trace metals elements (TME) encouraged many researchers to elucidate their toxicity and tolerance mechanisms. In Tunisia, *Tamarix gallica,* a halophytic shrub, is from coastal and desert regions, also living in some saline depressions, which are usually accumulation sites of industrial and urban effluents contaminated by TME. It has attracted attention for its specific biological and ecological characteristics adaptation to different and extreme environment. In the present study, authors intend to determine the tolerance traits of *Tamarix gallica* to avoid TME toxicity.

In order to evaluate the mechanisms responsible for Arsenic (As) and aluminum (Al) tolerance, the plant were grown in semi-controlled conditions upon exposure to different metal concentrations (0, 200, 500 and 800 μM) supplemented or not with NaCl (0, 200 mM). Growth parameters, metal accumulations, proline and glycine betaine contents and antioxidant enzymes activities (SOD, CAT, GPX and APX) were measured using established techniques.

Plants demonstrated a good growth even after prolonged exposure to high metal concentrations. More than 75% of the plants that were exposed to As and Al (800 μM) survived until the end of the treatment. On the other hand, the proline and malondialdehyde content in the leaves of stressed plants increased significantly in all treatments. However, glycine betain showed an increase only in combined stress TME/NaCl. Activities of SOD and APX were increased with the TME concentrations.

Our results show that Al and As induce oxidative stress in *Tamarix gallica* and that enzymatic antioxidants (SOD, APX) and organic osmoticums (proline, glycine betain) play significant roles in tolerance to TME toxicity.

ST-09.04.4-002
Increased plasma 7-ketocholesterol levels may explain the hypocholesterolemia of sickle cell disease patients

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Introduction: Sickle cell disease (SCD) occurs due to a point mutation in the hemoglobin gene. It is characterized by anemia, vasooclusion and chronic inflammation. Hypocholesterolemia is a common finding in SCD patients; however, there are only a few studies to explain its etiology. We had previously shown that SCD patients had a negative correlation between their plasma cholesterol and erythrocyte malonyldialdehyde levels. We hypothesized that increased cholesterol oxidation may be a factor for hypocholesterolemia in SCD.
Material and Methods: The study consisted of pediatric SCD patients ($n = 22$) and healthy controls ($n = 8$). The patients hadn't had any crisis for the last three months except two. Blood samples were drawn into EDTA tubes to separate plasma. Levels of holesterol oxidation products, 7-ketocholesterol and Colestane-3β,5α,6β-triol were measured by LC-MS/MS with the method by Griffiths et al. Plasma total cholesterol levels were measured by commercial kits. Statistical analysis was performed with Graphpad Prism 6.0. This study was approved by the Institutional Review Board of Mersin University.
Findings: Mean 7-ketocholesterol levels were 10.47 ± 1.83 ng·mL^{-1} in patients and 8.97 ± 1.05 ng·mL^{-1} in controls ($P = 0.0298$). Mean Colestane-3β,5α,6β-triol levels were 6.49 ± 2.31 in patients and 5.69 ± 2.69 in controls ($P = 0.4283$). Mean Cholesterol levels were 106 ± 19.1 mg·dL^{-1} in patients and 149.6 ± 28.9 in controls ($P < 0.0001$).
Discussion: We found significantly lower plasma cholesterol and higher 7-ketocholesterol levels in SCD patients than controls. We suggest that increased oxidative stress should be considered as a factor contributing to hypocholesterolemia in SCD besides hemolysis and inflammation suggested by two previous studies. This is the first report investigating plasma oxysterol levels in SCD patients in literature. Further studies are needed to understand the effects of hypocholesterolemia and increased cholesterol oxidation products in SCD.

ST-09.04.4-003
Mental stress disorders and calcium induced changes in nitric oxide levels in rat hippocampus

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Stress as the influence of various external factors on body is one of the interesting topics of recent biology. Modern scientists are deeply involved in understanding more the biochemical bases of stress.

Recent study was about the impact of mental stress on nitric oxide (NO) content in rat hippocampus, as the inducer of oxidative processes. It was studied the levels of Ca^{2+} ions inside and

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outside of mitochondria and the activity of Nitric Oxide Synthase (nNOS) that is activated by it.

It was shown that under 30 day social isolation the concentration of Ca^{2+}, as well as of NO in hippocampal cells was increased for about 62% and 38% respectively, compared to control individuals, while the activity of Ca-ATPase was decreased (mitochondrial, ER and plasma membrane).

Simultaneously it was investigated the functional state of MPTP and the result proved that under the mental stress conditions the pore was activated and this could be due to the increase in inner mitochondrial calcium concentration.

Finally it can be declared that long-term social isolation has negative influence on hippocampus and could be thought as an inducer of pro-oxidative processes and somehow can stimulate pro-apoptotic pathways.

ST-09.04.4-004
Oxidative stress and antioxidant status in pseudotumor cerebri

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Aim: Pseudotumor cerebri is a clinical syndrome of unknown etiology compromising cerebrospinal fluid pressure over 200 mmH20 which accompanies with the absence of any abnormal findings detected in the brain and cerebrospinal fluid (CSF). The disease is often accompanied with obesity which is suggested by the effects of obesity metabolism. In this study, we aimed to investigate relationship of pseudotumor cerebri and the oxidation parameters.

Material and Method: Thirty eight pseudotumor cerebri patients whom administrated to neurology clinic of Bezmialem Vakıf University and 53 healthy volunteers were included in the study. Total oxidant status (TOS), total antioxidant status (TAS) and total thiol levels (TTL) were evaluated in serum. Oxidative stress index (OSI) parameter was calculated.

Results: Mann-Whitney U test was used for comparisons of two groups. Body mass index was the only demographical parameter different between two groups ($P < 0.001$). TOS (med; min-max, 10.35; (6.49–59.9) and OSI (med; min-max, 8.33; (4.3–37.6) of patient group were both significantly higher from healthy group (both; $P < 0.001$). TAS and TTL were not different between two groups. Regarding the papilledema of patient group, there were no difference of TOS, TAS, TTL and OSI. There was no correlation between BMI of the patient group and oxidative parameters. TTL were positivelly correlated with TAS ($P = 0.02$; $r = 0.376$), whereas negative correlated with TOS ($P < 0.001$; $r = -0.547$) and OSI ($P < 0.001$; $r = -0.623$).

Conclusion: TOS and OSI were both higher in pseudotumor cerebri patients than the control group, regardless of obesity levels. Pseudotumor cerebri disease may seem to be a physiopathological disease of the brain and spinal cord. However, in our study, it was also found to be associated to the oxidative metabolic load.

ST-09.04.4-005
Nitrite oxidation and tyrosine nitration by human myeloperoxidase

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Several reactive nitrogen oxide species are generated by autoxidation of nitric oxide (NO) under aerobic conditions. Though NO_2^- is a relatively unreactive end product of NO oxidation, NO_2^- can be converted into reactive species by hemoproteins including myeloperoxidase (MPO). Therefore, reactive nitrogen oxides generated during hemoprotein-catalyzed nitrite oxidation are held responsible for bactericidal and cytotoxic actions of NO_2^-. In the presence of H_2O_2 NO_2^- is oxidized to form NO_2 by the action of peroxidases, e.g. myeloperoxidase. NO_2, derived from inflammatory cells can mediate the nitration of tyrosine (Tyr) to form 3-nitrotyrosine (3-NT). Hence, 3-NT is likely not a footprint for peroxynitrite alone but more generally a marker of nitrative stress.

In this study we searched for the oxidation of NO_2^- to NO_3^- in the presence of H_2O_2 by purified human MPO and simultaneous nitration of Tyr to 3-NT. MPO catalyzed nitrite oxidation in a pH and time-dependent manner with optimum pH of 4.5. No nitrite oxidation was observed either in the absence of H_2O_2 or MPO, showing that the reactive species causing nitrite oxidation is an oxoferryl complex (compound I) formed from the reaction of H_2O_2 with MPO. Using nitrate reductase, we found that the NO_2^- disappeared from the medium is primarily oxidized to NO_3^-. K_m and V_m values for nitrite oxidation by MPO were measured as 0.78 mM and 5.5 mM, respectively. In the process of nitrite oxidation by MPO, the reactive species generated caused the nitration of Tyr. MPO-catalyzed tyrosine nitration was pH-dependent with the optimum pH of 5.5, in the presence of H_2O_2. In tyrosine nitration studies the K_m for tyrosine was calculated as 0.72 mM and V_m as 1.85 mM. We conclude that, in the presence of H_2O_2 the heme group of MPO is oxidized to Compound I, and Compound I oxidizes nitrite to nitrogen dioxide radical ($NO_2^{\cdot}$) that causes the nitration of tyrosine.

Keywords: Nitrite oxidation, tyrosine nitration, myeloperoxidase, NO_2^-, NO_2.

ST-09.04.4-006
The effect of PDE5 inhibitors on bone and oxidative damage in ovariectomy-induced osteoporosis

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Osteoporosis is a major public health problem associated with many factors, and it affects more than 50% of women over 50 years old. In our study, we aimed to investigate the effect of PDE5 inhibitors on osteoporosis via the NO/cGMO/PKG signalling pathway.

A total of fifty female albino Wistar rats were separated into five groups. The first group was appointed as the healthy control group with no ovariectomy. All animals in the other groups were bilaterally ovariectomized. Six month after the ovariectomy, vardenafil, udenafil and tadalafil were given to the third, fourth and fifth groups, respectively, but were not administered to the positive control group (10 $mg{\cdot}kg^{-1}$ per day for 2 months). The BMD values were determined using a densitometry apparatus for all groups pre-and-post ovariectomy and after treatment. The level of NO, eNOS, ADMA, cGMP, PKG, PDE5, PYD, DPD, CTX and PICP were determined using an ELISA. The levels of MDA, 8-OHdG, dG and CoQ10 were determined by an HPLC assay. Additionally, the right femoral trabecular bone density and the epiphyseal plate were measured in all groups. Angiogenesis was histologically observed in the bone tissue.

PDE5 inhibitors increased bone tissue angiogenesis through the NO/cGMP/PKG signalling pathway. Thus, we determined that the inhibitors caused a positive impact on the reduction of BMD and increased bone resorption markers. We also observed the positive effects of these inhibitors on oxidative stress.

In conclusion, these PDE5 inhibitors increase angiogenesis in bone tissue and increase the re-formation rate of bone in rats with osteoporosis.

ST-09.04.4-007
Effects of antiviral agent acyclovir on thymidine kinase activity of a model insect, greater wax moth, *Galleria mellonella* L.

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The effects of antiviral agent, acyclovir, on thymdine kinase (TK) activity of a model insect, greater wax moth *Galleria mellonella* L. were investigated by adding this agent into artificial diet.

Insect thymidine kinase activity was measured using reactions of two enzymes, lactate dehydrogenase-pyruvate kinase at 340 nm for 25 ^{0}C.

Thymdine kinase (TK) activities of seventh instars, pupae and adults were not significantly different from those of control insects at 0.001% concentration of acyclovir. At dietary concentrations of 0.01 and higher, acyclovir resulted in significant increase in TK activity in each developmental stage of *G. mellonella*. A most significant increase in the enzyme activity was recorded in adults at 3.0% concentration of acyclovir compared to control group. This concentration markedly increased the enzyme activity from 2.09 ± 0.10 in control adults to 4.02 ± 0.35 nmol·min^{-1}·mg^{-1} protein.

The results strongly suggest that insect thymidine kinase can be regarded as an attractive novel target for the development of potent and selective insecticides.

ST-09.04.4-008
Ischemic postconditioning inhibits ischemia-induced AKI-to-CKD progression via Akt/GSK3β pathway

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Ischemia-reperfusion (IR) injury, a relevant factor of acute kidney injury (AKI), will induce renal fibrosis and then, chronic kidney disease (CKD). Postconditioning can minimize the effect of IRI, while its function and related mechanism in the transition of AKI to CKD remains unknown. It has been reported previously that type II epithelial-to-mesenchymal transition (EMT) in tubular epithelial cells plays a vital role in the pathogenesis of renal fibrosis. This study aims to investigate the underlying molecular mechanism.

We established a single renal ischemia/reperfusion model with C57BL/6 mice. All mice with IR injury were divided into 2 groups, which are with (IR group) or without (PC group) additional ischemic interruption reperfusion before permanent perfusion. Renal function, fibrosis and EMT-related makers were detected and measured. A protein array was used to evaluate the expression and activation levels of signal node proteins in kidneys.

PC group was associated with reduced BUN of 54.0 ± 5.7 mg·dL^{-1} and Cr of 0.420 ± 0.033 mg·dL^{-1} ($P < 0.05$) as well as amelioration in acute tubular necrosis from 2 weeks to 8 weeks compared with (I/R) group. Immunohistochemistry and western blotting at both 4 weeks and 8 weeks indicated lower expressions of snail and EMT-related protein makers (α-SMA, fibronectin and S1004A) in PC group. The protein chip showed down regulation of phosphorylation levels of Akt (Ser473), Akt (Thr308), and GSK3β (Ser9), and verified by *in vivo* Immunohistochemistry and western blotting.

Postconditioning suppressed the EMT of tubular epithelial cells caused by IRI, and it related with a down regulation of Akt/GSK3β signal pathway, and this could be a therapeutic procedure to kidney IR injury and reduce the pathological progress of AKI to CKD.

ST-09.04.4-009
Investigation of free radical metabolism in septic rats' heart tissues treated with lipopolysaccharide; effect of vitamin D

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Introduction: Sepsis is a common cause of morbidity and mortality in the intensive care units. As a result of stimulation with infectious agent, proinflammatory cytokines increase in sepsis. Secreted proinflammatory mediators trigger the formation of free radicals and oxidative stress. Endotoxin shock, along with oxidative stress, exacerbates sepsis. On the other hand vitamin D increases the activity of antioxidant enzymes and reduces oxidative stress. We aimed to investigate the free radical metabolism and the effect of vitamin D on free radical metabolism in heart tissue of rats which are experimental sepsis model.
Materials and Methods: Twenty four female wistar albino rats were divided into randomely 4 groups: 1. SHAM, 2. Sepsis, 3. Sepsis+ vitamin D, 4. Vitamin D. Sepsis was induced with single intraperitoneal injection of Lipopolysaccharide (LPS) *E. coli* 16 mg·kg^{-1}. 25(OH) Vitamin D3 was given 2 mg·kg^{-1} dose for 3 days. Rats' rectal body temperature was measured. Antioxydant enzyme activities and lipid peroxidation levels of rats heart tissues were measured by spectrophotometrically. Additionally rat heart tissues were analysed histopathologically.
Results: Superoxide dismutase (SOD), Catalase (CAT) and Nitric Oxide Synthase (NOS) enzyme activities and Malondialdehyde (MDA) level were significantly higher in the Sepsis Group than SHAM Group (respectively $P = 0.027$; $P = 0.0488$; $P = 0.029$ and $P = 0.002$). CAT enzyme activity was significantly higher in the Vitamin D group than SHAM group ($P = 0.0047$). CAT enzyme activity was significantly elevated and MDA was significantly decreased in the Vitamin D+ Sepsis Group than Sepsis Group (respectively $P = 0.0372$ and $P = 0.02$).
Conclusion: It is also understood from these results that lipid peroxidation increases in sepsis due to insufficient elevation of antioxidant enzyme activities. Vitamin D treatment in sepsis can provide an increase of antioxidant enzyme activities and may reduce the level of lipid peroxidation in heart tissue.

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ST-09.04.4-010
Determination of stanozolol's effects on apoptosis mechanisms via oxidative stress in rat cardiac tissue

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Anabolic androgenic steroids (AAS) are synthetic derivatives of the male hormone testosterone. Stanozolol is a widely used 17α-alkylated AAS derivative. The effects of stanozolol on oxidative stress parameters and mitochondrial apoptosis pathway were investigated in heart tissue of rats. 34 male Sprague Dawley rats were divided into 5 groups (control, vehicle-control, steroid, vehicle control-exercise and steroid-exercise). Animals were subcutenously treated with 5 $mg \cdot kg^{-1}$ stanozolol for steroid groups and 1 $mL \cdot kg^{-1}$ propylene glycol for vehicle-control group. After 28 days, animals were sacrificed and oxidative stress and apoptosis parameters in cardiac tissue were evaluated. According to our results, vehicle control-exercise group had significantly decreased malondialdehyde (MDA) levels compared to other groups ($P < 0.05$), while no significant difference was detected in terms of glutathione (GSH) values. Increased protein carbonyl (PC) levels have been detected with stanozolol administration ($P < 0.05$). By using TUNEL assay in cardiac tissue, it has been demonstrated that stanozolol treatment triggers apoptosis, while this effect is significantly reduces in the presence of exercise. In the immunohistochemical assessment, there were no significant difference between groups in terms of superoxide dismutase (SOD) staining. It has been evaluated that cytochorome-c staining intensity was highest in steroid group and catalase (CAT) staining was evaluated to be moderate in steroid and steroid-exercise groups. Consequently, it can be concluded that stanozolol administration induces apoptosis in cardiac tissue without affecting GSH and MDA levels.

ST-09.04.4-011
Brain aluminium accumulation and oxidative stress in calcium silicate dental cement applied rats

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Mineral trioxide aggregate (MTA) is a hydraulic calcium (alumino) silicate dental cement used successfully for various dental applications. MTA-based dental biomaterials contain heavy metals which were shown to be released into simulated body fluids *in vitro*. However release of aluminium (Al), a neurotoxic metal, was not investigated before. The neurotoxicity of Al depends on its induction of oxidative stress. The present study was undertaken to test whether the presence of three calcium silicate dental cements in the dental extraction socket of an *in vivo* model would affect the brain Al levels and oxidative stress parameters.

Right upper incisor was extracted from rats. Polyethylene tubes filled with MTA Angelus, MTA Fillapex or Theracal LC were inserted into the depth of the extraction socket. The rats were killed 7, 30 or 60 days after the operation. Brain tissues were obtained before killing. Al levels were measured by atomic absorption spectrometry. Thiobarbituric acid reactive substances (TBARS) levels, catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities were determined using spectrophotometry.

Brain Al level was highest in MTA Angelus group on day 7. Brain Al concentration of MTA Fillapex group was also significantly higher when compared with controls at all times. Theracal LC group had significantly increased brain Al concentration on days 7 and 60. Brain TBARS level and CAT, SOD and GPx enzyme activities were significantly higher in MTA Angelus group; TBARS level and SOD activity were significantly higher in MTA Fillapex group, and TBARS level and GPx activity were significantly higher in Theracal LC group compared with controls on day 7. These parameters were similar in all four groups on days 30 and 60.

Our results show for the first time that Al accumulated in brains of rats having calcium silicate dental cements. Moreover, oxidative stress was induced and antioxidant enzymes were upregulated in the brain tissue.

Aging

ST-09.03.3-001
Glucose-6-phosphate dehydrogenase a novel hope on a blood-based diagnosis of Alzheimer's disease

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Alzheimer's disease (AD) is a multi-factorial neurodegenerative disorder that numerous factors have key properties in the development of this proteopathy. Glucose-6-phosphate dehydrogenase (G6PD) is the most common form of enzymopathy. We have examined G6PD enzyme activity levels in the serum of newly diagnosed AD patients compared with control subjects without dementia from the both sexes. Serum G6PD levels were found to be significantly higher (approximately two times) in AD patients compared to control geriatric subjects in both sexes. We have concluded that G6PD seems to play an integral role in the progress and/or prevention of AD.

ST-09.03.3-002
Ionizing radiation-mediated premature senescence and paracrine interactions with cancer cells provoke alterations in human stromal fibroblasts in favor of tumor growth

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Introduction: Accumulating evidence indicate the importance of aged stroma in tumor development. On the other hand, ionizing radiation (IR), can provoke premature senescence of human cells. Here we studied the effect of IR on lung and breast human stromal fibroblasts and their interactions with cancer cells.

Results: IR provokes premature senescence of lung fibroblasts *in vitro* in a p53-depenedent mode. These cells express of catabolic phenotype and enhance considerably the growth of human lung cancer cells *in vitro* and in SCID mice *in vivo*, partly due to the increased production of matrix metalloproteases. IR provokes premature senescence also in breast fibroblasts, both *in vitro* and *in vivo*. These cells are also characterized by an intense catabolic phenotype. They further overexpress the cell surface proteoglycan syndecan 1 (SDC1), a poor prognostic factor when expressed in the malignant breast stroma, enhancing tumor cells' growth. This overexpression is independent of the p53 and p38 MAPK – NF-kB pathways, and it is due to an autocrine TGF-β loop, acting via the Smad pathway. Finally, the highly invasive human breast cancer cells MDA-MB-231 increase further SDC1 expression by a paracrine action of TGF-β.

Discussion and Conclusion: The above suggest that the synergism of a side effect of radiotherapy (i.e. premature senescence) with aggressive tumor cells leads to a reactive stroma, which is in favor of an enhanced tumor growth.

Autophagy: Regulation mechanisms

ST-02.03.3-001
Tacrine-melatonin heterodimer-induced lysosomal rupture leads to modulation of autophagy at initiation and degradation stage

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Autophagy, as a process associated with degradation and recycling of dysfunctional cell components, plays diverse physiological and pathological roles. Recently, its regulation became a target of anticancer therapy, especially against cancer cells resistant to apoptosis induction.

We synthesized a tacrine-melatonin heterodimer (C10), which exerted a cytotoxic effect on MCF-7 breast cancer cells. We found that already 1 hour treatment with C10 led to autophagy induction and increase of autophagy markers such as Atg5, p62 and LC3B-II by attenuation of Akt-mTOR signalling pathway. However, C10 treatment for 1 day resulted in blockade of autophagic flux demonstrated by accumulation of autophagy proteins and vesicles as measured by mRFP-GFP-LC3 construct. The autophagy blockade may be caused by inhibition of vesicles fusion or blockade of cargo degradation. Both significant level of LAMP-1 and LC3 protein colocalization and accumulation of single membrane vesicles observed by electron microscopy confirmed proper fusion between lysosomes and autophagosomes. These results showed that C10 probably inhibited lysosomal degradation and suggested that C10 may attenuate lysosomal function. Analysis of physicochemical properties of C10 demonstrated that it is a lipophilic agent and as a weak base may have lysosomotropic activity and may cause lysosome rupture. Using the Galectin 3 (marker of lysosomal rupture), we showed C10-induced lysosomal membrane permeabilization, which explains induction of autophagy of lysosomes and impairment of lysosomal degradation.

Altogether, we have identified a novel agent that modulate autophagy at both initiation and degradation levels, which might result in metabolic stress and cell death. Thus, C10 seems to be more effective compound than commonly used autophagy inhibitors, as chloroquine, and appears to have the potential in anticancer therapy as autophagy modulator enhancing action of chemotherapeutics.

ST-02.03.3-002
A new non-canonical pathway of G(q) protein regulating mitochondrial dynamics

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A novel localization of heterotrimeric G proteins at the mitochondria and their implications on the physiology of the organelle has been recently reported. In particular, the Gq subfamily is required to keep the proper balance between mitochondria fusion and fission acting at both outer and inner membrane dynamics. Together with Gβ (that binds to Mfn1), Gαq stabilizes elongated mitochondria and cristae structure. Gq is also necessary for the maintenance mitochondrial membrane potential and the activity of the respiratory chain and mitochondrial ATP synthesis. Surprinsingly, Gq is necessary for the supercomplex assembly at the inner membrane. The molecular mechanism of action of heterotrimeric G proteins at the mitochondria is still unknown. A recent MS-proteomic analysis has helped us to decipher the Gq-interactome ("Gq-mitoproteome"). We have utilized mitochondrial enriched fractions from four different cell lines, among them the Gq/11-MEF knockout, the recover Gq-MEF-knockout, MEFs wild type and NIH3T3 cells, as well as, two different anti-Gq antibodies. The new candidate binding proteins are being analyzed by their capacity to interact to Gq. Among the candidates outer and inner mitochondrial binding partners are present, proteins necessary for mitochondrial protein import, as well as, proteins involved in the respiratory chain response, mitochondrial dynamics and mitophagy. In summary our group postulates a new non-canonical mitochondria-function of heterotrimeric G proteins that involves their translocation to the mitochondria and the interaction with several mitochondrial partners.

ST-02.03.3-003
Investigation of the role of cardiolipin on autophagy in the yeast model

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Cardiolipin (CL) is a phospholipid which is localized exclusively to the mitochondrial inner membrane (MIM). CL interacts with proteins involved in oxidative phosphorylation and also plays a role in the modulation of apoptosis and autophagy. We studied the role of CL on autophagy/mitophagy, in two mutant strains of *S. cerevisiae,* the Crd1Δ (cardiolipin synthase gene deleted) strain and the Taz1Δ (Tafazzin, a critical cardiolipin modifying enzyme gene deleted) strain and the wild-type *S. cerevisiae* strain GA74-1A. Autophagy induction was monitored by GFP expression using Western blot and flow cytometric analyses. We

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observed that autophagy induction increased in three strains which were grown in different media. Mitochondrial respiration, mitochondrial membrane potential and the organization of mitochondrial protein complexes were also studied. In the wild-type strain, while autophagy did not affect NADH-dependent mitochondrial respiration significantly, it positively affects succinate-dependent respiration. In contrast, in the Taz1Δ strain, autophagy negatively affects succinate-dependent respiration. We found that levels of complex IV subunit Cox2p changed in the in Crd1Δ strain upon starvation. Measurement of mitochondrial membrane potential (MMP) showed a decrease in Crd1Δ strain grown in fermentative and non-fermentative media. Prolonged autophagy induction diminished the level of MMP in all strains. In addition, mRNA levels of Atg32 and Atg17 that are essential proteins for mitophagy showed different profile in wild-type and mutant yeast strains. We further analysed changes in lipid composition of mitochondria upon autophagy induction. The data presented in this study on the possible roles of cardiolipin in autophagy and mitophagy will contribute to the understanding of the basic mechanisms of aging and neurodegenerative disorders.

Acknowledgement: This work was supported by The Scientific And Technological Research Council Of Turkey (Grant number: 212T027).

Personalized medicine

ST-08.02.5-001
Multi-level strategy for analysis of bioactive drug conformations

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The procedure of drug discovery is very time and resources consuming process. Computer-aided drug design (CADD) is one of the powerful tools which can be used to increase the efficiency of the drug discovery. Estimating the relative free energy of a ligand in its target-bound state (i.e. the bioactive conformation) is necessary to optimize the potency of bioactive molecules and to improve the accuracy of SDBB methods.

Our aim is to develop an efficient framework for finding the bioactive conformation of the flexible ligands.Since the bioactive conformation of the ligand may differ from the global minimum of the free ligand in the physiological environment, one has to evaluate the energetic cost required for adopting the bioactive conformation. A set of 100 crystal structures of pharmaceutically relevant drug-like molecules was tested using multi-level approach. We combined low-level method (LL) for sampling the conformational minima and high-level (HL) ab-initio calculations for estimating their relative stability.

The method was automated and tested on various ligands with different numbers of atoms, charge and rotatable bonds. The analysis show that is necessary to perform Hamiltonian Replica Exchange simulations in order to explore all possible states of energy landscape of given dihedrals. Our findings suggest that the method is an effective way to improve analysis of the bioactive conformations of drug-like molecules.

It is worth noting that present framework for multilevel strategy is a complex and long-term task, which requires a lot of rehearsals and implementations. Taking into account the flexible nature of molecules, protonation state and tautomeric forms, make our task even more challenging.

The proposed strategy may represent an efficient tool for predicting the conformational landscape of drugs while keeping a reasonable balance between chemical accuracy and computational cost.

ST-08.02.5-002
Assessment of clinical importance of molecular basis of neuroblastoma: can high risk group be categorized as high risk and ultra-high risk?

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Neuroblastoma is the most common pediatric neuroendocrine tumor that arise from the neural crest of sympathetic nervous system. Neuroblastic tumors exhibit extreme heterogeneity, which results in different therapy outcomes. Neuroblastoma is mainly categorized into three risk groups as low, intemediate and high. Molecular evaluation has come into prominence for determination of the risk categories. Recently, identification of new genes that may affect the therapy outcome, raised the possibility of the presence of sub-risk groups.

With this study, we aimed to assess the expression of some genes that may play role in identification of "ultra-high risk" group of patients. We analyzed, 25 and 29, low- and high-risk group of patients, respectively, who were chosen according to molecular and clinical data of routine Turkish Society of Pediatric Oncology (TPOG) 2009-protocol. Expression of ALK, ATRX, HIF1a, HIF2a (EPAS), H2AFX and ETV5 genes were evaluated among these patients by real-time PCR. Statistical analysis were performed by SPSS 15.0 program.

No significant relation was found between these genes and status of MYCN amplification, 1p loss, 11q deletion and 17q gain, except ALK, which is found to be highly expressed in patients with 17q gain ($P = 0.018$). All genes found to be highly expressed in high-risk group compared to low-risk group, except ETV5. When "ultra-high-risk" and high-risk groups were compared, ALK found to be highly expressed in "ultra-high-risk" group ($P = 0.027$) significantly, while ATRX found to be highly expressed in high-risk group ($P = 0.01$).

Our results show that, ALK can be a candidate gene that may be used to distinguish the "ultra-high risk" subgroup among high risk group of patients. Further, assessment of these genes is needed to clarify their roles in neuroblastoma progression.

ST-08.02.5-003
EGFR mutation status is associated with clinicopathological features in Turkey elderly, female, never smokers with NSCLC

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EGFR mutations in exons 19 and 21 results in sensitivity to TKIs and in exon 20 results in resistance frequently. The

mutation rates of EGFR show differences according to gender, smoking status and regions. The aim of this study is to detect relationship between EGFR mutation status and patient characteristics and to demonstrate their effects on survival.

401 lung cancer cases analyzed in this study. After DNA extraction EGFR mutations in exons 18–21 were evaluated by Pyrosequencing. Comparisons between groups were performed with chi-square test. Survival rates were estimated using the Kaplan-Meier method and log-rank test was used for comparisons P statistically significant.

EGFR mutations were detected in 15.96% of patients. EGFR mutations were 10% more frequent in females than males and 2.8-fold higher in never-smokers than smokers. Exon 19 mutations were 2-fold higher in females than males and 4-fold higher in never-smokers than smokers. Exon 21 mutations were 2.5-fold higher in females than males. L851R mutation causing drug sensitivity was 4-fold higher in females than males and 15-fold higher in never-smokers than smokers. Exon 21 mutations weren't detected in patients under the age of 50 and 8.2% of patients over the age of 50 had exon 21 mutations. Exon 20 mutations related with drug resistance were detected more frequently in males than females. Males had relatively higher survival rates than females, never-smokers had relatively higher survival rates than smokers and patients with EGFR mutations had relatively higher survival rates than wild types without any statistical significance.

EGFR mutation status, gender and cigarette smoking are factors affecting survival times in lung cancer. We showed survival rates according to this factors in our population and that mutation status changes according to gender and smoking status.

ST-08.02.5-004
Investigation of the peripheral blood gene-expression signature in patients with type-2 diabetes mellitus, using mRNA next-generation sequencing

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Type-2 diabetes (DM2) is a chronic metabolic disorder both environmentally and genetically regulated. Yet, its particular gene-expression profile has not been fully described.

Based on previous hypotheses that molecular profiling of circulating blood cells reflects pathophysiological events in affected tissues, we studied the expression pattern of 28 DM2-related genes in the peripheral blood (PB) of DM2 patients. RT-qPCR on total RNA from PB samples of 49 DM2 and 29 healthy donors showed that *CDK5* and *CDC123* expression levels were significantly higher in the DM2 compared to the control group ($P = 0.021$ and $P = 0.049$, respectively). Within DM2 group, untreated patients expressed lower *WFS1* ($P = 0.035$) and higher *PPARG* ($P = 0.006$) mRNA levels. Also, *CDC123*, *FTO*, *TCF7L2* and *LPL* mRNA levels were associated with certain disease parameters [BMI, hyperlipidemia, metabolic syndrome, HbA1c, fasting glucose and/or insulin levels ($P < 0.05$)]. Based on these results we conducted mRNA-seq analysis in PB-RNA samples of 4 patients and 2 controls, aiming at unraveling the complete disease-specific transcriptome signature. Poly(A)-RNA library construction, template preparation and next-generation sequencing were performed with appropriate kits on an Ion Torrent™ Personal Genome Machine® System. Focusing on the insulin pathway, differential mRNA expression was observed in 29 mRNA transcripts of 17 genes: *TCF7L2*, *IGF2BP2*, *KCNQ1*, *FTO*, *JAZF1*, *CDKAL1*, *NOTCH*, *PPARG*, *HHEX*, *CDK5*, *CDC123*, *THADA*, *CAMK1D*, *CAPN10*, *LPL*, *ADAMTS1* and *WFS1*. qPCR validation in the total cohort (49 DM2 *vs.* 29 healthy donors) as well as analysis of possible correlations with disease features are now under way.

Our data revealed that PB of DM2 patients exhibits a particular mRNA signature, further associated with clinical and biochemical parameters. The study of specific mRNA transcripts of the aforementioned, plus other, genes is anticipated to contribute to the generation of the complete DM2-related PB transcriptome profile.

Sunday 4 September 17:30–19:30, Hall C

Mechanism of neurodegenerative diseases

ST-09.02.2-001
Endoplasmic reticulum stress in case of hypothyroidism: focus on hippocampus and amygdala

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Thyroid hormone receptors are widely expressed in the adult rat brain. The aim of the present study was to clarify link between adult-onset hypothyroidism and endoplasmic reticulum stress in hippocampus and amygdala regions of rats. Male Wistar Albino rats (12 weeks) were randomly divided into two groups as control ($n = 6$) and thyroidectomized ($n = 12$). Hypothyroidism was induced by surgical thyroidectomy. Four weeks after thyroidectomy operation, hippocampus, amygdala and blood samples were taken from rats. Thyroid stimulation hormone (TSH) and free tri-iodothyronine (fT3) serum concentrations were assessed by autoanalyzer. The protein expressions of ER stress markers GRP78/BIP, PERK, ATF4, ATF6, and XBP-1 were determined by SDS/PAGE and western blot analysis. Images were analysed by using the ChemiDoc MP (Bio-Rad). *T*-test (SPSS 19.0) was used for statistical analysis. Hypothyroidism was confirmed in the thyroidectomized group by elevated TSH and decreased fT3 levels in serum ($P < 0.05$). GRP78/BIP and ATF6 protein expressions were found to be upregulated ($P < 0.05$), while PERK protein expression did not changed on both hippocampus and amygdala in the thyroidectomized group. Meanwhile, XBP-1 protein expression was upregulated on hippocampus and downregulated on amygdala ($P < 0.05$). Although ATF4 protein expression was tended to increase on hippocampus, it was upregulated on amygdala significantly. These results showed that dissociation of GRP78/BIP led to activation of ATF6, XBP-1 and ATF4 signaling pathways on hippocampus and amygdala in hypothyroidism. Based on this evidence, mood and memory impairments as a cause of hypothyroidism might be induced by ER stress. However, future investigations need at transcriptional level for clarify the underlying molecular mechanisms.

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ST-09.02.2-003
Murine studies regarding the regulatory effects of melatonin, tryptophan and arginine on brain neuromediators

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Physical and psychological stress is known to be a factor affecting synaptic plasticity, dendritic morphology and to induce neurotoxic damage in humans, through the generation of free radicals. Melatonin, the main pineal hormone, is an endogenous biomolecule with multiple roles: antioxidant, antiaging, anti-inflammatory, anticancer, DNA protector and anti-neurodegenerative.

Tryptophan is an essential amino acid with a role in protein synthesis and in the biosynthesis of serotonin. This biomolecule is involved in modulation processes connected to insulin sensitivity, regulation of ghrelin secretion and immune response development. Arginine is a semi essential amino acid studied for its effects on cell division processes, in the reconstruction of bone tissue, modulation of the immune system, regulation of hormone secretion and an antihypertensive agent precursor for NO synthesis.

The objective of our study was to determine the modulating capacity of these three endogenous biomolecules on the synthesis of some neurotransmitters: noradrenaline and dopamine.

The experimental model of Albino Swiss mice implies administering arginine, tryptophan and melatonin, single or in combination, in drinking water, the animals having free access to water and food. After three weeks of treatment, the 7 groups of animals were sacrificed and the brain tissue was used for obtaining the brain homogenate, further used for the determination of neurotransmitters cerebral concentration by an HPLC method.

The obtained results demonstrate the greatest effect of melatonin as a modulator of neuronal adrenergic and dopaminergic functions, stabilizing the thymic condition and the symptoms of psychiatric disorders by balancing norepinephrine and dopamine levels, providing a scientific confirmation for the use of these biomolecules as adjunctive therapy in bipolar disease and Parkinson syndrome.

ST-09.02.2-004
Comparative effects of nicotine and 6-hydroxy-L-nicotine against chlorisondamine induced memory impairment and oxidative stress in the rat hippocampus

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6-Hydroxy-l-nicotine (6HLN) is a nicotine metabolite resulted from nicotine degradation within *Arthrobacter nicotinovorans* with positive effects on spatial memory and oxidative stress damage. In the present study, the effects of 6HLN on spatial memory performance were assessed in chlorisondamine-treated rats.

Rats received a single injection of chlorisondamine ($10\ mg{\cdot}kg^{-1}$) 24 h before the behavioral testing within Y-maze and radial arm-maze tasks and were treated with 6HLN ($0.3\ mg{\cdot}kg^{-1}$), 30 min before the behavioral testing. Also, the antioxidant activity in the hippocampus was assessed using the superoxide dismutase, the glutathione peroxidase, and malondialdehyde levels. Statistical analyses were performed using one-way analysis of variance (ANOVA). Significant differences were determined by Tukey's *post hoc* test. *F* values for which p

Nicotine and chlorisondamine-induced memory impairments were observed, as measured by the Y-maze and radial arm-maze tasks. Decreased activities of superoxide dismutase and glutathione peroxidase were observed in the rat hippocampal homogenates of nicotine and chlorisondamine-treated animals as compared with control. Production of malondialdehyde (lipid peroxidation) significantly increased in the rat hippocampal homogenates of nicotine and chlorisondamine-treated animals as compared with control, as a consequence of impaired antioxidant enzymes activities. Additionally, in chlorisondamine-treated rats 6HLN significantly improved memory formation and decreased oxidative stress, suggesting memory-enhancing and antioxidant effects.

Our results suggest that administration of 6HLN ameliorates chlorisondamine-induced spatial memory impairment by attenuation of the oxidative stress in the rat hippocampus.

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ST-09.02.2-005
The role of ganglioside GM1 and sphingomyelin in the oligomerisation of beta-amyloid at physiological conditions: a single molecule study

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Oligomerisation of amyloidogenic proteins has been reported to play an important role in the development and progression of neurodegenerative diseases including Alzheimer's and Parkinson's disease. However, the molecular mechanism of how the oligomerization occurs and what are the triggering/inhibiting factors is still unclear. In this work, we investigated the impact of selected lipids on the beta-amyloid (Aß) aggregation and came to an interesting finding that oligomerisation is induced by sphingomyelin and inhibited by ganglioside GM1.

The oligomerisation was characterized by fluorescence (cross-) correlation spectroscopy, a single molecule technique which is able to follow diffusion and co-diffusion of fluorescently labelled molecules and by that to determine to which extent the molecules moved together. This study was performed on model free-standing bilayers, which have the advantage of modifying the lipid membrane composition in a controllable way.

By using nanomolar Aß concentrations we could show that Aß aggregated in the dioleolylphosphatidylcholine (DOPC)/cholesterol (Chol)/sphingomyelin (Sph) membranes but not in the bilayers lacking Sph. As molecular dynamic simulations indicated, change of Aß conformation in favour a conformation richer in beta-sheets might explain the observed behaviour. The oligomerisation was inhibited by adding physiological levels of GM1 to the membrane, perhaps due to specific interactions of Aß with the GM1 headgroup. Therefore, the role of GM1 seems protective. Interestingly, GM1 levels in the brain were reported to decrease with aging, which might contribute to the onset of Alzheimer's disease.

ST-09.02.2-006
Dynamic conformation-based assembly of three synthetic human Tau amyloid strains

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In tauopathies such as Alzheimer's disease and the inherited dementia FTDP-17, Tau protein assembles into highly ordered and structurally distinct filaments called amyloid fibrils. New evidence suggests that various disease phenotypes in tauopathies may be determined by conformationally distinct self-templating Tau amyloid strains. However, molecular mechanisms by which Tau spreads strain-specific conformations have remained enigmatic.

We studied polymorphism of polyglutamate-induced fibrils of recombinant human full-length Tau (FL), the Tau fragment K18 composed of the microtubule binding domain, and K18 carrying the deletion ΔK280 linked to FTDP-17. We seeded rapid assembly of monomeric FL by sonicated amyloid fibrils (mother seeds) of FL, K18 and ΔK280. In order to study possible templating effects, fibrils obtained in the first seeding – daughter fibrils were used to seed FL in subsequent reactions which produced granddaughter fibrils.

By means of transmission electron microscopy, we visualized differences in the morphology of FL (ribbon-like), K18 (rod-shaped) and ΔK280 (helical) fibrils. Thioflavin T fluorescence assay demonstrated that the fibrilization rate of FL seeded by FL (FL/FL) was similar to FL/ΔK280. Further, FL/ΔK280 exhibited indistinguishable proteolytic patterns from FL/FL, whereas FL/K18 produced unique fragment sizes. Fourier transform infrared spectroscopy revealed that β-sheet-rich amyloid cores of obtained fibrils, in particular FL/K18, were protease-resistant. α-helix and/or random coil components were readily digested. We also observed increased fidelity of the conformational heritage from daughter to granddaughter fibrils, suggesting that strain adaptation may occur over subsequent generations. We are currently studying neurotoxic effects of the obtained strains in rat hippocampal neurons.

Our findings provide molecular insights into the complex biology of Tau amyloid strains and suggest molecular events that likely happen in tauopathies.

ST-09.02.2-007
Serum nitric oxide, lipid hydroperoxide levels, nitric oxide synthase activity and total serum antioxidant capacity in patients with Parkinson's disease

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Parkinson's disease (PD) is one of the common neurodegenerative disorders. Oxidative stress is considered as a contributing factor to the development of PD. Present study aims to investigate serum oxidative stress status in patients with PD.

32 patients with PD and 32 healthy controls were recruited from neurology clinic of Ankara Education and Research Hospital. Severity of PD was estimated by modified Hoehn and Yahr scale and Unified Parkinson Disease Rating Scale (UPDRS). Oxidative stress was assessed by measuring serum nitric oxide levels, lipid hydroperoxide concentrations and, nitric oxide syntase activity. Additionally total serum antioxidant capacity (TAC) was evaluated by using serum 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method. Comparison of biochemical parameters between patients with PD and controls were carried out by Student's t test and Mann-Withney U test as appropriate. Spearman and Pearson correlation tests were used to establish possible relationship between variables.

Our results indicated that serum nitric oxide ($P = 0.045$) and lipid hydroperoxide levels ($P < 0.0005$) were significantly lower in patients with PD than controls. Moreover nitric oxide levels were found to be negatively correlated with Unified Parkinson's Disease Rating Scale (UPDRS) ($R = -0.041$, $P = 0.021$). However no statistical difference was observed in total serum antioxidant capacities and nitric oxide syntase activities between patients and controls.

The present study indicates that although antioxidant capacity was not changed, lipid hydroperoxide level was found decreased. This might show pre oxidative process in these patients. Additionally, decreased NO level and negative correlation observed between NO level and disease rating scale implicated a role for NO in the diseaese process.

Miscellaneous

ST-Mis-009
An investigation of the effect of tamoxifen on potassium channel gene expression in breast cancer

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Ion channels play a vital role in basic physiological functions such as generation of electrical activity in nerves and muscle, intracellular signaling, hormone secretion, cell proliferation, cell volume regulation. K^+ channels are a most diverse class of ion channels in the plasma membrane. It is shown that voltage dependent K^+ channels are associated with tumor cell proliferation in particular if they are epithelium originated. One of the voltage activated K^+ channels is HERG1 that plays important roles in regulating tumor cell proliferation and cell cycle progression. K^+ channel blockers inhibit cell proliferation. Tamoxifen being used for the treatment of breast cancer significantly inhibits K^+ current and cell proliferation. We investigate the effect of tamoxifen on HERG1 K^+ channel gene expression in MCF-7 breast cancer cell. Cytotoxic effect of tamoxifen at different concentrations was evaluated for MCF-7 breast cancer cell line using MTT assay. Cells were incubated 24 h and 48 h. IC_{50} value is measured as 31.9 for 48 h and 20 μM concentration. For electrophysiological analysis patch-clamp experiments were conducted. The maximum reduction in K^+ channel current was observed at 5 μM concentration of tamoxifen. The levels of HERG1 K^+ channel gene expression are analyzed by using Real-Time PCR method. While the gene expression levels observed to be decreased with 5 μM tamoxifen concentration, depending on its increasing concentrations the levels of gene expressions increased. Although, decreased activity and gene expression of K^+ channel at low concentration (5 μM) of tamoxifen give insight into tamoxifen's inhibitory effect on HERG1 K^+ channels, this inhibitory effect of low

concentration of tamoxifen on HERG1 K^+ channels was not statistically significant compared to control group. Suppression of expression at a lower concentration, raises the hope on the control of proliferation on the HERG1 genes that is thought to play an important role in breast cancer.

Computational biology

ST-03.02.2-001
Protein engineering insights from metagenomes of diverse bacteria

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Considering the fact that only small portion of bacterial species are culturable, the metagenome sequences derived from environmental samples harbour yet to be discovered insights. With the emergence of next-generation sequencing technologies the metagenomic studies has been accelerated. Not only 16S rRNA but also whole metagenome extracted from an environmental sample is subjected to analysis. Such a depth in analysis allows multiple sequence comparisons for a selected gene across metagenomes.

In this study, we used publicly available whole metagenome sequencing data from which sequences for industrial enzymes were extracted and subjected to multiple sequence alignment. The sequence differences for a given enzyme were overlaid on top of three dimensional structure of that enzyme.

Out method reveals the amino acid residue changes at certain locations in select enzymes. The sequence changes we have identified are novel and has not been described in bacterial enzyme databases.

Such an approach has potential to pave the way for better understanding of enzyme structure and protein engineering implications.

ST-03.02.2-002
Structure-based and ligand-based docking, molecular dynamics and free binding energy studies of falcipains against South African natural compounds

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Malaria is the most devastating parasitic disease of the current era, causing over half a million deaths per annum and a great economic burden in many countries in Africa, South America and Asia. It is caused by parasites of the genus *Plasmodium*. One of the main reasons for the persistence of malaria is the continuous emergence of resistant parasites to antimalarial drugs. Thus, the identification of novel drug targets and potential inhibitors to develop new drugs remains an urgent task. Falcipain-2 (FP-2) and falcipain-3 (FP-3) of *Plasmodium falciparum* are validated drug targets. The development of peptide based drugs against these enzymes has not been successful due to degradation by host enzymes. Considering the importance of natural products in drug discovery, this study aimed to identify potential non-peptide inhibitors from South African (SA) natural compounds with inhibitory potency against FP-2, FP-3 and homologs from other *Plasmodium* species. We also aimed to determine the selectivity of identified hits on the host homologs, human cathepsins. Structure based virtual docking approach was used to screen a small non-peptidic library of natural compounds from South Africa (SANCDB; https://sancdb.rubi.ru.ac.za/) against 11 proteins. A potential hit, 5α-Pregna-1, 20-dien-3-one (5PGA), with inhibitory activity against plasmodial proteases and selectivity on human cathepsins was identified. Ligand based virtual screening of 186 ZINC compounds, analogs to 5PGA, resulted in identification of five further potential hit compounds based on their docking energies. The key residues of proteins responsible for interactions with compounds were identified by means of free binding energy calculations and molecular dynamics. These compounds have cholesterol-like nuclei and showed distinct inhibitory effect against malarial proteins. Thus, they provide a starting point for further design of more effective derivatives.

ST-03.02.2-003
Computational modeling of iron binding and release in human serum transferrin

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We study distal site communication and dynamics for human transferrin (hTf). hTf is a bilobal transport protein which circulates iron in the blood and delivers it to tissues. It displays highly pH dependent cooperativity between the two lobes. Iron binds tightly to hTf and yet is promptly released at endosomal pH.hTfalso forms a tight complex with the receptor during endocytosis and recycling back to the serum. We aim to capture the local motions of hTf that affect the global conformational change while binding the iron. Meanwhile, iron dissociation mechanisms from deeply buried binding cavities are revealed.

We have explored short-term dynamics of hTf to identify functional information relevant to long-term dynamics of iron-binding event via Perturbation Response Scanning (PRS) method. To explore the dissociation of iron in various conformational states of hTf, random acceleration molecular dynamics (RAMD) and steered molecular dynamics simulations are performed on the two lobes of hTf.

We apply PRS to extract essential components that contribute to large-scale conformational transition of hTf in complex with its human and bacterial receptors.We find that iron binding processis mainly coordinated by residues at the synergistic anion uptake sites, a finding also corroborated by multiple all-atom molecular dynamics simulations. The examination of local dynamics in the hTf–receptor pair reveals cooperativity in the quaternary structure and explains resistance to iron release in the complex. The analysis of the hTf complex with a bacterial receptor identifies two putative regions that mechanically manipulate dissociation from the pathogen.

Our study provides a molecular level understanding of the full cycle of hTf iron-binding release dynamics while resolving residues directly participating in each event in the cycle.

Plant biochemistry and molecular biology

ST-02.08.5-001
Optimization of plant cell suspension cultures for the production of recombinant proteins

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Molecular Farming – the use of plant based systems for production of recombinant proteins – is an emerging field with a high potential impact in the biopharmaceutical industry. In the last years, plant production platforms emerged as promising

alternatives to traditional expression systems, since they offer significant advantages namely in terms of safety and cost. However, a major challenge remains so that plants become truly competitive, which is to improve the low yields of product accumulation that are generally obtained.

Plant cell suspension cultures are of great interest since they allow GMP compliance and an easy recovery of the secreted recombinant protein from the culture medium. Nonetheless, recombinant protein yields observed so far in plant cell suspensions are still low when compared to mammalian cell cultures.

We have developed strategies for the improvement of human recombinant protein production in plant cell suspensions. Our aim is to reduce the proteolytic activity in this system, since it affects final recombinant protein yields and quality. To accomplish this goal, we studied the proteolytic profile of proteases present in our system by spiking target proteins in cell medium and analyzing the degradation pattern caused by endogenous proteolytic activity. With the addition of specific protease inhibitors, we could detect which protease classes are present in our system. Furthermore, we used activity based protein profiling (ABPP) probes to identify specific protease, within the protease class identified by the spiking assays, that is affecting the target proteins. In parallel, we performed a mass spectrometry analysis of the plant suspension media. Finally, we used N-terminal sequencing to assess the protease specific cutting sites on the target proteins.

Our main goal is to establish new production plant suspension lines with reduced proteolytic activity.

ST-02.08.5-002
The anticholinesterase activity of two *Ajuga* species

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Ajuga vestita Boiss and *Ajuga xylorrhiza* Kit Tan are endemic species occurring in a narrow area in South East Region of Turkey. Lack of the knowledge about these *Ajuga* species attracted our attention for carrying out the present study which deals with the anticholinesterase activity of petroleum ether, acetone and methanol extracts of these species.

Aerial parts of the plants macerated by petroleum ether, acetone and methanol. The anticholinesterase activity determined by a spectrophotometric method indicating the acetyl- and butyryl-cholinesterase inhibitory effects.

The petroleum ether and acetone extracts of *A. vestita* and the methanol extract of *A. xylorrhiza* exhibited the same powerful inhibitory activity against acetyl-cholinesterase enzyme (72% inhibition). This activity was very close to galantamine's activity (74% inhibion) which was used as positive control. The highest butyryl-cholinesterase enzyme inhibition was recorded by acetone extracts of the both species (*A. vestita* 63% inhibion, *A. xylorrhiza* 59% inhibition).

ST-02.08.5-003
Phenolic content and potential antı-inflammatory effects of *Myrtus communis* L. products

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Myrtle (*Myrtus communis L.*) is an aromatic plant having strong antioxidant and anti-inflammatory properties due to its phenolic content. We aimed to investigate the effect of different infusion and decoction methods on the anti-inflammatory activities of Myrtle plant products (whole raw berry, dried berry fragmented raw berry, berry shell and berry seeds).

Teas were produced via brewing (infusion) and boiling (decoction) in different time periods (1 min, 3 min, 5 min, 10 min and 30 min). The concentrations of 14 different phenolic compounds were examined by LC-MS/MS and the anti-inflammatory effects were determined by the percentage inhibition of hyaluronidase by the Morgan–Elson reaction.

In all brewed tea products were determined less than 15% inhibition on hyaluronidase activity for all studied time intervals. The highest inhibitory activity of 91% in 1st minute was found in tea processed by boiling of aged natural fruit (1 year) having the highest content of ferulic acid, cafeic acid, catechin and epicatechin concentraions. The second highest inhibitory activity (85%) was observed in products of boiled dried leaves in 3rd minute correlated with highest content of cafeic acid and gentisic acid. The mean value of inhibitory activity in whole product was as 73% in 5th minutes. The gentisic acid (2,5-dihydroxybenzoic acid) was found to have the highest value concerning all products and time intervals.It's highest concentration was stated in fragmented-dried-boiled product as 60 187 $nmol \cdot mL^{-1}$. As the second phenolic compound with highest value was determined cafeic acid with concentration of 3061 $nmol \cdot mL^{-1}$ in product of dried leaves.

Results indicated that, the anti-inflammatory activities of myrtle tea products have are closely related to gentisic acid, ferulic acid, cafeic acid, catechin, epicatechin and cafeic acid contents. We suggest that, the anti-inflammatory activity could be optimized by production techniques such as extraction/time procedures.

ST-02.08.5-004
Isolation and characterization of genetic elements related to periodicity and fruit detachment in olive

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Periodicity and difficult fruit detachment in olive cause significant yield loss for olive growers. These features greatly differ from cultivar to cultivar pointing the genetic factors' roles with these processes. The objective of this investigation was to isolate and characterize genetic elements associated with periodicity and those with fruit detachment. For this purpose, multiple cDNA libraries were constructed from the leaves of "on year" trees and those of "off year" in July and November. For fruit detachment analysis, a cDNA library from the pedicels of an easy detaching cultivar and another library from that of a tight holder cultivar were prepared. Several hundred arbitrarily selected colonies from each library were analysed and the inserts from the positive clones were sequenced. All the cDNA seqeunces were first analyzed and those that seemed to be associated with alternate bearing and/or fruit detachment were further characterised with respect to temporal/spatial expression patterns, polymorphism, genomic copy number and biochemical function. Most of the insert sequences from each library were homologs of organelle sequences. rDNA sequences were abundant while their base compositions were sigficantly different depending of their origin. cDNAs specific to each library were further analysed for their functions. Two cDNAs were determined as dehydrins with increased expressions under temperature stress. A metallothionein isolated from fruited leaves in November accumulated multiple metals when expressed in bacteria. Another cDNA isolated from

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unfruited leaves in November displayed multiple open reading frames. cDNAs from pedicels yielded homologs of genes with cell wall degrading functions along with many transposons. Detailed functional analysis of these genes and their transcription control mechanism will greatly help devicing genetic solutions against alternate bearing and hard fruit detachment that are significant economical value for olive growers.

ST-02.08.5-005
The structure of the alpha subunit and its role in G protein signaling in plants

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In plants the heterotrimeric guanine nucleotide-binding proteins (G proteins) regulate signaling pathways in developmental processes including seed germination, early seedling development, and organ shape and size determination. The complex consists of the alpha, beta, and gamma subunits. The alpha subunit has GTP binding and hydrolysis activity and the beta and gamma subunits interact with downstream effectors as a heterodimer. Although the activation mechanism for the mammalian complex is well understood, recent studies point to significant differences for the plant proteins. Two major points are the lack of G protein coupled receptors in plants and the constitutively active (GTP-bound) state of the alpha subunit, which has to be suppressed by a membrane protein when there is no signal. We investigate the structurural features of heterotrimeric G proteins from *A. thaliana* to gain insight into its activation mechanism and to develop a better understanding of molecular interactions of G protein related signaling pathways in plants.

We cloned and expressed the wild type alpha subunit (AtGPA1) and an N-termina 36 aa truncated version (GPA1t) in yeast and bacteria respectively and the recombinant proteins have been purified for biochemical and strructural characterization. Analyses using absorbance spectroscopy, Circular Dichroism Spectropolarimetry (CD), Dynamic Light Scattering (DLS) confirm nucleotide binding as well indicating some structural and stability differences. DLS and native-PAGE analyses combined with small angle X-ray scattering (SAXS) measurements reveal that AtGPA1 has a tendency to form trimers in solution. SAXS data also shows that AtGPA1-GDP has a globular structure with some flexibility.

SAXS structural models will be compared with the crystal structure of GPA1t and results will be discussed, in the framework of current models of G-protein activation mechanism in plants based on this crystal structure.

ST-02.08.5-006
Engineering of glutathione transferases for bioremediation of environment

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Glutathione transferases (GST) mainly involved in detoxication almost in all organisms can also be used in phytoremediation processes to clean-up the environment contaminated with heavy metals, herbicides or explosive compounds. Recently Mannervik et al. reported that some GSTs members (GSTE6, GSTE7) have high activity with TNT (2,4,6-trinitrotoluene) whereas poplar GSTU16 and GSTU45 have lower activity. In this study a library of poplar GSTU45 variants including wild type and engineered forms were used mainly to increase the activity for TNT and also with most common used substrates CDNB (1-chloro-2,4-dinitrobenzene), PEITC (phenethylisothiocyanate) and TNT.

Histine-tagged GSTU45 clones were heterologously expressed in *Escherichia coli* and purified using Ni Sepharose activated His GraviTrap columns. All clones were obtained from the wild type sequence engineered by DNA 2.0 Inc. with 3 amino acid substitutions away from the active site. Clones were compared by means of kinetic studies with substrates CDNB, PEITC and TNT and modeled protein structures.

Wild type GSTU45 showed CDNB as the most active substrate (3.3 $\mu mol \cdot min^{-1} \cdot mg^{-1}$) and PEITC with lower activity (0.43 $\mu mol \cdot min^{-1} \cdot mg^{-1}$). As compared to GSTU45, one of the clones showed activity increased up to 6 times (19.2 $\mu mol \cdot min^{-1} \cdot mg^{-1}$) for CDNB and around 8 times for PEITC (3.4 $\mu mol \cdot min^{-1} \cdot mg^{-1}$). Although TNT is a structural analog of CDNB, activity measurements are in nanomol $min^{-1} \cdot mg^{-1}$ ranges in all the clones. The modelled structure of the most active clone shows that mutations are away from the active site.

Thus, GSTU45 and engineered clones cannot play a major role in the degradation of TNT but have to be improved by enzyme engineering of active site residues.

ST-02.08.5-007
Differentiation of bread wheat transcription factors against major abiotic stresses

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Transcription factors (TF) are the master regulator of gene expression belonging to types of families shown to be involved in response to various abiotic stresses in plants. Yield potential of cultivated crops are negatively influenced by drought and high-temperature (heat) stresses. As one of the main staple crops, bread wheat (*Triticum aestivum* L.) is faced with drought and high temperature especially at grain filling stage resulting significant yield loses.

Here, the high-throughput next generation sequencing data obtained by different tissues of the tolerant and sensitive bread wheat cultivars treated with drought, high-temperature and both stresses were analyzed to identify and measure the TF transcripts. Root, leaf and grain of the 14-day stressed and control samples were sequenced by a next-generation platform and comparatively analyzed by bioinformatics tools. Then, differentially regulated TFs against drought and/or heat stress in the tissues of the tolerant and sensitive cultivars were identified from the RNAseq data.

Differential expression of the identified TFs against high temperature and/or drought stress varies among tissues, cultivars and stress specific manner. The integrated effects of high temperature and drought stress were found to be higher in the tissues than the additive effects of high temperature or drought stress alone in terms of the number of differentially regulated TFs. The expression levels of TF transcripts in leaves were significantly affected by high temperature. TF-encoding genes showed significant responses to drought stress in grain. Among the differentially regulated TFs in root, leaf and grain of drought-sensitive cultivar subjected to combined stress, 149, 88 and 8 TF-encoding genes were found to be identical to those in drought-tolerant cultivar, respectively. Our findings provide more insight into the cultivar specific and spatial responses of TFs to abiotic stresses and their regulatory roles on stress responsive genes.

Structural biology: Membrane complexes and supercomplexes

ST-03.03.3-001
Snake venom E-NPP: structure and function of cobra venom toxin binding to insulin receptor

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Snake venoms consist of ecto-enzymes capable of hydrolyzing nucleotide, but their structure and function remain poorly understood. In this study, we clone an ecto-nucleotide pyrophosphotase-phosphodiesterase (E-NPP) cDNA from Taiwan cobra venom gland to obtain an 830 amino acid sequence and purify E-NPP toxin from crude venom to determine its 3D structure at 2.5Å resolution. The purified E-NPP hydrolyses ATP, ADP into AMP and PPi or Pi and the obtained crystals bind to AMP at nucleotide-binding pocket near a characteristic bimetallic (two zincs) active site. In addition to the expected enzymatic activity, solid-phase binding assay shows that E-NPP could also bind to the ecto-domain of human insulin receptor. Our results suggest for the first time that cobra venom also consist of toxins capable of targeting glucose homeostasis to exhibit symptoms of muscle weakness and thirst, in addition to the previously well-known neurotoxic, inflammatory and thrombotic effect for the snakebite victims.

ST-03.03.3-002
Structural studies on gamma subunits of heterotrimeric G proteins in *Oryza sativa*

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Heterotrimeric G proteins are composed of alpha, beta and gamma subunits and they act as molecular switches to turn on intracellular signaling upon arrival of external stimuli at the membrane receptors. Plant G-proteins are involved in multiple developmental processes ranging from seed germination, early seedling development and organ shape determination to hormone perception and ion-channel regulation.

Although the activation mechanism is well understood in animals, recent studies show that the intrinsic signaling and network mechanisms in plants have noticeable differences. Unlike animals, plants lack the canonical G-protein-coupled receptors, thus the plant alpha subunit is self-activating and has to be repressed by a membrane protein in the absence of a signal. Recent studies also indicate that plants have a complex family of gamma subunits which may be directly involved in signaling and play a role in specificity of signaling. Future studies are expected to reveal more components of the heterotrimeric G-protein signal transduction pathways and to identify the mechanisms by which G-proteins regulate plant phenotypic and developmental plasticity. The present study, is a part of a larger investigation on the activation mechanisms of G-protein signaling pathways in plants. Here results from studies on rice (*Oryza sativa Indica)* gamma subunit-1, RGG1, and gamma subunit-2, RGG2, will be reported. Both proteins were expressed in *E. coli*. RGG1 was purified from bacteria for biochemical and structural characterization. Structural features of RGG1 obtained from dynamic light scattering (DLS), circular dichroism (CD), and small angle X-ray scattering (SAXS) data analysis will be discussed. Additionally recent results from RGG2, will be presented for comparison with RGG1. Structural features RGG1 and RGG2 will be discussed in the context of their respective function(s).

Systems biology

ST-03.01.1-001
Chemical sympathectomy fails to alter deleterious effects of unilateral vasectomy on contralateral epididymal sperm characteristics and *in vitro* fertilizing capacity

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Introduction: Unilateral injuries of vas deferens may also cause contralateral testicular damages. The purpose of the present study was to determine whether the sympathetic nervous system plays a role in the contralateral epididymal sperm in vitro fertilizing capacity reduction following unilateral vasectomy in mice.
Materials and Methods: Neonatal male mice were randomly divided into four groups of six mice each. Within 24 hours of birth, control mice were received 0.1 $mL{\cdot}day^{-1}$ normal saline for 7 days intraperitoneally and sham operated at 42 days of age. Chemical Sympathectomy (CS) group received intraperitoneal injections of 6-Hydroxydopamine at a dose of $0.075\ mg{\cdot}gr^{-1}{\cdot}day^{-1}$ for 7 days after 24 hours of birth. In the left vasectomy (LV) group, the mice were unilaterally vasectomized at 42 days of age and in the CS + LV group, the animals were treated by CS plus unilateral vasectomy. At 11 weeks of age all the mice were euthanized and contralateral epididymal sperms characteristics and in vitro fertilizing capacity were evaluated. The data were analyzed by one-way analysis of variance followed by Tukey test for post hoc comparisons.
Results: LV significantly decreased contralateral epididymal sperms motility, viability and concentration as well as fertilization and blastocyst development rates. No significant difference was detected between LV and CS + LV groups regarding abovementioned parameters.
Discussion and Conclusion: These findings suggest that sympathetic nervous system may not play the main role in the contralateral testicular damages following unilateral vasectomy.
Keywords: Chemical Sympathectomy, Vasectomy, Sperm, In Vitro Fertilization, Mouse

ST-03.01.1-002
The effects of lornoksikam and intravenous iboprofen in lower extremity ischemia reperfusion injury

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We aimed to investigate the effects of lornoksikam and ibuprofen on paraoxonase activity assay (PON) and Ischemia−modified albumin asssay (IMA) levels in lower extremity ischemia reperfusion injury.

24 Wistar Albino rats were grouped as; Control (C), ischemia reperfusion (I/R), ischemia reperfusion + lornoksikam (I/R + L), ischemia reperfusion + ibuprofen (I/R + I). I/R group underwent laparotomy and cross-clamping of the infrarenal abdominal aorta (IAA) for 120 min then 120 min of reperfusion; I/R + L and I/R + I group received lornoxicam (2 $mg \cdot kg^{-1}$) or ibuprofen (30 $mg \cdot kg^{-1}$), after 120 min of ischemia. All rats sacrificed, renal tissue specimen was collected.

PON and IMA levels were statistically different between the groups. PON levels were high in lornoksikam and ibuprofen groups compaired to the I/R groups. IMA levels were significantly high in I/R group compaired to control group, but low in I/R + L and I/R + I groups compaired to I/R groups.

Renal regions were evaluated via hematoxyline-eosin staining method. It has been observed that lornoksikam treatment prior to ischemia considerably decreased hemorrhage, but ibuprofen treatment has no effect.

It can be suggested that, lornoksikam has more antioxidant and antihemorrhagic effects than ıntravenos injections of iburofen in renal damages.

ST-03.01.1-003
A glance at smoking effect on preeclamptic placenta with transcriptional regulation approach

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Preeclampsia (PE) is a pregnancy specific disease can cause adverse outcomes like prematurity and growth restriction of baby meanwhile hypertension and proteinuria for mother. Smoking affects baby by plecanta previa, low birth weight but paradoxically risk of PE is lower in smoker mothers than non-smoker ones. Exact mechanism and relation between cigarette smoking and preeclampsia has not been enlightened yet.

Objective: To search whether a preventive side of smoking on preeclampsia. If so, to investigate a relation between cigarette smoking and preeclampsia by using transcription factors (TFs).

Methods: To elucidate gene expression differences, three high throughput screening datasets downloaded (GSE44711-preeclamptic plancenta, GSE7434- cigarette smoking placenta, GSE48424 smoking-preeclamptic placenta). Differentally expressed genes (DEGs) are sorted out and transcription factors that regulates these DEGs are determined. An algorithm TRANSREGNET was coded includes 57 157 genes and 348 TFs that allows the user finding of TFs which is responsible for regulation of query genes. By using this program TFs were found for DEGs from each three cases.Then these TFs were classified by their job.

Results: Three types of placentas share common TFs of AR, E2F4, ESR1, FOXA1, FOXP3, GATA1, GATA2, GATA3, MYC and YBX1. Among these TFs ESR1 and GATA3 regulates uterus development, MYC is responsible from Wnt signaling which is essential for female reproduction, uterine function and organ development. ESR1 and E2F4 have roles in epithelial cell development which is important for PE.Because epithelial dysfunction plays an essential role in pathogenesis of PE, also.

Conclusion: The system behind smoking in pregnancy and reduced PE risk is not fully clear in literature yet. While the hierarchy between genes and cross-talk of TFs in our model, there could be a logical relation between smoking during pregnancy, lowers PE risk by regulation of oxidative stress and prevention of vascularization.

Sunday 4 September 17:30–19:30, Hall D

Host–pathogen interactions

ST-04.01.1-001
Ancient tuberculosis DNA analysis: possible impact of the burial environment

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Tuberculosis (TB) is one of the most infectious and deadliest diseases worldwide that threatened human health and life throughout centuries. Biomolecular approach now makes it possible to detect and examine ancient pathogens, such as *M. tuberculosis*, from archaeological samples and to discover the spread of TB during human evolutionary history. However, most of the sequences isolated from ancient human remains do not originate from the specimen of interest, but instead are found to be typical of soil environments. For instance, archaeological bone samples can be contaminated with environmental mycobacteria and thus give false positive results of the presence of the *M. tuberculosis* complex.

The aim of this study was to evaluate the impact of the burial environment and environmental mycobacteria upon the analysis of ancient *M. tuberculosis* in archaeological bone samples.

In this study, Medieval cemeteries in Latvia were used to collect archaeological bone samples and corresponding soil samples from each burial environment. Bone samples were examined in order to detect characteristic TB lesions. Those, which possessed the lesions, were selected for ancient DNA (aDNA) isolation. Further, isolated aDNA was used for the initial detection of *M. tuberculosis* aDNA via full microbiome sequencing and analysis (Ion Torrent technologies). Soil samples underwent same procedure of DNA isolation and full microbiome sequencing and analysis in order to identify environmental mycobacteria.

Our results show that soil microbiome of burial environment can be considered as an important contamination factor for archaeological bone samples and thus environmental mycobacteria can lead to false positive results when examining *M. tuberculosis* in bone remains and disturb the interpretation of TB aDNA data.

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ST-04.01.1-002
Unravelling the molecular basis of adhesion to the host via Als1, a major virulence factor from *Candida albicans*

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Candida albicans is a human opportunistic pathogen responsible of the most prevalent fungal and a major source of life-threatening nosocomial infections. Members Als (agglutinin-like sequence) family from *C. albicans* mediate adhesion of the fungus host-cell surface proteins and the formation of biofilm and amyloid fibres, resulting key virulence factors in this pathogen. Structural data available for Als3 and Als9 show that adhesion to host-proteins happens via the N-terminal domain of Als adhesins (NT-Als; up to 314 amino acids). This region contains immunoglobulin-like domains and exhibits a large cavity capable of binding flexible C termini of peptides in extended conformation. The protein-peptide interactions happen via the side chain of an invariant lysine found at the end of the cavity and which recognizes the C-terminal carboxylate of peptide. In addition, complex formation is held by H-bonds formed between main-chain atoms of the Als and ligand. Here we present the structural/functional characterization of one of the major *C. albicans* adhesins of this family, Als1 using a biophysical approach combining protein biochemistry, X-ray crystallography, fluorescence polarization and ITC. Our results provide, on one hand, insights into Als1 substrate recognition and specificity compared to Als3, the other major adhesin of this family. On the other hand, we have performed the screening of a library of C-terminal peptides of proteins from human saliva, serum and extracellular matrix, which provides evidences *in vitro* of Als1 interaction with reported human ligands and uncover new potential human targets for this adhesin. Thus our work deciphers key aspects of Als' adhesion essential for understanding their interactions with host-molecules but also for the design of novel antimicrobials impairing Als' adhesion function to treat and prevent candida infections.

ST-04.01.1-003
Eukaryotic model development for therapeutic efficacy assessment of bacteriophages against antibiotic resistant microbial pathogens

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Urgent needs for alternative antimicrobial agents' development caused by increased resistance of pathogenic bacteria to the most of known antibiotics. The use of bacteriophages is a promising way solving this problem based on phage ability to regulate the microb's population. However, this approach has not found yet the wide application in human health care because of lack of scientific evidences of phage therapy efficacy, including therapeutic dose and safety of phages.

We developed a model of human cells, which allows quantitatively assessing the level of infection aroused by clinical and laboratory *Pseudomonas aeruginosa* strains followed by 90% of cells recovery after treatment with phages. The morphological changes of human cells before and after treatment by phages were detected using ImageXpress Micro XL System. By CellTiter – Glow viability assay and NucGreen Dead 488 ReadyProbes assay we estimated the therapeutic effect of phages through the growth of healthy cells population. The declined apoptotic changes inside on infected cells were confirmed by reduction of chromatin condensation and nuclear exclusion, and the caspase-3 activation. Anti-inflammatory effect of phages revealed the decreased level of cytokines IL-6 and TNF-alfa production in human cells.

During development of simulation in vitro of acute and chronical infections, we gathered set of quantitative data of phage therapy efficacy, including the optimal dose and incubation time with phages, inflammatory and apoptotic factors production during the infection and post-infection processes. Bacteriophage safety was confirmed by human cells toxicity assessment.

By this study, we demonstrated the scientific evidence of the bacteriophage ability to decline pathogenic microbial population in infected human cells. Our model might be used in the future, as a first screen for efficacy of new anti-microbial therapeutics prior to proceed the expensive and time-consuming pre-clinical trials in animal models.

Mechanisms of pro-inflammatory diseases

ST-04.02.2-001
Flax lignan's activity towards platelet aggregation

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Introduction: Mammalian lignans enterolactone (EL) and enterodiol (ED) are metabolites of the dietary precursors secoisolariciresinol diglucoside (SDG) present in flaxseed. They are known to act as anti-inflammatory molecules. The aim of present work was to investigate the effects of EL and ED on activation, degranulation and aggregation of platelets.

Methods: Human platelet rich plasma in the presence of ED/EL was activated by ADP, collagen or PAF. In spectrofluorimetry study pH-sensitive fluorescent dye acridine orange loading and release by platelets after stimulation with ADP were registered. Flow cytometry was used to detect the changes of the granularity and shape of the platelets.

Results: Both ED and EL in concentration of 0.8 mM decreased the level of ADP- and PAF-induced platelet aggregation by 50 and 75% respectively. They also prolonged the lag-period of collagen-induced aggregation from 10 s up to 40 s. The effects of EL on ADP-induced platelet aggregation of healthy individuals and patients with chronic inflammatory disease which could be accompanied by PAF accumulation was compared. EL inhibitory effect at lower concentration (0.08 mM) was much prominent in the case of disease PRP (22.8% vs. 3.2% of inhibition). Flow cytometry results showed that both lignans did not affect the shape or granularity of resting platelets but prominently protected platelets from shape and granularity changes induced by 20 μM ADP. This data was also supported by spectrofluorimetric registration of AO secretion which showed that 0.8 mM ED/EL inhibited ADP-induced degranulation of platelets by 48%).

Conclusions: ED and EL inhibited platelet aggregation affecting mostly platelet activation process that was directly shown by flow cytometry and spectrofluorimetry. Especially significant effect of lignants on platelets aggregation was found in the case of arthritis pathology with PAF accumulation.

ST-04.02.2-002
Investigation of effects of epigallocatechin-3-gallate on a scleroderma model of fibrosis

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Objective: The aim of this study was to investigate the potential effects of epigallocatechin-3-gallate (EGCG) against fibrosis, using an experimental bleomycin (BLM) model.
Material and Methods: 32 *Balb/c* female mice were randomly selected into four groups. For 21 days:

Control group ($n = 8$) was given 100 μL *subcutan (sc)* saline (SF) once a day; 100 μL *intraperitoneal (ip)* SF twice a week, BLM group ($n = 8$) was given 100 μL (100 μg) *sc* BLM once a day; 100 μL *ip* SF twice a week, BLM + EGCG group ($n = 8$) was given 100 μL (100 μg) *sc* BLM once a day; 100 μL (100 μg) *ip* EGCG twice a week, EGCG group ($n = 8$) was given 100 μL *sc* SF once a day; 100 μL (100 mg) *ip* ECCG twice a week.

EGCG's effects on fibrosis were investigated through a physical examination prior to sacrificing of animals. Histochemical (hematoxylin & Eosin) and Masson Trichrome staining of dermal areas were performed. Anova-Sidak test made for significant differences between groups. Statistical significance was $P < 0.05$.
Results: When compared to sham and control groups, experimental group (EGCG-treated group) was observed to have reduced connective tissue fibrosis in dermis area ($P = 0.000$) according to Masson Trichrome results. EGCG group showed a significant reduction in fibrosis at the dermal surface area ($P = 0.022$) using hematoxylin measurements.
Conclusion: This study shows that EGCG has protective effects against fibrosis in an experimental model of scleroderma.
Keywords: Bleomycin, experimental scleroderma model, epigallocatechin-3-gallate, fibrosis.

Molecular mechanisms of inflammation

ST-04.04.4-001
Endoplasmic reticulum stress decreases hepatic polyunsaturated fatty acids and induces a proinflammatory state in rat liver

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In this study, we sought to evaluate the effect endoplasmic reticulum (ER) on hepatic polyunsaturated fatty acids (PUFAs) and inflammatory response through cyclooxygenase mediated pathway. Male Wistar rats were divided into control, tunicamycin (TM) treated and TM + tauroursodeoxycholic acid (TUDCA) treated groups. Hepatic ER stress was induced by i.p. injection of TM and the ER stress inhibitor TUDCA was injected 30 min before hepatic induction of ER stress. The presence of ER stress was confirmed by increased intracellular levels of C/EBP-homologous protein (CHOP) and 78-kDa glucose-regulated protein (GRP78). Necroinflammation was evaluated in liver sections stained with hematoxylin-eosin using the Ishak-modified hepatic activity index. Levels of arachidonic acid (AA, C20:4n-6), dihomo-gamma-linolenic acid (DGLA, C20:3n-6), eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) in liver tissue were determined by an optimized multiple reaction monitoring method using LC MS/MS. Phospholipase A2 (PLA2), cyclooxygenase (COX) and prostaglandin E2 (PGE2) were measured in tissue samples to evaluate changes in the inflammatory pathways. Tunicamycin treatment significantly decreased all measured PUFAs and increased AA/EPA ratio in liver tissue compared to controls. Activity of PLA2, COX and PGE2 levels were significantly increased in liver tissue of TM treated rats compared to controls. Tauroursodeoxycholic acid lead to a partial restoration of liver PUFA levels and significantly decreased PLA2, COX and PGE2 levels compared to TM treated rats. The results of this study reveal the presence of a proinflammatory state in hepatic ER stress as shown by significantly increased AA/EPA ratio. To our best knowledge, this is the first study reporting altered PUFA levels in ER stress and supports the use of omega-3 fatty acids as adjuvant treatment in liver diseases demonstrating ER stress.
Acknowledgement: This study was supported by a grant from TUBITAK; # 214S223.

ST-04.04.4-002
Gene expression of MICA and MICB in sepsis patient

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Introduction: Major histocompatibility complex class I-related chain A and B (MICA/B) act in the regulation of protective responses due to the stress induced expression. Sepsis is one of the major causes of the morbidity. It has gained considerable of interest to evaluate the evidence of relation between sepsis and stress biomarkers.
Objective: It was aimed to measure the mRNA levels of MICA/B genes in the control and sepsis patient groups to evaluate the potential of them as sensitive biomarkers and provide a fundamental data for developing the new treatment approaches in relation to sepsis.
Materials and Methods: Individuals were separated into two groups as control and sepsis patients according to the decision of Ethic Committee of Çukurova University at Balcali Hospital. Blood specimens (3 mL) were collected from the venous blood into EDTA containing tube and WBCs were isolated. mRNA was isolated from WBCs using the High Pure RNA Isolation Kit (Roche Diagnostics, Mannheim, Germany). Quantitative PCR was performed using the LightCycler® instrument. Differences between control and patient group were compared statistically by both One way ANOVA and paired Student t ($P < 0.05$).
Results: According to the data, the mRNA concentration of MIC A showed a 2.0×10^6 fold increase in the patient group in contrast to the control group. Similarly, MIC B mRNA concentration also increased whereas this level was found higher than MIC A mRNA concentration as 1.4×10^{12} fold in the sepsis patient group.
Conclusions: This the first report according to our knowledge representing the significant increases in the MIC A and MIC B expressions in the patient group. Therefore this might provide a useful data for emphasizing these molecules as sensitive

biomarkers. Nevertheless, there is required further research to enlighten the detail mechanisms of the MIC A and MIC B roles and also to develop new treatment approaches in this area.

ST-04.04.4-003
Impact of rFSH treatment on Sertoli cell cytokine expression

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Objective: To evaluate the differences for the expression of cytokine profiles in Sertoli cells of NOA (non obstructive azoospermia) and OA (obstructive azoospermia) patients and the effects of FSH treatment on cytokine gene expressions.
Material and Methods: A total of 15 azoospermic men diagnosed as obstructive azoospermia (OA) ($n = 5$) (control group) and NOA ($n = 10$) were included in the study. NOA patients were split into two further subgroups: normal (nFSH) and high (hFSH) serum FSH levels. Primary Sertoli cell cultures were prepared from the testicular tissue samples collected during the micro-TESE procedure. Expression of cytokine gene panel (88 genes), FSH receptor (FSHR) and androgen binding protein (ABP) were evaluated by real-time PCR array analysis. FSHR protein level was measured by the Western blot.
Results: In primary cultures of Sertoli cells 7 genes were found to be increased and 13 were decreased in NOA group, when compared to OA ($P < 0.05$). When rFSH was introduced into the culture media, expression of 12 genes in the NOA group restored a comparable level to those of the control OA group. Sertoli cells in all groups responded rFSH administration with increased expression of ABP.
Conclusion: Our study was the first to measure the expression levels of these cytokine genes in Sertoli cells in relation to their influence on male infertility Our results suggest that FSH treatment may have positive effects on Sertoli cells of non-obstructive azoospermic patients via changing the expression levels of certain cytokine genes and restoring their levels in normal Sertoli cell population.

Some cytokine levels can be considered as a potential candidate for detecting NOA patients. ABP is a good marker for cell viability and functionality in primary Sertoli cell culture.
Keywords: Cytokines, FSH, Sertoli Cells, Male Infertility
Acknowledgement: This research has been supported by The Scientific and Technological Research Council of Turkey (No: 214S532).

New optical methods for studying neuronal structure and function

ST-09.01.1-001
Anorexigenic neuropeptides

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The Hypothalamus in the regulation of appetite and metabolism plays a critical role in the complex neuroendocrine system operation for the life. Human beings are in to appetite control, weight loss-gain. Sufficient and well-balanced Nutrition helps the body for its energy homeostasis. The cocaine and amphetamine-regulated transcript peptide and melanocortins such as alpha-melanocyte-stimulating hormone (αMSH) are anorexigenic and increase energy expenditure, melanin-concentrating hormone, neuropeptide Y and endogenous melanocortin receptor antagonist agouti-related protein (AgRP) are orexigenic-appetite stimulant and anabolic peptide. Circulating levels of αMSH and AgRP of which the potential role especially in childhood malnutrition or being fat have been studied lately. The alterations at levels of these peptides have been studied with regard to the homeostatic model assessment of insulin resistance (HOMA-IR). This term has been demonstrated for evaluated beta cell function and insulin resistance. HOMA-IR is a feature of risk cardio-metabolic, metabolic syndrome, fatty liver disease, diabetes mellitus. To eat unhealthily in early life has an effect development obesity.

The study groups have been comprised of two groups of normal to over-weight people. It was concluded that differences were not found between normal or over-weight children relating to AgRP levels but αMSH levels were decreased in over-weight children than in normal weight children. HOMA-IRs were positively correlated with glucose and insulin levels in groups.

It appears that αMSH levels could be helped understand the metabolic regulation and energy balance. Further research in the area would lead to the development of new treatment strategies for weakness and obesity. The interaction between appetite, dietary behaviour and skills in childhood and adolescence has influence on growth and psychosocial development are of interest in the world population.

Proteins in action

ST-02.02.2-002
Molecular characterization of organic cation transporter 1 (Oct1) active region in zebrafish (*Danio rerio*)

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Organic cation transporters (Octs) are membrane proteins from large SLC (Solute Carriers) family which play key role in absorption, distribution, metabolism and excretion of xeno- and endobiotic compounds. These polyspecific transporters are present in all vertebrates, with three human co-orthologs dominantly expressed in liver and kidney. There are two Oct co-orthologs in zebrafish, Oct1 and Oct2. Oct1 is dominantly expressed in zebrafish kidney and liver, which indicates its potential compensatory role related to human OCT1 and OCT2. Our initial functional characterization based on heterologous expression in HEK293T cells and the use of novel fluorescent zebrafish Oct1 substrates revealed potent interactions with numerous compounds, ranging from physiologically important steroid hormones to potent environmental contaminants such as organotin compounds and various pharmaceuticals. The aim of the present study was to elucidate type of interactions of Oct1 with identified interactors. Michaelis-Menten transport kinetics and homology modeling with

molecular docking revealed the complexity of Oct1 active region, with more than one active site and several crucial substrate-interacting amino acid residues. Identified amino acid residues are located in four of twelve transmembrane domains (TMDs), TMD2, TMD4, TMD10 and TMD11, which together form active cleft of the transporter. Additional investigation of Oct1 active region using site-directed mutagenesis revealed W218 and D475 as important amino acid residues for interaction of substrates and Oct1 active site(s). Both mutations, W218Y and D475E, decreased the activity of Oct1 transport by lowering the V_{max} value and increased the affinity for model substrate ASP^+. Further study will be focused on site-directed mutagenesis of other identified amino acid residues which are potentially relevant for transport activity of this physiologically and toxicologically important membrane transporter.

ST-02.02.2-003
Overexpression, purification and preliminary biochemical analysis of the C-terminal region of ecdysteroid receptor from *Aedes aegypti*

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Female *A. aegypti* mosquitoes are the major vector of dengue and Zika diseases. They require vertebrate blood in order to carry out reproduction process – vitellogenesis, controlled by the presence of 20-hydroxyedysone (20E). High concentration of 20E enables creation of the active complex of ecdysteroid receptor (EcR) and Ultraspiracle (Usp). This complex binds to the response elements in the promoter sequence of vitellogenin gene, stimulating its expression. Exploring the subject of EcR may deepen the knowledge about *A. aegypti* reproduction and potentially help in developing efficient strategies to control these mosquitoes population.

Our studies concern the C-terminal region of EcR form *A. aegypti* (AaFEcR). This unique region is not possessed by all nuclear receptors and the insight into its function remains vestigial. AaFEcR cDNA was cloned in pCold vector and overexpressed in *Escherichia coli* strain BL1(DE3)pLysS. Homogenous trigger factor–AaFEcR (TF-AaFEcR) recombinant protein was obtained by two-step purification procedure: metal ion affinity chromatography (IMAC) and gel filtration. AaFEcR (131 amino acids encoding a polipetyde of 13.295 kDa) was subsequently removed by HRV3C protease and separated from TF with gel filtration.

Molecular weight of homogenous AaFEcR was confirmed by ESI-TOF MS. Using size-exclusion chromatography (SEC) we observed ca. 1.5 times larger Stokes radius in comparison to the globular protein of the same molecular weight. In SDS-PAGE electrophoresis we also observed atypical behavior of AaFEcR. Preliminary *in silico* and circular dichroism (CD) analyses of the secondary structure suggest that of AaFEcR exhibits properties characteristic for intrinsically disordered proteins (IDPs).

ST-02.02.2-004
Random mutagenesis of the C-domain in firefly luciferase *L. mingrelica* reveals new aspects of its domain alternation mechanism

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Firefly luciferase is a two-domain enzyme catalyzing a bioluminescent oxidation of firefly luciferin. The enzyme uses two catalytic conformations for the luciferin adenylation and the half-product oxidation. For this, the C-domain rotates around the N-domain by a 140-degree angle. The C-domain contains two of the catalytic residues but its role in the modulation of bioluminescence color is overlooked.

Using random mutagenesis, we searched for color-shifting C-domain mutations in wild-type *L. mingrelica* luciferase. Mutants were screened in vivo; prospective enzymes were purified to homogeneity and characterized. Using known crystal structures of homologous luciferases, we modeled the protein in different catalytic conformations.

We identified three strong color-shifting single mutations: Phe467Ser, Glu490Val, and Glu490Lys. They changed the pH-dependence of the bioluminescence spectra but had little effect on other properties (K_m values, specific activity, and thermal stability) of the enzyme. Structural analysis showed that these mutations play no part in maintaining the C-domain structure. They belong to the C-domain region that approaches the N-domain in adenylation conformation but the subsequent domain rotation moves them 4 nm away.

Bioluminescence spectra reflect the state of the exited product within the active site during the light emission. In the C-domain, residues Phe467 and Glu490 are not structurally significant; their mutations cannot affect the active site via long-range interactions. The color-shifting effects must result from direct interactions with the active site or its neighboring residues, which is impossible in the oxidation conformation.

The effect can be explained if the residues Phe467 and Glu490 reapproach the active site by the emission stage: the oxidation conformation initiates the oxidation, but the reverse rotation of C-domain starts before the exited product is formed. The light emission most likely occurs in the adenylation conformation.

ST-02.02.2-005
Functional and structural implications of phosphorylated cytochrome *c*

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Protein function is frequently modulated by post-translational modifications. Cytochrome *c* is phosphorylated *in vivo* at threonine 28, serine 47 and tyrosine 48. Phosphorylation of tyrosine 48 of cytochrome *c* is related to a wide range of human diseases due to the pleiotropic role of the heme-protein in cell life and death. However, the effect of modifications of threonine 28 and serine 47 on the physiological functions of cytochrome *c* – namely, the transfer of electrons in the respiratory electron transport chain and the triggering of programmed cell death – is still unknown. The study of phosphorylated cytochrome *c* are difficult to study as its yield from cell extracts is very low and its kinase remains unknown. Firstly, we analyzed tyrosine 48 by a close phosphomimic of cytochrome c, developed by optimization of the synthesis of the non-canonical amino acid p-carboxymethyl-l-phenylalanine (*p*CMF). It is noteworthy that the Y48pCMF mutation significantly destabilizes the Fe-Met bond in the ferric form of cytochrome *c*, thereby lowering the pK_a value for the alkaline transition of the heme-protein. This finding reveals the differential ability of the phosphomimic protein to drive certain events.

On the other hand, to study the modifications of threonine 28 and serine 47, we replaced them by aspartate, in order to mimic phosphorylation, and report the structural and functional changes. We found that the T28D mutant causes a 30-mV

decrease on the midpoint redox potential and lowers the affinity for the distal site of *Arabidopsis thaliana* cytochrome c_1 in complex III. Both the T28D and S47D variants display a higher efficiency as electron donors for the cytochrome *c* oxidase activity of complex IV. In both protein mutants, the peroxidase activity is significantly higher, which is related to the ability of cytochrome c to leave the mitochondria and reach the cytoplasm. We also find that mutations at serine 47 impair the ability of cytochrome *c* to activate the caspases.

ST-02.02.2-006
Improving the heterologous expression of cytochromes from Gram-positive bacterium *Thermincola potens* JR

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Microbial electrochemical technologies (METs) are emerging as environmentally friendly biotechnological processes. Recently, a thermophilic Gram-positive bacterium capable of producing electricity in a microbial fuel cell was isolated. *Thermincola potens* JR contains several multiheme *c*-type cytochromes that are key players of extracellular electron transfer pathway. In order to understand the molecular basis by which Gram-positive bacteria perform this type of respiration, these proteins need to be characterized in detail. Towards this end we developed a strategy to heterologously express and purify the relevant proteins. By constructing a chimeric gene that contains the signal peptide from Shewanella oneidensis MR1 small tetraheme cytochrome (STC) and the gene sequence of the target proteins we successfully overexpressed and purified from *Escherichia coli* the decaheme periplasmic protein TherJR_0333 and the nonaheme outer-surface protein TherJR_2595. Spectroscopic techniques are being used to characterize structurally and functionally these cytochromes. This knowledge will be of significant importance to guide the rational improvement of METs for energy harvesting and wastewater treatment. Moreover this methodology is highly versatile and it can be tune towards the heterologous expression of other Gram-positive proteins.

ST-02.02.2-007
Insights into the mechanisms of RNA secondary structure destabilization by the HIV-1 nucleocapsid protein

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The mature HIV-1 nucleocapsid protein NCp7 (NC) plays a key role in reverse transcription facilitating the two obligatory strand transfers. Several properties contribute to its efficient chaperon activity: preferential binding to single-stranded regions, nucleic acid aggregation, helix destabilization, and rapid dissociation from nucleic acids. However, little is known about the relationships between these different properties, which are complicated by the ability of the protein to recognize particular HIV-1 stem–loops, such as SL1, SL2, and SL3, with high affinity and without destabilizing them. These latter properties are important in the context of genome packaging, during which NC is part of the Gag precursor. We used NMR to investigate destabilization of the full-length TAR (trans activating response element) RNA by NC, which is involved in the first strand transfer step of reverse transcription. NC was used at a low protein:nucleotide (nt) ratio of 1:59 in these experiments. NMR data for the imino protons of TAR identified most of the base pairs destabilized by NC. These base pairs were adjacent to the loops in the upper part of the TAR hairpin rather than randomly distributed. Gel retardation assays showed that conversion from the initial TAR–cTAR complex to the fully annealed form occurred much more slowly at the 1:59 ratio than at the higher ratios classically used. Nevertheless, NC significantly accelerated the formation of the initial complex at a ratio of 1:59.

Keywords: HIV-1 nucleocapsid protein; nucleic acid dynamics; imino protons; NMR spectroscopy; kinetic model.

ST-02.02.2-008
Structural biological aspects of *Staphylococcus aureus* pathogenicity island bioregulation

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It was recently shown that mobilization of *Staphylococcus aureus* pathogenicity islands (SaPIs) is controlled by a phage-mediated molecular switch. Although these genetic elements are included in the horizontal gene transfer of several toxins and virulence factors, still, no structural study has yet been reported on this regulation process. Besides the possible perspective of designing new antibacterial drugs after the detailed mechanism is revealed, our group has shown that this system has an additional potential to design human dUTPase inhibitors, which are now of key biomedical interest.

We set out to investigate the structural background of this transcriptional regulation process with various state-of-the-art molecular biology and in vitro techniques (cloning, mutagenesis, native gel electrophoresis, electrophoretic mobility shift assay, steady-state and transient kinetics, VIS and fluorescence spectroscopy, mass spectrometry, small-angle X-ray scattering, protein crystallization and crystallography).

First, by using systematic and knowledge-based design of various oligonucleotides in electrophoretic mobility shift assays we identified short regulatory regions in the SaPI genome that interacts the Staphylococcal repressor, Stl. We could also show by native mass spectrometry that Stl binds to these DNA oligos as a dimer. In parallel, we aimed to explore the dimerization surface of Stl. Applying a chemical crosslinking – mass spectrometry analysis we proved that mostly the carboxy terminal part of the protein is responsible for protein dimerization.

Our results revealed key structural information about the life-cycle regulation mechanism of the Staphylococcal pathogenicity islands. To further extend our knowledge on the initiation of this special horizontal gene transfer process we are analyzing the structure of Stl repressor and its complex with the phage-related derepressor protein by small angle X-ray scattering (SAXS) measurements.

ST-02.02.2-009
The role of NMDA receptors on renal fibrosis and its mechanism

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Renal fibrosis occurs virtually as a common end point in almost every type of chronic kidney disease (CKD). Herein, finding potential approaches to ameliorate or even reverse RF is crucial. N-methyl-D-aspartate receptors (NMDARs), known as key glutamate-gated calcium ion channels, are found probably to play a role in unilateral ureteral obstruction (UUO)-induced RF.

Fibrosis severity and expression of NMDARS subunit NR1, α-SMA, fibronectin and S100A4 were evaluated in UUO C57BL/6 mice with or without lentiviral vector-mediated RNA interference of NR1 and/or CAMKII inhibitor KN93. The expression and activation of CaMKII and Erk were also evaluated.

UUO caused an up-regulated expression of NR1, α-SMA, fibronectin and S100A4 compared to the control group ($P < 0.05$); The expression of NR1, α-SMA, fibronectin, S100A4, CaMKII and Erk in NR1-shRNA transfected group is lower compared to UUO and Scr-shRNA transfected groups ($P < 0.05$). KN93 significantly suppressed RF as well as the expression and activation of Erk, but not decreased the expression of NR1. KN93 or KN93 + NR1-sh showed no significant difference on fibrosis severity and Erk expression ($P > 0.05$).

NMDARs participate in the pathogenesis of RF at least partly through CaMKII activated Erk in UUO mice, which could be a potential therapeutic target for Renal fibrosis.

ST-02.02.2-010
Discovery of novel selective carbonic anhydrase IX inhibitors for anticancer therapy

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Carbonic anhydrase (CA) enzyme catalyzes a simple but highly important reaction of CO_2 hydration to acid and bicarbonate, thus every living cell contains this enzyme. Humans express 12 catalytically active highly homologous isozymes. Different isoforms participate in numerous pathological processes, especially cancer. For example, CA IX is expressed in tumor tissue and its selective inhibition could potentially treat solid hypoxic cancers.

We have synthesized and tested over 700 CA inhibitors and discovered highly selective ones that inhibit isoform IX, but do not inhibit other vital isoforms. Rational design required over 50 high resolution X-ray crystallographic structures of inhibitors bound to most CA isoforms. Furthermore, a detailed analysis of intrinsic thermodynamics of binding was necessary to understand the structure-activity correlations of these inhibitors. CA-compound interaction was measured by the fluorescent thermal shift assay (DSF), isothermal titration calorimetry and enzymatic stopped-flow CO_2 hydration assay. The dissociation constant reached 50 pM for the strongest binders, some of the tightest known protein-ligand binding reaction.

There is abundant evidence that CA IX, nearly absent in healthy human body, is overexpressed on cell surface of hypoxic metastatic highly invading cancers. The action of CA IX caused the pH to decrease from 7.4 to 6.2. Our inhibitors fully stopped this acidification of the environment in hypoxic HeLa cell cultures, prevented hypoxic spheroid formation for numerous cancer cell lines and reduced the number of migrating highly metastatic cells. Heterologous human CA expression in *Xenopus* oocytes demonstrated nanomolar affinity and high selectivity towards CA IX over other isoforms. Compound toxicity was tested in zebrafish embryos and was found to be lower than CA inhibitors-drugs used in clinic. Thus this series of compounds are promising candidates for animal testing and development into anticancer drugs.

ST-02.02.2-011
Isothermal titration calorimetry for measuring enzyme kinetics and inhibition in crowded solutions

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Cells are crowded. This fact has many consequences; crowding affects thermodynamics and kinetics of association of molecules and their biochemical reactions. One of the optimal methods to study processes in crowded solutions may be isothermal titration calorimetry (ITC). ITC measures the change of heat during a studied process, typically a molecular binding event. The advantage is that ITC does not require transparent solutions and labeling of the reagents. However, up to now, ITC has been commonly applied to determine the thermodynamic parameters of association of molecules and has not been appreciated to investigate chemical reactions catalyzed by enzymes.

Our aim was to figure out the benefits and limitations of the ITC technique for enzyme kinetic analysis. We performed ITC experiments for various types of trypsin-catalyzed reactions in diluted solutions and under crowded conditions. For the latter we have been using macromolecular agents (PEG and BSA) of different molecular weights, charges and concentrations. The amide hydrolysis was assayed with casein as a substrate. Additionally, casein is an insoluble macromolecule substrate, which allowed us to examine the ITC technique for a turbid solution. The ester hydrolysis was monitored with *N*α-benzoyl-DL-arginine β-naphthylamide (BANA) as a substrate. Moreover, a small BANA substrate containing a fluorescent naphthylamide group allowed us to compare the efficiency of ITC with a classical fluorometric enzymatic assay and confirmed ITC accuracy. Furthermore, we explored the potential of ITC in estimating the inhibition of the reaction by both reversible and irreversible inhibitors of trypsin.

Our experiments showed that the ITC is a powerful method to study enzyme kinetics and inhibition for complex solutions and different types of substrates and inhibitors.

ST-02.02.2-012
Regulation of siderophore biosynthesis by different transcriptional regulators in *Streptomyces clavuligerus*

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The best known antibiotic producing bacteria, *Streptomyces* sp. produce siderophores to utilize ferric ions of low concentrations in their environments. Siderophores are small weight molecules that also play important roles in sporulation and antibiotic

biosynthesis. This study aimed at to explain the regulation of siderophore biosynthesis in *Streptomyces clavuligerus* (*S. clavuligerus*) to find out possible connection between iron metabolism and antibiotic biosynthesis in the following studies.

The genes encoding Dtx-family like transcriptional regulator, Fur family like iron uptake regulatory protein and TetR family transcriptional regulator in *S. clavuligerus* were cloned to pET28a(+) expression vector and introduced into *Escherichia coli* (*E. coli*) BL21 cells via transformation. Optimization studies (different incubation temperature, IPTG concentration and induction time) were carried out for expression of recombinant proteins in *E. coli* BL21 cells. Induced proteins after IPTG addition were purified by using Protino Ni-Ted Columns (Macharel Nigel). Purified proteins were used in gel retardation experiments to determine their binding properties to the promoters found in siderophore gene cluster in *S. clavuligerus*.

Three regulatory genes were successfully cloned into pET28a (+) vector and expressed in *E. coli* BL21 cells after performing several optimization studies. By gel retardation assay, faint bands were detected in the promoter region of *desA* gene located in the siderophore gene cluster in *S. clavuligerus*.

This study reports for the first time the effect of global regulator and pathway specific regulatory protein in siderophore biosynthesis in *S. clavuligerus* thereby indicating a connection between antibiotic and siderophore biosynthesis in this bacterium.

Acknowledgement: This study was supported by TUBITAK project number KBAG-114Z948.

ST-02.02.2-013
Characterization of PATZ1 protein structure and its interaction with the tumor suppressor protein p53

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PATZ1, a POZ/BTB and AT-hook Zinc-finger (ZF) containing transcription factor, was recently shown to inhibit the function of the p53 tumor suppressor. Although a region on the C-terminal of PATZ1 was identified for the binding to p53, the mechanism of the interaction is not yet clear.

We obtain crystal structures of different regions of PATZ1, including the N-terminal BTB domain, the ZFs and the putative p53 binding domain for the first time. The present work involves both experimental and computational approaches to the study of the structure of the PATZ1 protein for the achievement of three main goals.

The first is the generation of the bacterial expression constructs from the mouse *Patz1* gene, their expression in *E. coli* cells and the purification of the fragments of the protein by column chromatography.

The second goal is the collection of X-ray protein diffraction data on the crystals of the purified proteins.

The final goal is the analysis of the crystallographic 3D protein structures using computational tools (molecular dynamics simulations). Within this final goal, we specifically confine attention to the structure of the p53 binding region of the PATZ1 protein and focus on the characterization of the PATZ1/BTB domain.

This study enriches the dataset of the family of BTB domains, allows conducting computational studies on the protein-protein interaction surface and leads to generating a model to describe this binding site. The analysis of the structure of PATZ1 protein extends the current understanding of the interaction mechanism involving p53 with implications on the study of cancer-related molecular processes.

ST-02.02.2-014
Obtaining and FITC labeling of influenza A specific IgY antibody as diagnostic tool

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Immunoglobulin Y, known as IgY, are obtained from the avian egg yolk. IgY antibodies have many advantages over the mammalian antibodies. IgY obtaining methods is non-invasive, easier and cheaper than monoclonal techniques and provide high amount of antibody. Monospecific IgY antibodies can be obtained by immunization of hens with specific proteins or peptides of related infection agent. In this study, Influenza A specific IgY antibody production was preferred regarding the global importance of diagnosis, prevention and immunotherapy of this disease.

In experiments, hens were immunized with conserved region of Influenza A M2e protein. After immunization, IgY antibodies were obtained from eggs and purified by PEG 6000 extraction method. Then the activity of M2e-specific IgY antibodies was calculated by ELISA. After this process, fluorescein isothiocyanate (FITC), which is amine reactive fluorescent dye, was used for labeling IgY. Optimum dye quantity and incubation time were identified. Optimization of labeling experiments was carried out with fluorescence spectrometer. Results of ELISA showed that M2e peptide-specific IgY antibody was obtained. Florescent labeling method was also optimized in this study.

For the first time in literature, FITC labeled-M2e peptide specific IgY antibodies was obtained and this developed formulation may contribute the diagnosis and immunotherapy strategies of Influenza A virus.

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Sunday 4 September 17:30–19:30, Hall E

DNA repair and cancer

ST-05.01.1-001
NELF-E facilitates transcription silencing at DNA double-strand breaks and promotes DNA repair

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DNA damage triggers rapid and transient transcription pause to prevent collisions between repair and transcription machineries at DNA breakage sites. Little is known about the mechanisms that ensure transcription block after DNA damage. Here we reveal dual functions of the negative elongation factor (NELF) in blocking transcription after damage and double-strand break (DSB) repair. We show that NELF-E subunit is rapidly recruited to DSBs in a PARP1-dependent manner to shutdown transcription. Remarkably, using *I-Sce-I* endonuclease and CRISPR-Cas9, we demonstrate that NELF-E is preferentially recruited to DSBs induced upstream transcriptionally active genes. Furthermore, we describe a non-canonical function of NELF-E in promoting BRCA1 recruitment to damage sites to foster homology-directed repair of DSBs. Altogether, our data

reveal a hitherto unknown pathway by which PARP1 promotes DSB-induced transcription silencing and identified NELF complex as the first component of the DNA damage response that selectively accumulates at DSBs surrounding transcriptionally active genes.

ST-05.01.1-002
Structural basis for the recognition and processing of DNA containing bulky lesions by the mammalian nucleotide excision repair system

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Mammalian NER eliminates the broadest diversity of bulky lesions from DNA with wide specificity. At that the double incision efficiency for structurally different adducts can vary over several orders of magnitude. Therefore, great attention is drawn to the question of the relationship among structural properties of bulky DNA lesions and the rate of damage elimination. The synthetic DNA structures (model DNA) which imitate NER intermediates and substrates, e.g. double-stranded DNA bearing an appropriate modification are widely used instruments of NER investigations *in vitro*.

Our present work concerns the properties of several structurally diverse model DNAs containing bulky modifications. We evaluated the impact of these lesions on spatial organization and stability of the model DNA. Their affinity for the damage sensor XPC was also studied.

According existing concepts, it was expected, that the values of melting temperature decrease, bending angles and K_D values clearly define the model DNAs substrate properties, but the experimentally estimated levels of the substrate properties were far away from these expectations.

Molecular dynamics simulations have revealed structural and energetic basement of the discrepancies observed.

A several lesion-specific regions of DNA secondary structure stabilization and destabilization were found, and their possible impacts on efficiencies of DNA damage recognition and subsequent excision was suggested.

ST-05.01.1-003
Adipocytokine levels in benign prostate hyperplasia and prostate cancer patients

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Objective: The occurrence of prostate cancer in men is one of the most common types of cancer. Recent studies have found important links between cancer and adipocytokines. Adipocytokines are thought to be factors in the occurrence of a variety of diseases. In addition, adipocytokines studies in cancer patients showed that these hormones may have an effect in the formation of cancer. In this study we aimed to evaluate the relationship between adiponectin, resistin, and leptin levels in BHP and prostate cancer patients.
Methods: This study was conducted from September 2012 to April 2013 at the Department of Medical Biochemistry and Department of Urology of Celal Bayar Univ. Medical Faculty, Manisa, Turkey. Blood samples were collected from 20 people in the same age range who had been diagnosed by examination and biopsy as BPH (benign prostatic patients) and prostate cancer patients but not operated on. Leptin, adiponectin, resistin, human serum levels were measured using ELISA kit.
Results: In the prostate cancer group, serum adiponectin and resistine levels were significantly decreased when compared to the BPH group. However, in the prostate cancer group serum leptin, levels were not significantly different from those in the respective BPH group.
Conclusion: This information and our own findings show that adiponectin and resistin, from the adipocytokine family, may play an important role in the progression of prostate cancer, and thus it may be possible to use them as diagnostic markers. Therefore, similar studies should be considered with a greater number of patients at different stages.

ST-05.01.1-004
A study on antiproliferative and genotoxic potentials in L929 and HeLa cell lines – the mutagenic activities in Salmonella strains of novel 2,5-disubstituted-benzoxazole derivatives

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Cancer is a mortal disease worldwide. The discovery and development of new treatments for cancer are urgently needed because of problems with current treatments such as toxicity and drug-resistance. Thus, research is directed towards novel drug designs with lower side effects and increased chemotherapeutic efficacy. Benzazoles, which are the substituted benzoxazole and benzimidazole derivatives, have been targeted by much research for many years because they constitute an important class of heterocyclic compounds that exhibit substantial chemotherapeutic activity.

In this study, some novel fused heterocyclic compounds of 2,5-disubstituted-benzoxazole derivatives, which were previously synthesized by our group, were evaluated from anticancer perspective by using various assays. Ames/*Salmonella* assay was used to examine mutagenic potentials of the compounds. Sulforhodamine B (SRB) cytotoxicity test was performed to assess growth inhibition of L929 and HeLa cancer cell lines treated with this compounds. DNA-damaging genotoxic potantials of the compounds were evaluated by using the comet assay.

By using Ames/*Salmonella* assay in the presence of S9 fraction, compound B22 (5-nitro-2-(p-nitrobenzyl) benzoxazole) was found to be mutagenic in both *S. typhimurium* TA98 and TA 100 strains at all tested doses. IC_{50} values which were evaluated by SRB cytotoxicity assay revealed that compound B11 (2-(p-nitrobenzyl) benzoxazole) (IC_{50}=99.16 μM) was the most antiproliferative compound on HeLa cancer cells, and it might cause DNA damage such as single and double-strand breaks in cancer cells. The comet assay results showed that B11 produced DNA damage at lower concentrations than the other compounds tested on HeLa cancer cells. Among the tested compounds, B11 was found to be a remarkable compound.

In conclusion, B11 could be a good candidate as a new anticancer agent. The present findings may provide future opportunities to design and develop more effective new chemotherapeutic drugs.

ST-05.01.1-006
Functional regulation of hypoxia induced genes in human brain cancer

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Hypoxia is a crucial factor in tumour aggressiveness and reponsible of its treatment resistance, particularly in human brain cancer. Tumour resistance against radiation- and chemo-therapeutic treatment approaches is facilitated by oxygenation reduction at tumour areas. HIF-1α regulated genes are mostly responsible for this resistance type. Among these hypoxia induced genes is Carbonic anhydrase isoform IX (CA9) which is highly overexpressed in many types of cancer especially in high grade brain cancer like Glioblastoma. Other HIF-1α regulated genes like *N*-Myc Downregulated gene 1 (NDRG1), Epo and to a certain extent VEGF show similar hypoxia induced regulatory mechanisms in human gliblastoma. CA9 expression as well as differenet hypoxia induced and HIF-1α regulated genes like EPO, NDRG1, VEGF in addition to HIF-1α and Egr-1 expression under different hypoxic and reoxygenation conditions were analysed, *in vitro*, in 4 glioblastoma cell lines under a broadrange of oxygenation conditions including hypoxia and reoxygenation after experimentaly induced hypoxia in a hypoxia chamber and also *in vivo* in brain tumor tissue specimens from 2 diiferent human brain tumor type patients groups, one patients group ($n = 15$) was suffering from low grade astrocytoma (LGA) while the second group ($n = 15$) was suffering from the high grade glioblatoma (GBM) in both on protein and mRNA levels, respectively. NDRG1, EPO, VEGF and CA9 overexpression pattern detected was phenotype associated in brain tumors occurring at a high frequency both, on protein or mRNA level, rendering both CA9 and NDRG1 as potential targets for tumor therapeutic approaches as well as diagnostic markers for brain cancer development detection. HIF-1α regulated NDRG1 expression in brain tumors under hypoxia. Expression inhibition in glioblastoma *in vitro* by siRNA or tumor cell glycolysis interference are potential therapeutic tool for gene expression regulation in glioblastoma. HIF-1α regulated NDRG1 under hypoxia.

ST-05.01.1-008
Ape1/Ref1 facilitates gene conversion in DT40 cells

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Antibody affinity maturation vertebrates is enabled by mainly two different mechanisms, gene conversion and somatic hypermutation. They initiate by a common molecular intermediate on DNA including abasic sites. It has been envisaged that strand breaks were also required, however it was not confirmed yet. In the present study, we addressed this question in DT40 cells by suppressing Ape1/Ref1, an endonuclease which processes abasic sites into strand breaks. We used a dominant negative form of this protein called ED and it was reported that ED binds to DNA much stronger than its wild type form and prevents strand break formation when it is over-expressed. By transfecting ED into DT40 cells by electroporation, observations with flow cytometry indicated that gene conversion was indeed blocked up to 90%, suggesting Ape1/Ref1 is required for gene conversion in chicken DT40 cells. In conclusion, our results strongly support the activity of Base Excision DNA Repair in an error prone manner in defense against invaders during B cell mediated adaptive immunity.

Miscellaneous

ST-Mis-014
Glucocorticoid receptor gene polymorphisms are related with the development of retinal vein occlusion

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Retinal vein occlusion (RVO) is the second most common type of retinal vascular disorder. Polymorphisms of the NR3C1 gene which encodes the glucocorticoid receptors may cause a resistance or hypersensitivity against endogenous or exogenous steroids. The aim of the present study is to evaluate the relationship between the development of RVO and N363S A>G, rs33388 A>T, BclI C>G, GR-9β A>G, Tth111I C>T polymorphisms in the NR3C1 gene.

187 RVO patients and 167 controls were enrolled. Hypertension was defined as systolic blood pressure >140 mmHg and diastolic blood pressure >90 mmHg. Genotypes were detected by Real-Time PCR. Statistical analysis were performed with SPSS v21.0.

Frequency of hypertension in RVO was higher than the control ($P < 0.001$). RVO risk was significantly higher in the individuals with heterozygous (CG) and homozygous (GG) BclI polymorphism then noncarriers of BclI G allele (CC) (OR = 1.96, $P = 0.004$; OR = 3.29, $P < 0.001$, respectively). BclI G allele frequency was higher in RVO group ($P < 0.001$). In addition rs33388 heterozygous (AT) and homozygous (TT) genotypes had increased risk for RVO (OR = 1.89, $P = 0.017$; OR = 3.22, $P < 0.001$, respectively). rs33388 T allele frequency was also higher in RVO group when compared with the control group ($P < 0.001$). Genotypes and allele frequencies of the N363S, GR-9β and Tth111I polymorphisms were not significantly different between the RVO and control groups.

Systemic hypertension is one of the primary risk factors for RVO. Previous studies found that NR3C1 polymorphisms leads to steroid hypersensitivity and hypertension. Our data suggest that carriers of the BclI G or rs33388 T alleles as heterozygote or homozygote have more risk for RVO. In conclusion, NR3C1 gene polymorphisms (BclI and rs33388) are risk factors for the development of RVO.

ST-Mis-015
Age, gender and season dependent changes in parathyroid hormone and vitamin D3 levels: a data mining study

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Introduction: 25(OH)D3 and parathyroid hormone (PTH) are important regulators of calcium homeostasis. The aim of the present study was to retrospectively analyze seasonal, age and

gender effects on serum 25(OH)D and PTH levels and to assess the suitability of critical decision threshold level for 25(OH)D3 deficiency in a four season region.

Materials and Methods: The study involved laboratory results from 9890 female and 2723 male individuals aging 38.8 ± 22.1 years who had simultaneous measurements of 25 (OH)D3 and PTH. Serum 25(OH)D3 and PTH levels were measured by mass spectrometry method and by electrochemiluminescence immunoassay, respectively.

Results: Mean serum 25(OH)D3 levels showed a sinusoidal pattern throughout the year and were significantly ($P < 0.01$) elevated between June and December. PTH levels were significantly higher ($P < 0.01$) in women and showed an inverted pattern of seasonal variation relative to 25(OH)D3. Age-dependent, significant low levels of 25(OH)D were seen between ages 20 and 40 years old, while the PTH hormone levels gradually increased by years. A significant inverse relationship was found between PTH and 25(OH)D3 ($r = -0.277$, $P < 0.001$), where PTH levels were significantly high when 25(OH)D3 levels were below 30 $ng{\cdot}mL^{-1}$.

Discussion and Conclusions: Use of 30 $ng{\cdot}mL^{-1}$ as clinical decision threshold level for 25(OH)D3 based on PTH levels was also supported by our retrospective study based on a large dataset. Nevertheless, the issue of assessing Vitamin D deficiency remains difficult due to seasonal variations in serum 25(OH)D3. Thereby, PTH measurements should complement 25(OH)D3 results for diagnosing Vitamin D deficiency. It is imperative that seasonally different criteria are applied in future.

ST-Mis-016
Dual inhibition of sodium chanel and Notch 4 receptor inhibits mTOR activity

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Receptors and signal trunsduction pathways play crucial roles in malignant transformation and cancer progression. A thorough understanding of these pathways and elucidation of the cross-talk between them may help the design of new treatment strategies for cancer.

Here, the effects of sodium channel and notch-4 receptor signal on mTOR (mammalian target of rapamycin) activity is analyzed using MDA-MB-231 metastatic human breast cancer cells. The neonatal variant of sodium channel nNav 1.5, which is known to be expressed in these cells were inhibited by Phenytoin. Notch-4 receptor, of which membrane signal is over expressed, was blocked by DAPT, a g- secretase inhibitör. Then, the phosphorylated forms of mTOR substrates, namely, S6K and 4E-BP1 was detected by western blotting.

Phenytoin decreased the expression of p-70s6k and p-4E-BP1 21% and 23% respectively in 24 h. While, γ-secretase resulted in 66% and 25% decrease in their expression after 72 h of treatment. However, inhibition of nNav1.5 did not effect the MDA-MB cell proliferation but, γ-secretase decreased cell proliferation by 50% after 48 h.

It is concluded that, nNav1.5 and Notch-4 may be cooperating in their actions. Thus, an effective treatment strategy may be based on targeting of mTOR, nNav 1.5 and Notch 4 simultaneously.

Epigenetics and cancer

ST-05.02.2-001
A keto-carotenoid astaxanthin from shrimp efficiently inhibits MCF-7 cells proliferation synergistically with β-carotene and lutein

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Aim of this study was to investigate the efficiency of shrimp astaxanthin (AST, a keto-carotenoid) with β-carotene (BC) and lutein (L) from green leafy vegetables on molecular/biochemical events of MCF-7 cells proliferation.

Cells (5×10^3) treated with 20 μM either with AST or BC or L or equimolar carotenoids combination or shrimp carotenoids extract for 48 h and subjected for cell based assays and cell proliferation parameters. Synergistic influence of AST with BC and L exhibited higher cytotoxicity and cell death than shrimp carotenoids extract or individual carotenoid. The IC_{50} and combination index suggested, AST induces anti-proliferation more effectively with BC and L at lower concentrations. Further, cells attained higher oxidative stress and correlated with AST accumulation levels. Likewise, AST combination with BC and L efficiently modulates the Bcl-2, Bax and phospho-p53 expressions and induced apoptosis by arresting cell cycle than individual carotenoids and shrimp extract.

Astaxanthin is the predominant active constituent found in shrimp and acts effectively with other major dietary carotenoids to inhibit proliferation of MCF-7 cells. This study provides insight on the importance and nutraceutical value of shrimp AST and green leafy vegetables carotenoids.

ST-05.02.2-002
Up-regulation of HK2 expression is associated with poor prognosis of breast cancer luminal B type

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Breast cancer is a complex and heterogeneous disease. There are four subtypes of breast cancer that are determined by morphological, molecular and genetic features. Breast cancer luminal B type (BCLB) is characterized by increased growth and proliferation rates and poor prognosis. It is well known that glycolysis and lactate fermentation promote the proliferation of cancer cells. Thus, we hypothesized that "Warburg effect" as well as up-regulation of glycolytic gene expression might be strong characteristic of BCLB. Using next-generation sequencing we have carried out transcriptomic analysis of two groups of breast cancer luminal B type with high and low Ki-67 proliferative index. We detected deferential expression of several genes involved in energy metabolism. These data were confirmed by qPCR and immunostaining. We revealed increased expression of HK2 gene at mRNA

and protein levels. The Kendall rank correlation coefficient between HK2 mRNA and protein expression was $\tau = 0.62$ ($P = 0.003$). Frequency and expression level of mRNA HK2 were higher in sporadic BCLB than in hereditary BRCA1-positive BCLB ($P < 0.005$). Moreover, we demonstrated the reliable association between Ki-67 and HK2 expression in majority of samples ($\tau = 0.55$, $P = 0.004$). The Spearman's rank correlation coefficient between the mRNA HK2 and HIF1a expression was $r = 0.6$ ($P < 0.05$). Evaluation of the 5-year survival rate demonstrated significant decrease in survival of patients with increased HK2 protein. BRCA1 mutation is apparently an alternative mechanism of activation of glycolysis through HIF1a in breast cancer. Our results suggest elevated expression of HK2 is a factor of poor prognosis of BCLB and should be taken into account to choose treatment strategies.

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ST-05.02.2-003
In vitro cytotoxic effect of water extracts of Turkish propolis on human laryngeal epidermoid carcinoma cell line

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Propolis is a natural resinous substance collected by bees from various types of trees and plants and has antibacterial, antiviral and antitumoral features depending on its antioxidant properties. Major aim in the present study is to investigate cytotoxic effect of Turkish propolis on human laryngeal epidermoid carcinoma (HEp-2) cells.

1.250–20.000 HEp-2 cells/well were loaded on RTCA system (XCELLigence, Roche) and the cell index was followed up during 48 h. Water extract of Turkish propolis (WEP) of 1.250–50.000 $\mu g \cdot mL^{-1}$ concentrations and ethanolic extracts of Turkish propolis (EEP) of 10–2.400 $\mu g \cdot mL^{-1}$ concentrations were treated with HEp-2 cells and followed by RTCA system. The cell indexes and IC_{50} (inhibiting viability by 50%) values were determined. HEp-2 cells were incubated with WEP (1.000–3.000 $\mu g \cdot mL^{-1}$ concentrations) and EEP (75–300 $\mu g \cdot mL^{-1}$ concentrations) during 48, 72 and 96 h. Proliferation was followed by flow cytometric DNA cycle analysis.

The most suitable HEp-2 cell count was found to be 5.000 per well. WEP of 1.250–20.000 $\mu g \cdot mL^{-1}$ concentrations, EEP of 100–2.400 $\mu g \cdot mL^{-1}$ concentratios were found to be cytotoxic to HEp-2 cells. When WEP 3000 of $\mu g \cdot mL^{-1}$ concentration, EEP, whereas, of 150 $\mu g \cdot mL^{-1}$ concentration were incubated with HEp-2 cells during 72 h, the highest antiproliferative effect was seen by interfering DNA cycles.

Turkish propolis extracts were found to be cytotoxic and antiproliferative to HEp-2 cells in the present study, therefore, it was concluded that it may fall within chemotherapy or target therapies for larynx cancers.

ST-05.02.2-004
GCN5 participates in ADA3 partnership as revealed with yeast hybrid

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Introduction: Eukaryotic cells organize their genetic material into a DNA–protein complex, called chromatin, which regulates the accessibility of genome thereby creating inherent barriers to nuclear events such as replication, transcription and repair via intensive protein-protein interactions. On the other hand, post-translational modification of histones has been reported to play crucial roles in chromatin regulation that insures the fidelity of opened chromatin structure. Among different epigenetic modifications, the increased global histone acetylation degree always correlates with transcriptional regulation in euchromatin and heterochromatin. The General Control Non-derepressible 5 (GCN5), a known partner of Alteration/Deficiency in Activation 3 (ADA3) protein, is the catalytic subunit of several related histone acetyltransferase (HAT) complexes. It adds acetyl groups to target lysine residues within histones and performs both global and locus-specific histone acetylation, as well as acetylation of non-histone proteins.

Materials and Methods: Previously, we identified four new novel interacting partners of human ADA3, namely AATF, PHF21A, and regulatory subunits of the protein phosphatases PP1 and PP2A (PPP1R7 and PPP2R5D, respectively) via yeast 2 hybrid (Y2H) library screening. In this study, we tested these interactions with another HAT member, GCN5, using Y2H to address its functional significance in ADA3-containing complexes.

Results: dataGCN5 also participated in ADA3 interaction network. Moreover, we mapped the domains required for GCN5 interactions to AATF, PHF21A, PPP1R7 and PPP2R5D. The regions of 'Acetyl Transferase' between 553. and 628. amino acid residues and 'Bromodomain' at C-terminal were critical in GCN5 interactions.

Discussion and Conclusion: Reported partnership with AATF, PHF21A, PPP1R7 and PPP2R5D extends beyond ADA3 that reveals important implications for the discovery of regulators against GCN5 enzymes and related HAT family.

Functional genomics and proteomics

ST-08.01.4-001
Molecular basis of resistance of chondrosarcomas to cisplatin

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Chondrosarcomas are malignant tumors of bone. They are considered as resistant to both chemotherapy and radiation. This emergent study aims to unravel the molecular mechanisms involved in the resistance of chondrosarcomas to cisplatin treatment by an innovative strategy of comparative functional genomics.

We observed, using five cell lines derived from human chondrosarcomas, that they had distinct response to cisplatin treatment. To understand the molecular basis of these different sensitivities, we performed whole-exome sequencing on the cell lines.

After strict filtration, 245 to 476 rare coding or splice variants per cell line were predicted to have a deleterious functional impact on the protein. We applied targeted, then pangenomic approaches to select relevant variants. We identified 66 mutated genes potentially implicated in the response to therapy. Interestingly, recurrent loss of function mutations of a tumor suppressor gene were identified in the three most resistant cell lines in which no apoptosis is induced by cisplatin. This gene is actionable by targeted chemotherapy.

Functional analyses are in progress to validate the role of this very promising gene mutations and of the 65 other genes involved in resistance to treatments.

In conclusion, chondrosarcoma cell lines respond differently to cisplatin therapy. In addition, our study is the first one which extensively characterizes commonly used human chondrosarcoma cell lines by whole-exome sequencing. Our preliminary results provide essential genetic information on resistance mechanisms through the identification of genes potentially involved in the response to cisplatin.

ST-08.01.4-002
Arthrobacter plasmids: molecular classification and conserved gene clusters

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Members of the *Arthrobacter* genus are ubiquitous in polluted and toxic soil samples. Despite their potential in environmental biotechnologies, theirs practical applications are hampered due to the scarce availability of useful tools for genetic engineering. More than a decade have past since the sequencing of the most known *Arthrorobacter* plasmid – pAO1, but very little in known about the core functions – replication and partition of *Arthrobacters* plasmids.

In this study, the available *Arthrobacter* plasmids sequences were analyzed by BLAST in order to identify their putative replication origin. Gene syntheny and genome wide comparisons were performed and visualized with progressiveMauve. Evolutionary relationships were inferred using the Maximum Likelihood method. Proteome wide comparations for core-genome plot analysis was performed with CMG biotools.

Based on parA homologs sequence, the *Arthrobacter* specific plasmids have been classified into 4 clades. Iteron like sequences were identified on most of the plasmids indicating the position of the putative *Arthrobacter* specific *ori's*. A cluster of 12 ORFs predicted to encode the components of a T4-secretion system involved in bacterial conjugation was identified as highly conserved and syngenic among a subset of 14 *Arthrobacter* plasmids. Also, a DNA repeat of about 370 nucleotides was found to be present 5' to the ORFs of DUF4192-, DprA- and ParB-like proteins on 12 additional *Arthrobacter* plasmids. The DNA repeats contain alternating GC and AT reach sequences, potential protein DNA-binding sites and purine reach stretches. A core-genes common for all the *Arthrobacter* plasmids could not be identified, indicating that the plasmid diversity within this genus exceeds what can be inferred from the study of the available sequences.

It is hoped that the findings presented here will stimulate further experimental work aimed at the elucidation of the ORFs implicated in the regulation of the life cycle of these plasmids.

ST-08.01.4-003
Differential gene expression in *Halolamina* sp. in response to excess salt

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Introduction: Extreme halophilic archaea survive in excess salt environments such as rock salt mines or salt lakes. Therefore, these microorganisms are good sources to study resistance to high salt concentrations. In the present study, global differential gene expression (DEG) was evaluated in the extreme halophilic archaeon *Halolamina* sp. isolated from Yozgat salt mine, Turkey.
Materials and Methods: The microorganism was grown in SG medium containing 2.7 M or 5.5 M NaCl. RNA samples were isolated using Genejet RNA Purification Kit (Thermo Scientific) and treated with DNase (Ambion). RNA sequencing analysis was performed via Illumina HiSeq 2000 platform. The differentially expressed genes in *Halolamina* sp. between 2.7 M and 5.5 M NaCl treatments were determined using bioinformatic tools. The expression of 13 genes were verified via qRT-PCR analysis.
Results: As compared to 2.7 M NaCl, expression of 2149 genes were induced and that of 1638 genes were decreased in *Halolamina* sp. grown in 5.5 M NaCl. Approximately 1000 genes were not expressed in 2.7 M NaCl while expressed in 5.5 M NaCl, and reverse was observed for ca. 1200 genes. The DEGs were determined to be related with mainly cell membrane and transporters, binding, rodox, and gene expression.
Discussion and Conclusion: In accordance with the previous studies, it was observed that expression of the genes related with the survival of *Halolamina* sp. in excess salt, such as cell membrane stability or transport was induced. On the other hand, expression of genes related with the process such as protein expression or folding was decreased to save energy.
Acknowledgements: This study was supported by TUBITAK project number KBAG-214Z171 and as the thesis of Büşra Abanoz by TUBITAK 2210-C Scholarship Program for Domestic MSc Studies on Priority Subjects.

ST-08.01.4-004
Fluid flow mediates epithelial-mesenchymal transition and negative regulation of apoptosis in colorectal cancer cells

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The formation of cancer and gaining an aggressive phenotype, have a complex matter, which is not simply based upon genetic mutations. Physical conditions, such as existence of peritoneal

fluid flow, shear forces, asidic level of microenvironent or aberrant interstitial fluid flow patterns driven by un-controlled tumor bulk; impose significant pathophysiologic functions against cancer treatment modalities, due to affecting progression, immune-escaping, and metastasis. Hence novel strategies should target phenotypic changes driven by physical parameters of tumor microenvironment. In this study, we used microfluidic culture techniques to understand the effect of continuous fluid flow on metastatic behaviour of cancer cells.

First, we characterized the epithelial and mesenchymal phenotypes (with E-cadherin, N-cadherin and Vimentin markers) of colorectal cancer cells (HCT-116 and HT-29) under continuous microfluidic flow. Target prediction and pathway analysis were further performed to better understand the effect of continuous microfluidic flow at the trancriptome and miRNA level.

Cancer cells under flow condition demonstrated a statistically significant decrease in E-cadherin expression and an increase in N-cadherin and vimentin expressions. Under fluid flow conditions angiogenesis, regulation of apoptosis, antigen presentation processes were observed as statistically significant with the bioinformatic analysis performed with mRNA and miRNA data.

Continuous fluid flow driven dynamic microenvironment, affects the main processes of cancer cell such as epithelial-mesenchymal transition, angiogenesis, regulation of apoptosis and antigen presentation. Although interstitial fluid flow is not fully understood, it can be manipulated for the positive outcomes of cancer management.

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ST-08.01.4-005
Comparisons of metastatic and non-metastatic breast carcinoma cells using proteomic analysis of exosomes

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Cancers are formed by heterogeneous group of cells of which only small percent can form distant metastasis. Metastatic cells are often resistant to conventional treatment. We previously isolated liver, brain and heart metastatic cells of 4T1 murine breast carcinoma and named them as 4TLM, 4TBM and 4THM, respectively. Liver metastatic cells were the most aggressive ones demonstrating organ-specific phenotype. The goal of this study was to examine secrotomes of breast carcinoma cells metastasized to different organs as well as non-metastatic 67NR breast carcinoma cells. 67NR cells were originally obtained from spontaneously formed breast tumor from which 4T1 murine breast carcinoma cells were obtained. Exosomes were prepared using serum-free conditioned medium obtained after 36 h incubation. OASIS HLB 6 cc (200 mg) extraction cartridges were used to concentrate the proteins. Peptides were analyzed in the Proteomics and Bioinformatics Center of Case Western Reserve University. Over 1800 peptides were found in exosomes. Of these peptides, 50 of them, which corresponded to 24 proteins, were significantly altered in metastatic cell lines compared to 67NR cells. Four of these proteins (osteonectin, Alpha-2-HS-glycoprotein, Chemokine (C-X-C motif) ligand 6, N-cadherin) were significantly decreased in metastatic cell lines compared to non-metastatic. Level of MMP9 was significantly higher in brain and heart metastatic cells compared to non-metastatic cell. Interestingly, MMP9 levels in liver metastatic cells were markedly lower compared to heart metastatic cells. The rest of the proteins including MMP3 was significantly higher in metastatic cell lines. EGF containing fibulin-like extracellular matrix protein 2, a protein recently found to associate with breast cancer progression, was markedly higher in liver metastatic cells, demonstrating a novel role for this protein in organ specific phenotype.

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Miscellaneous

ST-Mis-020
Tissue transglutaminase induced integrin trafficking in kidney cancer metastasis

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Integrins plays an important role in cancer metastasis by orchestrating the cell adhesion and cytoskeletal dynamics together with the surrounding extracellular matrix. Tissue transglutaminase (TG2) is the ubiquitously expressed member of transglutaminase family which is able to catalyze Ca^{+2} dependent cross-linking by forming an intermolecular ε-(γ-glutamyl)-lysine isopeptide bond between proteins. However regardless from its enzymatic activity, the upregulation of TG2 along with ITGβ1 was detected as a new diagnostic marker in the metastatic state of renal cell carcinoma (RCC). Since integrin targeted therapeutic agents is not able to stabilize the metastatic progress of RCC, this study seeks to adress whether TG2 effects on the endo/exocytic cycle of β1 integrin (ITGB1).

The crosstalk between TG2 and ITGβ1 was investigated in RCC cell lines using co-immunoprecipitation (IP) assay followed by the measurement of cell adhesion potential of TG2 downregulated RCC cells on ITGB1 substrates. In order to clarify the role of TG2 in the integrin endo/exocytic cycle, a biotinylation-based IP and Flow Cytometry assay using active ITGB1 antibody was performed, respectively. The role of TG2 in ITGB1 trafficking was demonstrated by the lentiviral mediated downregulation and overexpression of TG2 in Caki-1 cells.

Results showed that although TG2 was associated with ITGβ1 in all primary and metastatic site RCC cell lines, the highest interaction affinity was detected for the metastatic Caki-1 cells. TG2 silencing resulted in a marked decrease in cell the adhesion potential of RCC cells on ITGB1 substrates. TG2 downregulation in Caki-1 cells led to a significant retardation of ITGB1 internalization whereas TG2 overexpressing Caki-1 cells displayed a 2 fold increase in the levels of internalized ITGB1 when compared to the control cells. Collectively, our results point to a critical role for TG2 in cell adhesion and ITGβ1 trafficking during the development of RCC metastases.

Monday 5 September 17:30–19:30, Hall A

Developments in biomaterials and tissue engineering

ST-07.01.3-003
Investigation of the biocompatibility of a novel multi walled carbon nanotube based scaffold in human breast cancer cell line MDA-MB-231

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The application of bioengineered scaffolds is recently one of the crucial factors in tissue engineering. Multi Walled Carbon Nanotube based nanostructured scaffold (MWCNTs) is a novel biomaterial and its effects in cell culture studies are not known yet. In this study we aimed to show biocompatibility of MWCNTs in human breast cancer cell line (MDA-MB-231).

MDA-MB-231 human breast cancer cells were cultered in cell culture medium RPMI 1640 containing 10% FBS, 1% L-glutamine, 1% penicillin-streptomycin. MWCNT based scaffold was washed with distilled water three times before the seeding cells onto the material. After that they were sterilized by ethylene oxide. MWCNT based scaffold was placed on one well of 12-well cell culture cluster and silicon reference was placed on another well and 1×10^5 cells were seeded in each well of 12-well cell culture cluster to obtain a confluent monolayer. The culture medium was changed every other day and the experiment was terminated one week after the start. The half of the incubated cells were collected by scraping for immunocytochemical staining. The cells were centrifuged and put on slides using drop method. Remaining cells on the surface of materials were fixed with glutaraldehyde to be imaged by scanning electron microscope.

The resulting out come from SEM analysis showed that the cells on the MWCNT-based scaffold proliferate widely. On the other hand the results of immunocytochemical staining of cells with Estrogen, Progesterone, MMP-2, MMP-9, PI3K, AKT and NF-KB primary antibodies showed that there was no significant difference between groups compared to hormone receptor status and their ability of proliferation, migration and metastasis. These results demonstrated that the MWCNT based scaffold is biocompatible for breast cancer studies so MWCNT based scaffold and these properties made it to plausible potential candidate for tissue engineering or other biomedical applications.

ST-07.01.3-004
Development of biocomposite material on the basis of bacterial cellulose and cells of *Bacillus subtilis*

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Bacterial cellulose (BC) have gained increasing attention due to the chemical purity and crystallinity, high strength and stiffness, biodegradability and renewability, and their production and application in development of composites. Furthermore, high adsorption properties of BC allow to introduce different biologically active substances in it. That could be bacteria of *Bacillus* genus with high antimicrobial and proteolytic activity, possessing the local treatment of wounds and prevention of septic complications.

BC/*Bacillus subtilis P-2* biocomposite was prepared by their coaggregation on HS medium. Sorption efficiency of BC was evaluated by number of immobilized bacteria determined by optical density of culture suspension at 650 nm. The surface morphology of composite material was examined by a scanning electron microscopy (Quanta 3D 200i Dual system). The antimicrobial activity was determined by measuring the clear zone around the BC/*Bacillus subtilis* P-2 biocomposite against *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa.*

Thus, biocomposite material on the basis of BC and *B. subtilis P-2* cells was obtained. The optimal time for immobilization of *B. subtilis P-2* cells on BC membrane was 24 hours. The number of living bacilli in biofilm reached (2.4–3.1) 10^9 CFU·g^{-1}. The BC film with immobilized *B. subtilis P-2* cells possessed high antimicrobial properties against skin pathogens. Created biocomposite material may be used as a new drug form of transdermal therapeutic systems.

Stem cells and cancer

ST-05.03.3-012
Isolation and characterization of cancer stem cells in YKG1 glioma cell lines to evaluate the anti-tumor effect of thymoquinone vs mitoxantrone

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Introduction: In terms of treatment of tumors, surgery, chemotherapy and radiotherapy are not exactly efficient and recurrence could occur. It has been found that cancer stem cells (CSC) are resistant to chemotherapy and radiotherapy. Thymoquinone which had been isolated from Nigella sativa, investigated as antioxidant, anti-inflammatory and anti-cancer. We have proposed to reveal the role of thymoquinone on glioma cells by comparing with mitoxantrone and aimed to find more effective and less toxic treatment method.

Materials and Methods: YKG1 cell line has been thawed and proliferated. CSC has been successfully isolated by using Fluorescence Activated Cell Sorting (FACS) with the antibody CD133. Neurosphere formation has been observed and these spheres are well characterized by staining with Nestin. Various concentrations of thymoquinone (T1:40 μM, T2:80 μM, T3:160 μM) and mitoxantrone (M4:0.5 μg·mL^{-1}, M3:0.05 μg·mL^{-1}, M2:0.005 μg·mL^{-1}, M1:0.0005 μg·mL^{-1}) were prepared by diluting in DMEM solution. The cells were exposed to thymoquinone and mitoxantrone alone and finally with both of mitoxantrone and thymoquinone series together.

Results: Antiproliferative effect of thymoquinone was seen only at T3 doses while both M3 and M4 doses of mitoxantrone showed toxic effect to the cells. When combined use of mitoxantrone and thymoquinone were done, a significant antitumoral effect was seen in T2M4 combination.

Conclusions: The effect of T2M4 combination on the cell suppression was almost twice according to M4 doses of mitoxantrone. We thought this result shows synergistic effect of thymoquinone with mitoxantrone.

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ST-05.03.3-013
Cell cycle controlling of cancer stem cells in primary and metastatic colon carcinoma

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Cancer is a disease on which the incidents and mortality rates have increased in recent years. Today, it is considered that cancerous cells are created by the instability of stem cells during their proliferation and differentiation. The formation and controlling of cancer stem cells in the tumor microenvironment are not fully understood. The cells may be delayed in the G1–S transition and defectivity of cell-cycle control. Diverse biological functions regulated by cyclin D1 include the induction of cellular proliferation, angiogenesis, cellular migration, DNA damage repair, mitochondrial biogenesis, stem cell maintenance and miRNA expression. Cyclin D1 was also shown to regulate the miRNA expression.

In this study, the human metastatic colon carcinoma cell line, Colo 741, human primary colon cancer cell line, HCT 116, were cultured in RPMI-1640 culture medium including 10% FCS, 1% L-glutamine and 1% penicilin-streptomycine. Colon carcinoma stem cells characterized by CD133 surface protein were isolated from Colo 741 and HCT 116 cells by magnetic-activated cell sorting (MACS) technique. Magnetically labeled CD133+ and unlabeled CD133− cells were cultured and passaged after reaching 80% monolayer confluency. They were then fixed with 4% paraformaldyde and distribution of anti-cyclin D1, anti-c-myc, anti-β-catenin, anti-dicer, anti-drosha, and anti-eIF2α were investigated using indirect immunoperoxidase staining.

Results show that the separation was provided successfully by MiniMACS column. While Cyclin D1 was increased in both HCT116 and Colo741 cells, Dicer and Drosha was more detectable in HCT116 CD133+ cells. In addition, c-myc immunoreactivity was observed strongly in Colo741 CD133+ cells. In conclusion, our studies provide evidence for a novel level of fine tuning to regulate the functional interactions between cyclin D1, c-myc and miRNAs pathways. Different controlling pathways may play a role in the oncogenic stimuli for primary and metastatic cancer stem cells.

ST-05.03.3-014
Differentiation of adipose tissue derived stem cells to natural killer cells having anti-tumor activities *in vitro* in pancreatic cancer

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Introduction: Pancreatic cancer (PaCa) is one of the most lethal human cancers. Since these patients can only survive 6 months even with therapies there is a need of finding new therapeutic approaches and stem cells (SCs) can be good candidates. Adipose tissue is a rich source of SCs which can be obtained by a simple surgical procedure abundantly. Natural killer (NK) cells are critical mediators of host immunity against malignancy which lead to apoptosis of malignant cells. In this study we aimed to differentiate adipose tissue derived SCs (ADSCs) to natural killer (NK) cells and then to investigate their effect on human PaCa cells in vitro.

Materials and Methods: Human ADSCs were differentiated to NK cells by using haemopoietic induction and then differentiation protocols, and were characterized by flow cytometric and immunohistochemical analysis via using CD314, and CD90 antibodies. ADSCs derived-NK cells then co-cultured with pancreatic cancer cell line-Panc1 in vitro. While the apoptotic activity of NK cells on Panc1 cells were determined by Annexin V and TUNEL assays, alterations in oncogenic gene expressions were determined by RT-PCR.

Results: ADSCs derived-NK cells were identified by expressing high levels of NK specific cell marker-CD314, while not expressing the SC marker-CD90. These cells when cocultured with Panc1 in vitro caused to apoptosis of cancer cells. The efficiency of NK cells in leading pancreas PaCa cells to cell death was also demonstrated by the decrease in oncogene gene expressions.

Discussion and Conclusion: Allogenic NK cells, are not inhibited by self histocompatibility antigens like autologous NK cells. Hence, ADSC derived-NK cells will allow to prepare expanded, cryopreserved NK cells for instant administration without a need of ex vivo expansion in cancer therapy. Our results show that ADSCs can be converted into NK cells having anti-tumor activities. Thus, adipose tissue can be used as a source of SCs to produce NK cells to fight against cancer.

ST-05.03.3-015
Glioblastoma stem cells influence the permeability of blood-brain barrier

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Glioblastoma Multiforme (GBM) is a central nervous system (CNS) tumor with high aggressiveness and chemoresistance due to the presence of cancer stem cells (SCs). The blood–brain barrier (BBB), the microvascular endothelium surrounding CNS, limits the drug delivery to GBM due to the presence of tight junctions (TJs) and efflux transporters, like P-glycoprotein (Pgp), breast cancer protein (BCRP) and multidrug-related protein family (MRP). It has never been investigated whether the grade of differentiation or stemness of GBM cells at the tumor-BBB interface influences the permeability of BBB.

To address this issue, we set up co-cultures of human brain microvascular endothelial BBB cells and GBM cells obtained from primary surgical specimens. From each GBM we isolated and characterized the differentiated component (adherent cells, AC) and the stem cell (SC) component.

GBM SCs had higher expression of Pgp, MRP1, BCRP than AC. Pgp up-regulation was due to the activity of Wnt3a-dependent canonical pathway. Wnt3 also controlled the expression of Pgp in BBB cells. The presence of GBM cells or GBM-conditioned medium increased the permeability to substrates of Pgp and BCRP, and disrupted TJs, compared to BBB cells without GBM. The increased BBB permeability was stronger with GBM AC than with SCs, and was due to changes in the expression of

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efflux transporters and TJ proteins. Of note, GBM AC clones stably overexpressing Wnt3a acquired stemness properties and modified BBB permeability as SCs did.

Our results suggest that GBM cells modulate BBB permeability. GBM AC produce a leaker BBB than GBM SCs. GBM surface-associated factors or soluble factors released by GBM cells may be responsible for the changes in BBB permeability. Wnt3a may be a key actor in this process.

Our study may open new perspectives on the mechanisms regulating the drug delivery at GBM-BBB interface and may identify new therapeutic strategies tailored on AC and SC GBM.

ST-05.03.3-016
Targeting apoptosis resistance in cancer stem cells by the biphosphonate zoledronic acid

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In contrast to low-tumorigenic bulk tumor cells (non-CSCs), cancer stem cells (CSCs) are a subset of tumor cells with the potential to self-renew and differentiate into different cancer subtypes. Resistance to apoptosis is one of the most important features of CSCs. Zoledronic acid (ZA), a third generation and one of the most potent bisphosphonates, designed to treat osteoporosis and skeletal related malignancies, has been shown to has anti-tumor effects through cell death induction in some malignancies. However, the effects of ZA on CSCs remain unclear. The present study aimed to determine the apoptotic effect of ZA on CSCs using prostate CSCs as a model system.

Cluster of differentiation (CD) 133^{+high}/$CD44^{+high}$ prostate CSCs were isolated from the DU-145 human prostate cancer cell line. CSCs and non-CSCs were exposed to increasing concentrations of ZA for 24, 48 and 72 h and the viability was examined to determine the IC50 dose. Annexin-V analyses were performed for the detection of cell death. Each cell group was assayed by qRT-PCR array for the detection of 84 key apoptosis related genes.

Zoledronic acid caused a dose- and time-dependent decrease in cell viability. Treatment with ZA caused a concomitant increase in apoptosis. Significant over/under-expressions were detected in six of the studied genes of ZA-treated DU-145 CSCs cells. Expressions of *CASP8*, *CASP4* and *BAD* genes increased while the expressions of*BIRC3*, *BIRC2* and *BCL2* genes decreased. In the DU-145 non-CSCs, 5 genes showed changes in gene expression after ZA treatment, with 2 showing increased expression (*CASP3* and *BAX*) and 3 showing decreased expression (*BIRC3*, *BIRC2* and*BCL2*).

Our data revealed that ZA has the potential to overcome apoptosis resistance in prostate CSCs by induction of apoptosis through up-regulation of some pro-apoptotic and down-regulation of some anti-apoptotic genes. These data suggest that, ZA may be an effective therapeutic approach for targeting apoptosis resistance in CSCs.

ST-05.03.3-017
Three-dimensional vascularized self-assembled tumor spheroids

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It is known that *in vitro* three-dimensional (3D) engineered tissues mimic *in vivo* tissue structure better than two-dimensional (2D) culture systems. Well-simulated engineered tissue needs a microenvironment of the relevant cells and 3D culture system. In this study, we aimed to develop vascularized self-assembled tumor spheroids in order to mimic tumor structure, and provide an insight to develop cancer therapeutics and novel treatment systems. Since *in vivo* tumor tissues have cancer stem cells (CSCs), which are more resistant to cancer therapy, and thought to be responsible for maintaining tumor growth, our self-assembled tumor spheroids were chosen to include CSCs. $CD133^+$ cancer stem cells were sorted from human osteosarcoma cell line (SaOs-2) by magnetic-activated cell sorting (MACS) technique. Three different co-culture systems were organized in order to form vascularized tumor spheroids: SaOs-2 and human umbilical vein endothelial cell line (HUVECs), parental osteosarcoma stem cell line (OSCs) and HUVECs; sorted $CD133^+$ cells and HUVECs. Tumor spheroids were formed in 3D agar molds by seeding both cell types together at 5×10^4 cell number (Cancer cells:HUVECs, 1:1).

Self-assembly of the tumor structure and tube formation were observed in all three co-culture systems by immunohistochemistry methods. After immunohistochemical analyses, tube formation were detected in all group, however, in $CD133^+$/HUVEC co-culture group, this formation was more identical. In addition, intensity of VEGFRI, VEGFRII and CD31 were more detectable in $CD133^+$/HUVEC co-culture groups than other groups. Co-culture system with microtissue was supported tube formation, as a model for vascularization.

ST-05.03.3-018
Relationship between Annexin A2 levels and response to chemoradiotherapy in patients with local advanced stage non-small cell lung cancer

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Annexin A2 is a kind of phospholipid-binding protein over expressed in tumor cells and associated with poor prognosis in terms of tumor invasion, lymph node metastasis and overall and progression-free survival. In present study, the relationships between Annexin A2 levels and chemoradiotherapy response together with survival rates were simultaneously determined before and after chemoradiotherapy in patients with local advanced stage non-small-cell lung cancer (NSCLC).

Patients with NSCLC were subjected to 66 Gy radiotherapy together with weekly administration of 25 $mg{\cdot}m^{-2}$ docetaxel and cisplatin chemotherapy. Blood samples were taken a day before and after chemotherapy for studying Annexin A2 levels. Histopathologically, patients were classified as adenocarcinoma

(23%, $n = 9$), epidermoid carcinoma (71%, $n = 28$), adenosquamous (%2, $n = 1$), and sarcomatous type (2%, $n = 1$).

Complete response in 5% and partial response in 59% of the cases were recorded with the median follow-up period of 13 months. Overall survival rates were 74% for one year and 61% for two years. Mean overall survival rate, hydrangea of local control, and that of progression-free survival rates, were respectively observed as 18, 10, and 9 months. While the average level of Annexin A2 prior to treatment was 23.94 $ng{\cdot}mL^{-1}$, it was estimated as 17.66 $ng{\cdot}mL^{-1}$ after treatment. There was no correlation between Annexin A2 levels and chemoradiotherapy-induced response. However, pre-treatment evaluation of Annexin A2 levels in response to chemoradiotherapy revealed a slightly significant trend. No relationship was detected between Annexin A2 levels and overall survival, local control and progression-free survival rates.

In conclusion, a significant decrease was observed between Annexin A2 levels before and after treatment in patients receiving chemoradiotherapy due to local advanced NSCLC, but it was not reflected in evaluation of response to therapy and survivals.

ST-05.03.3-019
Mesenchymal stem cells reduce bone specific alkaline phosphatase (BALP) and terminal cross-linked telopeptide of type I collagen (CTX) in bisphosphonate induced osteonecrosis model

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Bisphosphonate group drugs are currently being used in many diseases associated with disorders of bone metabolism. But, these drugs result in osteonecrosis even with minor trauma in jaw bones having high bone turnover. The purpose of the study was experimentally to investigate the efficacy of stem cells for preventing the osteonecrosis caused by zoledronic acid.

Trials were carried out on 38 rats in 6 groups. These groups were planned as; "simply tooth extraction", "bisphosphonate application and tooth extraction", "dental pulp originated mesenchymal stem cell (MSC) therapy together with tooth extraction", "dental pulp sourced MSCs one day before the dental extraction", "bone marrow-derived MSC treatment together with tooth extraction" and "bone marrow derived MSC treatment a day before the tooth extraction". Bisphosphonate model was formed in rats through intraperitoneal injection of zoledronic acid in 3 doses per week throughout 60 days. Bone-specific alkaline phosphatase (BALP) and terminal cross-linked telopeptide of type I collagen (CTX) were biochemically evaluated.

Flow cytometric characterization indicated the expression of studied markers in bone marrow and dental pulp originated MSCs. CTX value in all MSC applied groups were significantly higher compared to only bisphosphonate treated group with the highest average value in "bone marrow-derived MSC treatment together with tooth extraction" group. High CTX biomarker value is an indication of increased bone turnover. The decreased value of markers in control group increased again in experimental groups due to enhancing effect of stem cells on turnover.

In conclusion, the clinical and biochemical aspects of MSCs significantly enhanced bone healing. It was concluded that bisphosphonate dependent development of osteonecrosis could be prevented through administration of MSCs. However, to elucidate the case better, comprehensive researches should be carried out with various other doses and sources of MSCs.

ST-05.03.3-020
The effects of hypoxia on cancer stem cells in mouse neuroblastoma cell line

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Neuroblastoma (NB) is the most common extracranial solid tumor in childhood. Malignant NB cells have been shown to possess cancer stem cell (CSC) characteristics. Decreased oxygenation (hypoxia) is a well-known feature of solid tumors and is a key prognostic factor for tumor progression and poor clinical outcome. A number of studies have reported that hypoxia has potential to regulate tumor cell differentiation thereby facilitating maintenance of CSC characteristics that can lead to malignancy. This study aimed to investigate hypoxic effect on cancer stem cells in neuroblastoma cell line.

The mouse neuroblastoma cells (NA2B) were cultured in DMEMF-12 and divided into two groups. For hypoxic condition group; cells were exposed to 3% O2, 92% N2, 5% CO2 gas mixture to create 3% hypoxic condition for 36 h in a hypoxia chamber. For control group; cells were incubated under normal culture conditions in humidified atmosphere at 37°C in 5% CO2. After 36 h incubation cells fixed in 4% paraformaldehyde and were stained with indirect immunoperoxidase technique in order to determine distributions of CD133, Oct-4 and Ki-67. Results evaluated by H-SCORE in comparison with One-Way ANOVA statistical test.

In hypoxic and control groups CD133 immunoreactivity was very strong but positive stained cells were more in hypoxic group when compare to control group. Ki-67 immunoreactivity was moderate in hypoxic group while it was weak in control group. Oct-4 immunoreactivity was strong in hypoxic group while it was moderate and positive stained cells were less in control group.

Hypoxia is an important environmental factor that regulates cell differentiation of many stem and progenitor cells. In our study we demonstrated that hypoxia can induce proliferation and stemness of cancer stem cells with triggered expression of CD133, Oct-4 and Ki-67. High malignancy potential of NB can be correlated with the cancer stem cells which are induced with the hypoxic microenvironment of the tumor.

ST-05.03.3-022
Effects of stem cell-based therapeutic approaches in wound healing in a burn wound modelof STZ-diabetic rats

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Introduction: Diabetes mellitus is a chronic metabolic disease and is one of the major causes of chronic wound healing problems where in severe cases, it leads to amputations. Therefore, in order to provide rapid treatments to these patientsit is useful to evaluate the effects of current regenerative medical approacheson burn models of diabetic rats.

Material and Methods: Rat adipose and bone marrow tissues were excised and mesenchymal stem cells (MSCs) were isolated, cultured and characterized.Adipose tissue-MSCs (AD-SCs), bone marrow MSCs, and keratinocytes differentiated from AD-SCs

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were injected to the thermal 2nd degree burn wound models of streptozotocine (STZ)-induced diabetic rats. The effect of cell applications in wound healing process was evaluated by measuringwound healing markers (MCP-1, Collagen-1, EGF, TGFB-1, FGF-2 and VEGF) immunohistochemicallyin the biopsy samples taken from burn woundsof diabetic rats on 3rd, 7th, 10th and 14th days following the cell-based treatments.

Results: There was a time-dependent decrease in MCP-1 expression butincreasesin expressions of Collagen-1 and growth factors (EGF, TGFB-1, FGF-2 andVEGF) in all cell-based treated groups whencompared to untreated diabetic control rats. Among cell therapies keratinocytes were found to be the most effective.

Discussion: Growth factors (GFs) and collagen-I play an important role in wound healing process however their decrease in diabetes are associated with delayed wound healing problems. MSCs and keratinocytesmay have a critical role in wound healing process by secreting GFs, decreasing inflammatory phase and preventing regression of wound healing process leading to complications related to chronic wounds.

Conclusion: Cell-based therapies are all effective and would be proposed in clinical trials of diabetic wounds/burns in order to achieve rapid and proper wound healing with less scar formation.

Miscellaneous

ST-Mis-004
Whole-cell matrix-assisted laser desorption/ ionization mass spectrometry for rapid detection of enterocin A and B

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Bacteriocins are ribosomally synthesized antimicrobial peptides killing the strains of the same species or closely related species by formation of pores on cytoplasmic membranes which cause dissipation of proton motive force. The classical procedure for detecting and characterizing bacteriocins relies on evaluation of their inhibitory activity against a panel of indicator organisms and their purification to homogeneity. Yet all these time-consuming methods result in identification of already known bacteriocins. Matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) appears to have potential to detect bacteriocins in culture fluids based on molecular masses of previously identifed bacteriocins. Its application directly to the whole bacterial cells to obtain mass spectral fingerprints allows determination of cell surface peptides. In this study, we investigated the utility of MALDI-TOF-MS analysis to detect mass spectral fingerprints of enterocins adsorbed on the whole cells of *Enterococcus faecium* strains. Samples were prepared on MALDI-TOF plate by mixing the matrix solution with prespotted pinhead amounts of colonies, which were taken from different *E. faecium* strains grown on MRS plates at 37°C for 48 h. Mass spectra were acquired on a Voyager-DE™ PRO MALDI-TOF mass spectrometer equipped with a nitrogen UV-Laser operating at 337 nm. The method was capable of detecting enterocin A, B and P from whole cells. MALDI-TOF-MS detection of enterocin A, B and P from bacterial colonies is more efficient than MALDI-TOF-MS detection of them from culture supernatants, which can be explained by restricted diffusion and hence accumulation of bacteriocins on solid media as compared to liquid cultures. Furthermore, different incubation temperatures of cells affected intensity of enterocin signals. Once the colonies appear on agar surface, only a few minutes is required to prepare the sample and perform MALDI-TOF-MS analysis.

MicroRNAs and noncoding RNAs

ST-01.03.3-004
Determination of miRNAs of usnic acid lichen seconder metabolite using high-throughput technology

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Breast cancer is the most common type of cancer diagnosed in women. Since breast cancer has heterogeneous molecular structure a common treatment strategy for breast cancer is not available and most of the patients develop resistance through the course of treatment. Usnic acid (UA) that is one of the secondary metabolites of lichens has been used for different purposes in the field of medicine and cosmetics and its antiproliferative effect has been shown in some of the cancer types which makes it a potential treatment molecule. *Usnic acid* was isolated from the lichens and determination of antiproliferative effect in breast cancer cells (the MDA-MB-231, BT-20, MCF7, BT-474, SK-BR3) and normal cell (MCF12A) was performed by MTT analysis. Cells treated with the effective concentration of UA were conducted to microarray analysis to profile UA-responsive miRNAs. After bioinformatics analysis cancer cell specific miRNAs responsive to UA were identified. Their targets and the pathways they are taking part in were determined by using a miRNA target prediction tool; DIANA Tools. Microarray experiments showed that 56 miRNAs were specifically responsive to UA in MDA-MB-231 cells while it was 9 for BT-474 and 7 for MCF-7 cells. The targets of the miRNAs were found to be enriched commonly in hedgehog signaling pathway. TGF-Beta, MAPK and apoptosis pathways were also the prominent ones according to enrichment analysis. The pathway enrichment analysis results conducted with the UA-responsive miRNAs together with the antiproliferative effects of UA may make UA as one of the potential alternative therapeutics for treatment of breast cancer.

This study will be the first study in the literature by aiming to find out the molecular mechanism of usnic acid in miRNA level by revealing the differentially expressed miRNAs, their targets and the molecular pathways that may have roles in the breast cancer.

ST-01.03.3-005
Differentially expressed miRNAs in the G-CSF primed donor mesenchymal stem cells

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Background: In bone marrow transplantation (BMT) when sufficient number of cells can not be obtained from healthy donors, the granulocyte colony stimulating factor (G-CSF) can be applied. Following G-CSF treatment generally mild side effects are observed, but more severe side effects such as cancer, and splenic rupture have also been reported in healthy donors.Studies investigating G-CSF effects including miRNAs are limited and mainly focusing on hematopoietic cells.

Aim: We aimed to investigate the effects of G-CSF on the miRNA expression of BM derived mesenchymal stem cells (BMMSCs). MSCs are important part of the stroma supporting hematopoietic system.
Materials and Methods: Regarding the previous project microarray data, we classified miRNAs as G-CSF primed ($n = 5$) and non-primed ($n = 8$), then analyzed using Partek and Affymetrix Tac platform.Expression of the most significantly changed 8 miRNAs in G-CSF primed BM-MSCs were quantified by real time PCR (qPCR) relative to the G-CSF nonprimed BMMSCs. Potential shared targets of selected miRNAs were predicted using the bioinformatics databases such as Targetscan and Diana-Lab.-Following pathway analysis, three predicted target mRNA levels were investigated using qPCR.
Results: Data analysis revealed 58 differentially expressed non-coding RNA genes.Among 8 of these were investigated for validation and only miR-1275 level was found significantly decreased in the G-CSF group. miR-638, miR-1908-5p, miR-149-3p expression levels also tend to decreased. Among the predicted target genes, *SIKE1* expression was higher in the G-CSF primed donor MSCs than G-CSF non-primed donor cells while expression levels of *TRAF6* and *NUMB* were lower.
Discussion and Conclusion: Our study represents the first report of G-CSF on human healthy donors BMMSCs at the miRNA level. miRNA-1275 seems to target many of genes involved in several biological process. Aberrant miRNA-1275 in the BMMSCs may be clinical and biological effects which need to be further investigated.

ST-01.03.3-006
RNA sequencing (RNA-SEQ) reveals microRNA signatures involving in human T helper 17 cells differentiation

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Recently discovered IL-17 expressing T helper 17 cells (Th17) are known to play a critical role in various conditions including Multiple sclerosis and cancer. They also mediate immune response to bacterial and fungal infections. Factors of Th17 differentiation in human are still under investigation. It is proposed that microRNAs (miRNAs), are 17- to 23-nt RNA molecules, in addition to cytokines and transcription factors can also play a role in T helper differentiation. Therefore, the roles of microRNA's in Th17 differentiation is need to be identified.

In this study, naive CD4+ T cells were purified from human peripheral blood mononuclear cells (PBMC). Purified naïve T cells were cultured with various stimulants (IL-6, IL-23, IL-1 beta, TGF-beta, anti-IL-4, anti-IFN gamma, anti-CD3 and anti-CD28). Culture medium was changed on day 5th and IL-23 was added to cultures. At day 7, cultures were stopped and Th17 effector cells were measured by flow cytometry. Total RNA from Th17 positive and negative control cells were isolated by Trizol method. To profile microRNAs that play a role in Th17 differentiation, the RNA samples were isolated from Th17-positive and negative control cells. Illumina MiSeq small RNA platform was used to analyze microRNA profiles.

The results showed that naive CD4+ T cells were activated in the Th17-polarizing conditions (CD25+ T cells was 47%, $P < 0.05$). CD25 positive CD4 T cells were expressed Th17 phenotype signature cytokine IL-17 (33%, $P < 0.05$) and IL-17 expression was increased by the time. Bioinformatics data analysis showed that when Th17-positive cells compared to negative cells (at least 5-fold difference in expression), there were 41 microRNAs which were differentially expressed. 12 of those microRNAs were down regulated and 29 microRNAs were up-regulated. Our results demonstrate a key role for microRNAs in differentiation of T helper 17 cells.
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Cell cycle and circadian clocks

ST-02.10.4-001
E2F1 transcription factor is post-translationally regulated by Protein Kinase A (PKA)

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E2F1 is one of the most important transcription factors in cell cycle regulation.Its activity can be regulated by post-translational modifications, particularly phosphorylation.Although E2F1 bears 3 putative PKA phosphorylation sites, whether these are genuine substrates for PKA and if so, the details of E2F1 regulation by PKA remain to be determined.

PKA phosphorylation of E2F1 was shown by reciprocal co-IP and in vitro kinase reaction.Putative PKA phosphorylation sites were site-directionally mutated to alanine (A) or glutamic acid (E) on E2F1 plasmid, with which 293 cells were stably transfected.To elucidate how PKA activation influences E2F1 levels, E2F1 overexpressing cells were treated with forskolin or PKA inhibitor (PKI) for different time intervals.The impact of E2F1 mutants on cell proliferation was assessed by MTT and Ki67 staining, the expression levels of E2F1 target genes were measured by western blot and qPCR.Proliferation of LNCaP, PC3, 293T and H1299 cells transiently transfected with E2F1 mutants was evaluated by MTT assay.Cell-based glucose uptake was explored in 293 and LNCaP cells.

According to our results, E2F1 levels decrease after 8–16 h of forskolin treatment, yet return to normal at the 24th hour of treatment.Presence of PKI or lactacystine abrogates forskolin's effect.E2F1-PKA interaction was augmented by forskolin treatment, resulting in E2F1 phosphorylation.Overexpression of A mutants leads to an increase in proliferation rate, while E mutants cause a decrease in proliferation but increase in caspase 3 activation.In both LNCaP and 293 cells, proliferation rates correlate with glucose uptake.As expected, E2F1 mutants cause varying degree of difference in the expression levels of E2F1 targets.

In sum, E2F1 is phosphorylated by PKA and this results in E2F1 degradation.Since abrogation of this phosphorylation leads to an increase in cell proliferation and glucose uptake; phosphorylation of E2F1 by PKA seems to play an important role in cell fate determination.

Miscellaneous

ST-Mis-005
The role of ATP-sensitive potassium channels in the vasodilatory effect of N-acetylcysteine

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N-acetyl cysteine's (NAC) anti-inflammatory, antioxidant and vasodilator effects are well known. However, explaining the mechanism of the effects are not sufficient. In this study, on the vasodilatory effect of NAC, role of ATP-sensitive potassium (K_{ATP}) channel was investigated by using electrophysiological and molecular genetics methodologies.

In this study, aorta smooth muscle cell lines were used. One control and three dose groups were studied. The control group were not exposed any treatment. The dose groups were treated with 2, 5 and 10 mM NAC respectively, using the cellattached patch clamp K_{ATP} channel currents were measured for 10 min. Intracellular

calcium levels in the same groups has been monitored using confocal laser scanning microscope by taking images at 488 nm wavelength with 15 s intervals for 10 min. K_{ATP} channel gene expression levels were assayed using real-time quantitative reverse transcription polymerase chain reaction. K_{ATP} channel gene expression (Kcnj8, Kcnj11, Abcc8, Abcc9) levels for each group were determined.

K_{ATP} channel flow was significantly increased in all dose groups compared to the control group. However, significant differences were not found among dose groups. Although, intracellular calcium concentrations relative to control group did not change in 2 and 5 mM groups, significant reduction was observed in 10 mM group. Compared to control group, Kcnj8 gene expression levels of 2 and 10 mM groups observed to be decreased, while there was not a significant difference between groups in the proportion of Kcnj11 expression. Abcc8 gene expression rate decreased in only 2 mM group relative to control group. While, in the 2 and 10 mM groups, compared to the control group, Abcc9 expression levels were significantly increased, no significant change were observed in 5 mM group.

Results obtained in this study suggested that, NAC's vasodilatory effect may be associated with K_{ATP} channels. This relationship has been showed for the first time.

ST-Mis-006
Effect of endoplasmic reticulum stress on phosphorylation/O-GlcNAc modifications in insulin signalling pathway in obesity and insulin resistance

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Obesity, is a growing global public health problem. Excess fat storage in adipocytes leading to release of increased amounts of non-esterified fatty acids, glycerol, hormones, cytokines. The factors that are involved in the development of insulin resistance. Accumulating evidence suggests that endoplasmic reticulum (ER) stress is present in type 2 diabetes. Protein glycosylation enzymes controls polypeptide folding and many intracellular mechanisms in the ER and cytoplasm. Glycosylation is a necessary modification for determination of protein structure, function and stability. Cytoplasmic and nuclear proteins are modified by a single O-GlcNAc moiety at serine or threonine residues, termed O-GlcNAcylation. This modification has been demonstrated to play critical roles in numerous biological processes, including cell signaling, transcription, and disease etiology and regulated in response to nutrients, stress, and other extracellular stimuli. Altough the role of O-GlcNAcylation in insulin signaling and endoplasmic reticulum stress in liver, skeletal muscle are well established; the relationship between O-GlcNAcylation and adipose tissue is largely unkown.

We determined role of O-GlcNAcylation in genetically obese mice and wild type mice challenged with glucosamine (GlcN). Insulin signalling pathway, hexosamine biosynthetic pathway and endoplasmic reticulum stress markers were investigated in adipose tissues.

In increased insulin resistance conditions it has been shown that O-GlcNAcylation levels are decreased in adipose tissue of obese mice. Then we confirmed GlcN challenged group has a similar phenotype for insulin resistance and ER stress.

Our results suggest that O-GlcNAcylation of proteins in obese mice regulated by an end product of the hexosamine biosynthetic pathway uridine diphosphate N-acetylglucosamine (UDP-GlcNAc) inhibition. Our findings imply that O-GlcNAcylation has an important role for development of type 2 diabetes and metabolic syndrome in obesity.

Monday 5 September
17:30–19:30, Hall B

Miscellaneous

ST-Mis-008
A promising novel antidiabetic compound: Alysine-A

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Although insulin, as well as oral hypoglycemic agents such as sulfonylureas and biguanides, is still the most important agents in treating the DM, studies searching for a more efficient anti-diabetic agents are being carried out continuously. Based on that we aim to investigate the potential anti-diabetic effects of Alysine A that is isolated from Teucrium alyssifolium.

Alysine A exerted the following effects at submicromolar concentrations as none cytotoxic doses:

1 Increased glucose uptake at least at the level of insulin action in T3-L1 adipocytes, C2C12 and Chang liver cell
2 Increased the glycogen content in C2C12 and Chang liver cell much more than the insulin and metformin levels
3 Suppressed the alpha-glucosidase and the GLUT2 expression levels in Caco-2 cells
4 Suppressed the SGLT1 (sodium-glucose transporter 1) and GLUT1-5 expression levels in Caco-2 cells
5 Induced the expression levels of IRS-1 and GLUT2 in BTC6 pancreatic cells
6 Induced the expression levels of INSR, IRS-2, PI3K, GLUT4, AKR, PK, G6P in 3T3-L1 and C2C12 cells
7 Increased the glucose transport through Caco-2 cell layer

8 Did not affect insulin secretion in pancreatic BTC6 cells

As a result, these data strongly support the anti-diabetic action of Alysine A on the particularly important model mechanism that plays a role in glucose homeostases such as glucose uptake, use, and storage. Furthermore, Alysine A was found to affect the expression level of the critical genes in glucose metabolism and insulin signaling pathway so that the results would be anti-diabetic. Alisine A has shown significant and positive outcomes on the mechanisms in glucose homeostasis that it is a natural and pleiotropic anti-diabetic agent.

A combination of in-vitro and in-situ tests confirmed the anti-hyperglycemic activity of Alysine A and its mechanism. Further in vivo studies are is required to elucidate fully the effect this compound on the glucose homeostasis.

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ST-09.04.4-013
Association between hepcidin levels and iron metabolites, inflammatory cytokines and oxidative stress in patients with missed abortion

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The aim of this study was to determine the association between hepcidin levels and iron metabolites, inflammatory cytokines and oxidative stress in patients with missed abortion.

We studied healthy volunteers ($n = 27$) pregnant women that they were included in the study as control group. Hemoglobin and C reactive protein levels were measured in blood samples taken from the pregnant patient that Missed Abortion ($n = 60$) been diagnosed in the hospital. Serum hepcidin, total free iron, ferritin, transferrin, transferrin receptor, tumor necrosis factor alpha, interleukin-6 glutathione, malondialdehyde levels were measured in the research laboratory. Serum protein levels were measured. Serum superoxide dismutase activity was measured. The measurements were made with the enzyme-linked immunosorbent assay method.

Hepcidin, superoxide dismutase, glutathione, malondialdehyde, tumor necrosis factor alpha, interleukin-6, total free iron, ferritin, transferrin, transferrin receptor results were significantly different in patients from the healthy control group ($p < 0.05$). C Reactive Protein values between patients and control group significant difference was found, but there was no significant difference in hemoglobin values ($P > 0.05$). Ferritin, glutathione, superoxide dismutase and haemoglobin levels were negatively correlated with the hormone hepcidin. Malondialdehyde, C-reactive protein, transferrin receptor, transferrin, free iron levels were negatively correlated with the hormone hepcidin.

The results indicate that it might be the role of inflammatory cytokines, iron metabolism and the products of oxidative stress in the pathogenesis of missed abortion. Thus, we believe that the serum hepcidin levels might be related to iron homeostasis, inflammatory cytokines, and oxidative stress in patients with missed abortions.

ST-09.04.4-014
The beneficial effects of *Nigella sativa* oil on redox homeostasis in experimental multiple sclerosis model

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Multiple sclerosis (MS) is a chronic demyelinating disorder of the central nervous system (CNS) that has inflammatory and degenerative components with neuronal and axonal loss. Oxidative stress, which is a situation of distortion of balance between reactive oxygen species (ROS) and antioxidants, is thought to promote neuronal cell damage in MS. Recently, several compounds have been suggested as antioxidative treatment approaches, many with ambiguous clinical success. Studies have demonstrated that crude extracts of *Nigella sativa* (NS) seeds and some of it's active constituents, especially thymoquinone (TQ), might have protective effect against CNS diseases. The aim of this study was to investigate NS oil's (NSO) therapeutic effects on ROS damage in cuprizone (CPZ) induced MS model where rats were fed with CPZ, leading to oligodendrocyte death and a subsequent reversible demyelination. Wistar Albino rats were randomly divided into three groups and maintained for 5 weeks: Control group (1% carboxy methyl cellulose/day (CMC), i.g., $n = 6$); MS group (CMC + CPZ 70 $mL \cdot kg^{-1} \cdot day^{-1}$, i.g., $n = 7$); MS + NSO group (200 $mg \cdot kg^{-1} \cdot day^{-1}$ NSO, i.g., $n = 7$). Demyelinization was shown histopathologically in the brain tissue samples of the rats and TBARS levels indicating the oxidative membrane damage and PCO levels indicating protein oxidation and SOD enzyme activity and FRAP levels evaluating the antioxidant status were also determined. TBARS levels were found to be significantly higher in MS group when compared with the control and MS + NSO group ($P < 0.05$, $P < 0.05$), yet, no significant difference was seen in PCO levels. Both SOD activity and FRAP levels were found lower in MS group and higher in MS + NSO group than the control group ($P < 0.05$, $P < 0.05$). NSO seems to decrease oxidative membrane damage and ameliorate the depleted antioxidant status in MS group. The potential relevance of NSO in clinical management of MS can be suggested, yet further studies would be useful carried out also with its components such as TQ.

ST-09.04.4-015
Fluorescent probes for detection of oxidative stress-induced carbonylation in live cancer cells

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Oxidative stress is known as the steady state level of oxidative damage in a cell, tissue, or organ, caused by the reactive oxygen species such as free radicals. It is often detected in the progression of a disease such as cancer, neurodegenerative diseases and diabetes. Oxidative stress-induced carbonylation of biomolecules (e.g. proteins, lipids and DNA) in live cells can be monitored via fluorescence spectroscopy. Here, we describe a simple and fast synthesis of amine-based fluorophores for detecting cellular aldehydes and ketones in different cancer cells lines. Hydrazine or amine based fluorophores act as strong nucleophiles that react rapidly with carbonyl sections

in live cells. The reaction of aldehydes with hydrazines (or amines) is an effective very well-known bioorthogonal conjugation technique to be used in live cells at physiological conditions. We therefore develop hydrazine or amine containing fluorophores that undergo a spectroscopic change upon hydrazone formation. Although designed fluorophores do react with aldehydes at neutral pH, the reaction is very challenging. In this work we show the design and spectroscopic characterization of each fluorophore for detection of oxidative stress-induced carbonylation in cellular events. These fluorophores can be particularly useful for detecting cellular carbonylation by using bioorthogonal chemistry in biological systems.

ST-09.04.4-016
The protective effect of thymoquinone against olanzapine-induced metabolic adverse effects in liver

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Background: Olanzapine (OLZ) is the one of first line antipsychotic drug for schizophrenia and other serious mental illness. However, it is associated with troublesome metabolic side effects, particularly body weight gain and obesity. Thymoquinone (TQ), which has antioxidant activities, scavenging oxygen free radicals. TQ, could neutralize the negative influences of various damaging agents on liver tissue.
Introduction: The aim of this study was to investigate the possible protective effect of TQ, an antioxidant which is known to have liver protective effects, against side effects of OLZ in the liver. Accordingly, we aimed to investigate the side effects of OLZ, liver preventive effects of TQ in experimental animal model.
Materials and Methods: Thirty five female Sprague Dawley rats were divided into five groups as follows: group 1, control; group 2, OLZ; group 3, TQ-1 + OLZ; group 4, TQ-2 + OLZ; group 5, TQ-3 + OLZ. On treatment day 15, all rats were humanely killed, then blood and liver tissues were removed.
Results: The results showed that 2 weeks administration of OLZ (4 $mg{\cdot}kg^{-1}$, once a day for the first week, 8 $mg{\cdot}kg^{-1}$ once a day for the second week, p.o.) and treatment of TQ (25, 50, 100 $mg{\cdot}kg^{-1}$, once daily, p.o.) reduced weight gain induced by OLZ. The elevation of serum marker enzymes induced by OLZ was inhibited by the treatment with TQ. Our observations also showed that daily treatment with TQ increased antioxidant capacity. TQ treatment found to have an ameliorating effect on side effects of OLZ in the liver, according to histopathological and biochemical findings.
Conclusion: These results indicated that TQ improved the side effects of OLZ, reduced weight gain, contributed to the oxygen radical scavenging activity, increased antioxidant activity and had ameliorative effects on recovery of increased serum marker enzymes. Thus, these results indicate that TQ has protective and antioxidant effects against side effects of olanzapine in liver of rats.

ST-09.04.4-017
Oxidative stress in rats during exposure to cigarette smoke and after smoking cessation

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Introduction: The components of cigarette smoke lead to endothelial dysfunction by increasing reactive oxygen radicals in vessels. In this study, we aimed to evaluate changes in tissue catalase and MDA levels as well as assessing differences in tissue histopathology following passive smoking in healthy subjects, while also investigating any improvement in catalase and MDA levels after smoking cessation.
Materials and Methods: A total of 40 rats were used and they were split into 5 groups: (1) Control group ($n = 8$), (2) Group II, rats exposed to cigarette smoke ($n = 8$), (3) Group III, rats that discontinued smoking for one month ($n = 8$), (4) Group IV, rats that discontinued smoking for three months ($n = 8$), (5) Group V, rats that discontinued smoking for five months ($n = 8$). MDA and catalase ativities were evaluated in tissue samples that were collected from rats in these groups.
Results: In rats who were exposed to cigarette smoke for 8 months (Group II), cardiac tissue showed higher MDA levels as compared to control group (Group I) and these elevated levels were still present and statistically significant in Groups III and IV. Group V exhibited lower MDA level than that of the control group (p
Discussion and Conclusion: In conclusion, as a result of exposure to passive cigarette smoking, we found decreased hepatic and renal catalase activity, elevated splenic catalase activity, and higher MDA levels in cardiac tissue. Histopathological analysis revealed that the primary organ affected by this change was the lung, and while smoking cessation for 1 month did not affect the rising catalase and MDA levels, both were observed to return to the normal values after quitting smoking.

ST-09.04.4-018
Fustin-, sulfuretin- and gallotannin-rich infusions from leaves and heartwood of the Eurasian smoke tree (*Cotinus coggygria* Scop.) demonstrate antioxidant and anti-inflammatory effects in rats

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Decoctions from Smoke tree (*Cotinus coggygria*), well known as poisonous plant, are used topically by the Balkan and Anatolian folk medicine for their antiseptic properties. We studied phytochemical composition and biological effects of aqueous infusion from leaves (AICCL) and ethanol infusion from heartwood (EICCW) in rat models of oxidative stress and inflammation after internal administration.

Phytochemical analyses were done on UHPLC-ESI/ToF/MS and HPCCC. Models were 3: Paracetamol (P)-induced liver toxicity (PILT), Indomethacin (IN)-induced gastric ulcerogenesis (INIGU), and Carrageenan (Carr)-induced rat paw oedema (CIRPO). Eight groups of Male Wistar rats ($n = 64$; 200–250 g) were used in PILT: Water control (C), P, 1/100 AICCL + P, 2/100 AICCL + P, 4/100 AICCL + P, Ethanol control (Et), Et + P, 1/1000 EICCW + P. Similar 8 groups were used in INIGU and CIRPO (IN or Carr instead of P). Rats were orally pretreated with AICCL or EICCW (10 $mL{\cdot}kg^{-1}$) by intragastric gavage. Groups C and P/IN/Carr received water. Groups Et and Et + P/IN/Carr received 20% Ethanol. The pretreatment lasted 7 days in PILT, 3 days in INIGU, and 15 days in CIRPO. Antioxidant effects were evaluated by biochemical and histopathological methods. Anti-inflammatory effect was evaluated by Digital plethysmometer LE7500.

Major components were gallotannins and gallic acid (AICCL), fustin and sulfuretin (EICCW). Liver enzymes were significantly

lower in 1/100 AICCL + P, compared to P. The hepatic parenchyma of 1/100 AICCL + P was without necrosis. 2/100 AICCL significantly decrease malondialdehyde levels and ulcerosis. The average Paw oedema volume in 2/100 AICCL+ Carr was significantly lower, compared to Carr.

Our study demonstrated the hepatoprotective effect of 1/100 AICCL, gastroprotective effect of 2/100 AICCL and EICCW, and anti-inflammatory effect of 2/100 AICCL. The most probable mechanism of these beneficial effects is decrease of lipid peroxidation, due to the in vivo antioxidant properties of the infusions.

ST-09.04.4-022
Oxidative status in patients with Crimean-Congo hemorrhagic fever

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Background: Crimean-Congo Hemorrhagic Fever (CCHF) is caused by the tick-borne CCHF virus and has a reported fatality rate of 3–30%. The known target cells of the CCHF virus include monocytes, endothelial cells and hepatocytes. The pathogenesis of the disease has yet to be fully explained. The purpose of this study was to determine the levels of the oxidative stress markers total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI) in patients with CCHF and to reveal the effect of oxidative stress in the pathogenesis.
Method: Ninety patients and 41 controls were included in the study. Serum specimens were collected at time of and 6 months after hospitalization. TAS and TOS levels were studied, and the OSI was calculated using the formula OSI = TOS (μmol H_2O_2 Eq/l)/TAS (mmol Trolox Eq/l) × 10.
Results: Patient and control demographic characteristics were similar. Fever was present in 87.8% of patients, lethargy in 96.7%, nausea in 62.2%, myalgia in 60.0% and hemorrhaging in 14.4%. Thrombocytopenia developed in 85% of patients during hospitalization and leukopenia in 90%, although only six patients required platelet transfusion. No mortality occurred. Mean TAS level was higher than in the controls in the acute and monitoring periods ($P = 0.030$, $P = 0.023$). Mean TOS level was lower than in the controls in the acute period ($P = 0.002$), but no significant difference was determined during the monitoring period ($P = 0.054$). Mean OSI level was lower than in the controls in the acute and monitoring periods ($P = 0.002$, $P = 0.003$). No statistically significant relation was observed in TAS or OSI levels between the acute and monitoring periods, while TOS levels decreased during monitoring ($P = 0.035$).
Conclusion: The results from this study suggest that although a high TAS level and a low TOS level may appear paradoxical, the absence of mortality and severe picture in patients' clinical courses suggest that antioxidant elevation may have a positive effect on prognosis.

Education, training, and career planning in molecular life sciences

ST-Edu-002
How the use of active learning strategies improved students' interest and performance for an introductive biochemistry course in large classrooms

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We will present a three academic years transformation of our teaching practices for an introductive biochemistry course, with 160-students groups at the beginning of a biology curriculum. The principle in French education is that universities should be open to all who graduate from secondary school but not all of the students are motivated for high education studies and perhaps even less attracted by biochemistry. The purpose was to rectify the low level of interest and performance of the students. Indeed, only 2–3% of them had validated the module during the academic year 2013–2014.

First, we used the constructive alignment principle, refined the syllabus and re-drafted the teaching program to introduce active learning and an organization of the activities that promotes the participation of all the students and help their understanding. After the intended learning outcomes were defined we have gradually adapted the contents of the courses and included interactivity. We also created teaching resources available through the university intranet, namely mini-videos on difficult aspects of the course, links to web sites of interest, documents, overview tables, and sets of multiple choice questions in order to complete the classroom based work. The use of clicker-questions for formative assessment for large student groups completed the teaching approach during the current academic year.

A quantitative and qualitative methodology was implemented, showing that the success rate was significantly improved – 18% (2014–2015) and 28% (after the 1st session 2015–2016) – and the pedagogical approach greatly appreciated. The students became much more regular in class and motivated. Concerning the use of clickers-questions it is still too early to fully conclude on their usefulness but the improved class attendance, the active involvement of the students and the better results to summative assessments are altogether a strong motivation for teaching stuff to continue the efforts.

Autophagy: Regulation mechanisms

ST-02.03.3-004
Upregulation of transmembrane transcription factor, ATF6 and cAMP response element-binding protein 3, CREB3 gene expression levels by ruxolitinib leads to ER stress-induced autophagy in CML

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Chronic myeloid leukemia (CML) is a malignant disorder of the haematopoietic stem cell characterized by BCR/ABL oncogene. BCR/ABL forms hematopoietic cells independent from exogenous growth-stimulatory signals by engaging signaling pathways such as JAK-STAT signaling.

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Ruxolitinib is a small-molecule JAK-1/2 inhibitor, was approved by FDA. Endoplasmic reticulum (ER) stress is a potent inducer of autophagy.

In this study we aimed to evaluate the effects of ruxolitinib on the signaling pathways genes which are linking ER stress to autophagy in chronic myeloid leukemia cell line, K562 cells and possibilities for their clinical exploitation.

K562 cells were treated with ruxolitinib time and dose dependent manner and cytotoxicity was evaluated by using WST-1 assay. Autophagic effects of ruxolitinib were detected by measuring LC3B-II protein formation. The RT-qPCR is used for gene expression analysis. Gene expression levels were evaluated by using RT2 Profiler PCR Array.

IC50 value of ruxolitinib for K-562 was determined 20 μM at the 48th hour. Ruxolitinib enhanced autophagic cell death 2.11 fold in K-562 cell line, according to control group. Significant increases in ATF6 (transmembrane transcription factor) and CERB3 (cAMP response element-binding protein 3) gene expression levels were observed in K562 treated with ruxolitinib. Ruxolitinib upregulated ATF6 and CERB3 gene expression levels 8.6 and 4.4 folds according to the control cell line, respectively.

Our findings showed that downregulation of JAK/STAT pathway which is downstream of BCR-ABL genes by ruxolitinib leads to enhance of ER stress-induced autophagy in CML. K562 cells could have autophagic cell death because the cells could not remain the ER expansion. ER stress-associated increases in ATF6 and CREB3 could activate autophagy via JAK/STAT associated pathways such as AMPK and mTOR. Therefore targeting ER stress-induced autophagy genes might be a new strategy for CML therapy.

ST-02.03.3-005
Modulation of autophagy by MSM in K562 cells

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Organic sulfur containing compound methylsulfonylmethane (MSM) has been widely marketed and used as a dietary supplement for treating or preventing osteoarthritis. MSM has been shown to protect cells against oxidative stress and inflammation and act as an apoptotic or anti-apoptotic agent in different cell models. Recently, we reported that MSM induced significant growth inhibition in K562 cells. In this study, the involvement of apoptotic and autophagic pathways in the proliferation modulatory effects of MSM were analysed. Results of this study showed that MSM may modulate apoptosis and autophagy in K562 leukemia cells.

ST-02.03.3-006
Autophagic cell death features of periviseral fat body remodeling of greater wax moth *Galleria mellonella* during larval-pupal transformation

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Autophagy is a cellular pathway responsible for the degradation of proteins and organelles. This process is involved in the maintenance of cell homeostasis but also intervenes in the remodeling of larval tissues and organs in holometabolous insects. The fat body is a dynamic tissue involved in multiple metabolic functions. It undergoes a great change called remodelling during metamorphosis. The larval fat body clusters dissociates into individual cells and removed through programmed cell death under hormonal factors. Pupal fat body cells originating from primordial cells or remaining larval fat body cells reorganize and clump. We investigated the autophagic process in the fat body of *Galleria mellonella* during remodeling period by using morphological, biochemical and gene expressions analysis methods. General histological structure were demonstrated by using Hematoxylen-Eosin, carbohydrate contents were observed by using Periodic acid- Schiff and localization of acid phosphatase activity were analyzed by using Gomori (1950). TEM results showed the presence of cytoplasmic budding, giant autophagic vacuoles, the low numbers of mitochondria and protein granules of heterophagic origin during pupal period.

Lysosomal acid phosphatase enzyme activities were also measured by spectrophometrically and expression levels of autophagy related genes,-ATG 6 and ATG 8-, were analyzed by using qRT-PCR. It is found that during the feeding period the volume of the cells continuously increases. Participation of autophagy in remodeling of periviseral fat body has been also confirmed by the elevated level of the marker enzyme, acid phosphatase, and increasing expression levels of ATG 6 and ATG 8 mRNA. In conclusion, this study provides for the first time direct cellular, molecular, and biochemical evidence of the involvement of autophagy in the periviseral fat body remodeling process during larval–adult transformation of *Galleria mellonella*.

ST-02.03.3-007
The role of STAMP2 in the regulation of autophagy in prostate cancer cells

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Autophagy is a lysosomal degradation pathway which maintains cellular homeostasis in the face of stresses, such as nutrient deprivation and hypoxia. Androgen signaling, the central proliferative pathway in prostate cancer (PCa) cells, has been previously shown to inhibit autophagic activity, while androgen deprivation activates autophagy which confers resistance to androgen ablation therapies and contribute to disease progression. The six transmembrane protein of the prostate 2 (STAMP2), a protein whose expression is androgen regulated, has previously been implicated in PCa as well as metabolic diseases. Here we have investigated the role of STAMP2 in autophagy. The core autophagy protein LC3 becomes conjugated to phosphatidylethanolamine (now called LC3-II) upon induction of autophagy and therefore serves as a marker of the pathway. The depletion of STAMP2 in PCa cell line LNCaP led to an accumulation of LC3-II and increased LC3 puncta formation, while the ectopic expression of STAMP2 decreased LC3-II amounts. In contrast, there were no consistent changes in long lived protein degradation assayed by a modified pulse chase protocol, suggesting that STAMP2 might be involved in a different form of specific autophagy. To understand the molecular mechanisms governing the changes in autophagy observed, the phosphorylation events of AMPK-mTOR-ULK1 was investigated. Autophagy inducing AMPK-mediated phosphorylation of ULK1 at S555 was found to be increased upon STAMP2 depletion. In contrast, the mTOR-dependent p70S6K phosphorylation was also increased upon STAMP2 knockdown, indicating increased activation of mTOR, which represses autophagy. These observations suggest that STAMP2 may act as a negative regulator of the autophagic process, and may affect, at least in part, the androgen mediated repression of autophagy.

Personalized medicine

ST-08.02.5-005
Graphene-based nanoparticles: a novel nano-drug for cancer

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Graphene-based nanoparticles (GBN) may have a potential to be used as a nano-drug due to their unique physico-chemical characteristics including easy functionalization and good biocompatibility, large surface to volume ratio, strong light absorbing properties and relatively simple preparation. The aim of this study was to investigate the cytotoxic effect of GBN on the oral cavity cancer cell lines (UPCI-SCC-131).

GO was synthesized from the graphite powder with a method of Hummers. This method is based on oxidation of graphite powder in the presence of oxidants and acids. UPCI-SCC-131 cell lines were maintained in MEM at 37°C with 5%CO_2. Cells were seeded at 1×10^4 cells/well into 96-E plates and exposed to cisplatin (10 mM) and GBN at various concentrations (100, 50, 25, 20, 10 and 5 mg·mL^{-1}). Cell viability was monitored by x-CELLigence RTCA DP system for 48 h. The percentage of cell viability was calculated by the ratio of cell index of control cells to cisplatin and nano-drug applied cells. Moreover, cell cycle distribution was analysed by flow cytometry.

Our results demonstrated that G-COOH had no cytotoxic effect at all concentrations; however proliferative effect was determined at concentrations of 100, 10 and 5 mg·mL^{-1}. G-COOH-PEG had cytotoxic effect at 100 and 50 mg·mL^{-1}; on the other hand, proliferative effect was detected at 20, 10 and 5 mg·mL^{-1}. GO was not cytotoxic up to 100 mg·mL^{-1}, however, it had proliferative effect at 50, 25, 20, 10 and 5 mg·mL^{-1}. In consistent with this data, cell cycle distribution supported cell viability outcomes.

It was reported that both GO and rGO showed dose and time dependent cell viability. In addition, surface modification of GO may lead to a significant reduction in cytotoxicity. Our results obviously demonstrated that G-COOH was not cytotoxic irrespective of its concentration. On the other hand, G-COOH-PEG and GO may have a potential to be used as a nano-drug for the treatment of oral cavity cancers.

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ST-08.02.5-006
Pre-analytical and post-analytical phase in therapeutic drug monitoring

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The aim of the present study is to evalute the main errors in the pre-analytical and post analytical phase in therapeutic drug monitoring (TDM) in clinical laboratories.

TDM is used to guide the optimal dose of the drug for the patients, monitor the patients's compliance to the given drug, to investigate the drug–drug interactions and to monitor the decontamination in toxicity. In pre-analytical phase, we will describe the most important factors that affect TDM, compare the advantages and disadvantages of different sample types on TDM, recommended specimen, evaluate the importance of blood sampling time, sample stabiltity and the sample matrix effects.

In post-analytical phase we will discuss the importance of the panic ranges in TDM, the clinical availability of free drug monitoring, individual differences, drug-drug interferences, advers drug effects, and differents factors like diet, cigarette smoking, exercise affecting the drug effects in the body. TDM is an multidisciplinary work flow in the hospitals involving the doctors, nurses, phlebotomists, clinical chemists, pharmacologists.

In conclusion, it is important to educate the clinicians, nurses and the phlebotomists on pre-analytical phase and post analytical phase errors to avoid the possible errors in TDM.

ST-08.02.5-007
Preclinical characterization of a novel palladium complex as anticancer drug candidate

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A new cationic palladium (II) complex (C20 H26 CIN5 O4 Pd) exhibited growth inhibitory effect at low micromolar concentrations in various cancer cell lines including breast, colon, prostate and lung. This study aimed to evaluate the potency of this complex for being an anticancer drug candidate by investigating its safety, biodistribution, drug transporter interactions and tumor efficacy. The safety and biodistribution profile was assessed after administering mice with various doses of the complex in wild-type mice intravenously, orally or intraperitoneally. In order to test the interactions of the complex with the drug efflux and uptake transporters, MDCKII and HEK293 cell lines overexpressing various drug transporters and knockout mice for certain drug transporters were used. Tumor efficacy of the complex was evaluated using xenograft mouse models. In vivo pharmacokinetic studies showed that Pd (II) complex has a very low oral bioavailability and tends to be retained in organs for a long time once distributed after oral and intravenous administrations. In vitro cytotoxicity assays using MDCKII cell lines expressing various ABC transporters suggested that Pd (II) complex is a transported substrate of MRP2, and to a lesser extent of MDR1 and BCRP. Cellular uptake assays using HEK293 cell lines expressing OATP proteins showed that Pd(II) complex was specifically transported by OATP1A2, but not by OATP1B1, -1B3 or -2B1. Based on the in vitro results, we are currently assessing in vivo interactions of this complex with the selected drug transporters and its in vivo tumor efficacy in various xenograft tumor models. In conclusion, Pd (II) complex is a promising anti-cancer drug candidate, and its preclinical profile somewhat resembles platinum drug properties, maybe with a better efficacy in certain tumors.

ST-08.02.5-008
The contribution of elevated foetal haemoglobin levels to a relatively mild clinical phenotype in Turkish Cypriot patients with β-thalassaemia

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Symptomatic forms of β-thalassaemia, a monogenic disorder caused by the reduced/absent synthesis of the β-globin chains of haemoglobin, fall into two categories according to their transfusion dependence: β-thalassaemia intermedia (TI) and β-thalassaemia major (TM). It is often proposed that interpatient variation in postnatal foetal haemoglobin (HbF) levels is one of the main modifiers of disease severity between these two clinically significant phenotypes.

This cross-sectional analytical study was performed on 72 β-thalassaemic patients who were diagnosed with either TI ($n = 27$) or TM ($n = 45$) by a haematologist. 13 patients with TI were on hydroxyurea treatment at the time of the study. The relative percentage of HbF was determined by haemoglobin electrophoresis using cellulose acetate at alkaline pH and subsequent scanning densitometry. Serum ferritin levels and the number of transfusions, which were previously measured at routine visits during 2015, were also included in the study for purposes of testing for association with HbF levels.

The Kaplan–Meier estimator revealed that the mean HbF levels of patients with TI and patients with TM were 22.1 (95% CI: 12.37–26.25) and 4.67 (95% CI: 3.71–5.63), respectively. The significance of this difference was confirmed by a Peto–Prentice test (two-sided $P < 0.0001$). Kendall's tau correlation showed that HbF levels were negatively related to the total number of transfusions in β-thalassaemic patients ($\tau = -0.414$, $P < 0.0001$). There was no interrelationship between HbF levels and averaged (*i.e.* steady-state) serum ferritin levels.

Although persistent/induced HbF production is unlikely to be an indicator of the efficacy of iron chelation therapy in Turkish Cypriot patients with β-thalassaemia, high levels of HbF appear to ameliorate the severity of the clinical phenotype in the same population of patients, possibly through decreasing transfusion requirements. HbF thus represents a *bona fide* target for personalised medicine.

Monday 5 September
17:30–19:30, Hall C

Mechanism of neurodegenerative diseases

ST-09.02.2-008
The useful effect of β-glucan in a C57BL/J6 mouse model that has oxidative and neuronal damage caused by global cerebral ischemia/reperfusion

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Stroke is among the most common causes of death and disability in developing countries. Beta-glucans (βg), that have many useful effects on the human health, are natural polysaccharides. Our aim in this study was to determine useful effect of β-glucan opposite to oxidative and neuronal damage caused by global cerebral ischemia/reperfusion (IR) in stroke imitated mice via surgical operation.

In total 40 mice used in this study. The subjects divided four equal groups randomly. The groups were named group 1 (sham-operated (SH)), group 2 (I/R), group 3 (βg) and group 4 (I/R + βg). The group 1 (SH) was kept as control. The bilateral carotid arteries of subjects in group 2 (I/R) and group 4(I/R + βg) were clipped for 15 min and then while the mice in group 4 (I/R + βg) were treated with βg (50 $mg \cdot kg^{-1} \cdot day^{-1}$), the mice in group 2 (I/R) were treated with only vehicle for 10 days. The mice of group 3 (βg) were treated with βg for 10 days without carotid occlusion.

Global cerebral I/R significantly increased oxidative stress (thiobarbituric acid reactive substances (TBARS) level) and decreased members of anti-oxidant defense system (levels of reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx)). In addition, global cerebral I/R raised histopathological damage end apoptosis in brain tissue. However βg treatment ameliorated both oxidative and histopathological ill effects of global cerebral I/R.

Our present study showed that βg treatment significantly ameliorated oxidative and histological damage in the brain tissue caused by global cerebral I/R. The useful effects of βg probably result of its antioxidant, anti-inflammatory and immunomodulatory properties. Therefore βg treatment can be supportive care for ischemic stroke patients.

ST-09.02.2-010
Structural and functional properties of myelin basic protein charge isomers

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Myelin basic protein (MBP) is one of the candidate autoantigens of the human inflammatory demyelinating disease multiple sclerosis (MS). The charge effects modulate MBP functions and may play an important role in pathogenesis of MS. Macrophages are important effector cells involved in the pathogenesis of demyelination. Upon activation, they secrete a plethora of pro-inflammatory mediators, such as cytokines, nitric oxide and glutamate, which are able to induce tissue damage.

The association of myelin basic protein charge isomers and its peptides with the lipid part of the myelin was investigated at the molecular level in a model membrane system, using optical waveguide lightmode spectrometry to determine the kinetics of association and dissociation to planar phospholipid membranes under controlled hydrodynamic conditions and over a range of protein concentrations. To study how mGlur5 is involved in macrophage/MBP isomers interactions we use mGlur5-transfected and non-transfected Mouse RAW 264.7 macrophages.

We have shown that deiminated (citrulinated) MBP is less effectively adsorbed on the myelin natural lipid membrane, than MBP other isomers. Phosphorylation not only affects electrostatic charge, but also affects protein conformational stability. The transfected macrophages by action of MBP C1 (the most cationic) and C8 (citrulinated) isomers released more NO, than nontransfected cells. The amount of secreted high mobility group box 1 proteins (HMGB1) also are increased in transfected cells. mGlur5 transfection has influence on the secretion IL-10. MBP charge isomers increase the expression of excitatory amino acid

transporter-2 (EAAT2) and intracellular glutamate concentration in transfected macrophages.

In summary, our results suggest that heavy citrulination will tend to produce demyelination. mGlur 5 plays an important role in the realisation of MBP charge isomer functions.

ST-09.02.2-011
Dysregulation of calcium homeostasis and signalling in cultured hippocampal neurones from young rodent models of Alzheimer's disease (3xTg-AD mouse & TgF344-AD rat)

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Amyloid beta-linked calcium dysregulation may play a key role in Alzheimer's disease (AD) onset and progression. We have studied how the endoplasmic reticulum (ER) functions in both maintaining calcium homeostasis and mediating intracellular signalling processes, with a specific emphasis on how these functions may be disrupted in AD.

Cultured hippocampal neurons were prepared from control and transgenic 3xTg-AD mice and TgF344-AD rats between 3–6 days old. Intracellular calcium signals were determined following loading with fluo-2 AM (150 μM). A particular calcium loading protocol was adopted which involved pre-loading the ER with Ca^{2+} (using a depolarising stimulus; extracellular application of 15 mM K^+) followed by application of a specific group 1 metabotropic glutamate receptor agonist (I-mGluR), (S)-3,5-dihydroxyphenylglycine (S-DHPG; 50 μM). Such conditions are thought to crudely mimic a 'learning event'. Data, unless otherwise stated, were analysed using Wilcoxon matched-pairs signed rank test and expressed as mean ± SEM

In control neurons, from both murine models, I-mGluR activation combined with the loading stimulus, evoked enhanced somatic Ca^{2+} signals relative to I-mGluR activation alone (mouse model, 679 ± 128%, $P = 0.0013$, $n = 47$; rat model, 6948 ± 1821%, $P = <0.0001$, $n = 46$). In contrast, we did not observe enhanced responses in transgenic neurons (mouse model, 21 ± 14% *reduction*, $P = 0.0006$, $n = 36$; rat model, $P = 0.6482$, $n = 28$). Secondly, we observed significantly larger responses to I-mGluR activation alone, in transgenic neurons compared with control neurons (mouse model, $P = 0.0013$; $n = 36$; rat model, $P = 0.0044$, $n = 28$ unpaired t test), suggesting a pathological increase in ER calcium levels.

The fact that such stark alterations in calcium homeostasis and signalling have been observed in neurons from rodent models of AD at such a young age (<6 days), suggests that calcium dysregulation may occur at a much earlier stage in the disease progression than previously thought.

ST-09.02.2-012
Effect of fasudil on the expression of apoptotic proteins in amyloid-beta induced PC12 cells

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Alzheimer's disease (AD) is a neurodegenerative disorder that accounts for the majority of dementia cases. Amyloid-beta (Aβ) peptide accumulation and tau protein hyperphosphorylation are the basic pathologic changes in AD brain. Rho-kinase plays role in various processes of the central nervous system (CNS) such as neuronal survival and neurite growth. Although Rho-kinase signaling pathway is known to be involved in the pathogenesis of many CNS disorders including AD, the effect of Rho-kinase inhibitors on hyperphosphorylation of tau in AD has not yet been studied extensively. In this study, we investigated the effect of fasudil, a Rho-kinase inhibitor, on the expression levels of proteins related with cell survival in AD model cells using Western-blot.

PC12 cell line was treated with Aβ25-35 peptide to induce tau hyperphosphorylation. Controls and Aβ25-35 induced cells were both treated with fasudil. After cell lysis, proteins in soluble fractions were separated by SDS-PAGE and then transferred on membrane for antibody detection by chemiluminescence.

Treatment with Aβ25-35 peptide led to significant increase in the cellular levels of Bcl-2, Bax, total Tau, β-amyloid, pTau-S404 and pTau-T231 proteins in comparison to controls. Upon treatment with fasudil, the levels of Bcl-2, Bax and pTau-T231 were decreased back to control levels. We also observed slight decreases in the levels of total Tau, β-amyloid and pTau-S404 proteins with fasudil treatment.

The increase in the levels of Bcl-2 and Bax upon Aβ25-35 induction confirms the role of apoptotic pathway in Aβ induced neurotoxicity as described previously. Our results show that Rho-kinase inhibitor fasudil can prevent this apoptotic effect of Aβ induced tau hyperphosphorylation by lowering Bax and Bcl-2 back to normal levels. This suggests that fasudil has a protective effect in Aβ induced cell death and can be a promising agent for AD treatment.

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ST-09.02.2-013
Knockdown of interleukin-10 induces the redistribution of sigma1-receptor and increases the glutamate-dependent NADPH-oxidase activity in mouse brain neurons

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In the central nervous system, interleukin-10 provides trophic and survival effects directly on neurons, modulates neurite

plasticity, and has a pivotal importance in the neuronal regeneration in neurodegenerative and neuroinflammatory conditions. This cytokine is primarily produced by glial cells and has beneficial effects on the neuronal viability. However, the mechanisms of IL-10-elicited neuroprotection are not clear.

Membrane preparations, isolated from wild type and IL-10 knockout mice brain were used in this study. It has been shown that compared to wild-type mice, in IL-10 KO mice brain, the amount of Immunoglobulin Binding *Protein* (BiP) is greatly increased, whereas the content of sigma receptor (SigR1) is not changed significantly. Coimmunoprecipitation experiments have shown that the association of SigR1 with small GTPase Rac, NR2B subunit of NMDA-receptor (NMDAR) and inositol-3-phosphate receptor (IP3R) is higher in the IL-10 KO mice brain than in the Wt mice brain. Besides, we have found that either glutamate or sigma ligands, separately or together, do not change glutamate-induced NADPH-oxidase (NOX) activity in Wt-type mice brain membrane preparations, whereas in IL-10 KO mice high concentration of glutamate markedly increases the NOX-dependent production of reactive oxygen species (ROS). Glutamate-dependent ROS synthesis was decreased to the normal levels by the action of sigma-agonists.

It has been concluded that IL-10 deprivation, at least in part, can lead to the induction of ER-stress, which cause BiP expression of and SigR1 redistribution between components of endoplasmic reticulum (ER) and plasma membrane. Moreover, IL-10 deficiency can change the specific organization of NMDAR, increasing the expression of SigR1-sensitive NR2B subunits. In the IL-10 deficient conditions, glutamate-dependent ROS production, as well as the release of Ca^{2+} from ER are greatly increased leading to the initiation of apoptosis of neurons.

ST-09.02.2-014
Amino-terminal wild type huntingtin as a protein folding aid – A function unexplored

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Huntington's disease (HD) is caused due to expansion of CAG repeat [encoding polyglutamine (polyQ)] in IT-15 gene, which encodes huntingtin (htt). Aggregation of mutant huntingtin (mhtt) is modulated by 17 amino acids N-terminal to the polyQ tract (N17), especially L4, L7 and M8 residues. The function of wild type huntingtin (whtt) is relatively less understood. We have shown that N-terminal-whtt inhibits protein aggregation in vitro and in vivo (Under Review). The aim of this study was to investigate if the anti-aggregation property of whtt is modulated by the N17 region. Mhtt with different polyQ lengths (51Q, 103Q) were expressed in *E. coli*, purified and their aggregation was monitored in the presence of N-whtt. Three amino acids of N17 region were mutated (L4A, L7A, M8A) and aggregation of luciferase was investigated using a published assay. Molecular modeling studies were done to understand the effect of mutations on structural properties of N-whtt. N-whtt showed a polyQ length-dependent (51Q and 103Q) inhibition of aggregation of mhtt, which correlated well with our earlier data. Effect of mutations in N17 region of N-whtt was monitored by aggregation of luciferase. While L7A and M8A showed the same inhibitory effect as the wild-type, L4A mutant was unable to inhibit aggregation of luciferase. Computational studies showed that while other constructs adopted a helical conformation, this ordered arrangement was disrupted in case of L4A and its structural stability was compromised. This may be responsible for the loss of activity of N-whtt. Our results establish a clear role of the N17 region of N-whtt in maintaining its structural and functional integrity. Due to lack of sequence homology, the functions of whtt are difficult to predict. C-terminal whtt may be involved in autophagy. Our results show that N-whtt, formed naturally in the brain as a result of proteolytic cleavage of the full length protein, may be a component of the cellular proteostasis network.

Miscellaneous

ST-Mis-010
Tyrosine phosphorylation of tumor necrosis factor receptor-1 (TNFR1) by Janus Kinase 2 (JAK2) influences TNFR1 signalosome

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TNFR1, upon binding TNF, can induce activation of NF-κB pathway (Complex1) or apoptosis (Complex2).Besides, TNF is known to activate ERK, p38, JNK and Akt pathways. Although TNF mediated apoptosis and NF-kappaB signaling is extensively studied, the signalosome activating ERK, p38, JNK and Akt pathways is not characterized. TNFR1 is known to interact with JAK2 and thus, we hypothesized that, TNFR1 can be tyrosine (Tyr) phosphorylated, triggering the activation of proliferative or stress-induced pathways.

In vitro kinase reaction was performed to demonstrate JAK2 mediated TNFR1 phosphorylation.Two putative Tyr phosphorylation sites (Y360 and Y401) were substituted with alanine (A) or aspartic acid (D) by site directed mutagenesis.Mutated or wild type TNFR1 were transfected into 293T cells.The effect on ERK, JNK, p38, Akt, and Stat3 activation was determined by western blot. TNFR1 interaction with c-Src, p85, Stat3, Grb2, JAK2, FADD, TRADD and RIP were investigated by co-IP. NF-κB activity was measured by luciferase assay and Stat3 DNA binding activity was determined by EMSA. MTT and colorimetric caspase 8/3 assays were used for measurement of proliferation and apoptosis.

JAK2 and TNFR1 interact with each other in a TNF dependent manner, resulting in TNFR1 Tyr phosphorylation. Y360A and Y401D mutants led to activation of Stat3, Akt and ERK pathways, while the opposite was observed for JNK pathway.These mutants improved the interaction of TNFR1 with c-Src, p85 and Stat3 but Grb2 binding was not influenced. NF-κB activation correlated with p85-TNFR1 interaction.Combined phosphorylation of both Y360 and Y401 leads to maximum p38 activation while combined inhibiton of both phosphorylations results in increased apoptosis and FADD-TNFR1 interaction.

In sum, TNFR1 is Tyr phosphorylated by JAK2. Phosphorylation of Y401 augments TNFR1 mediated proliferative pathways as well as complex1 formation, while inhibiton of phosphorylation at both Y360 and Y401 resulted in increased complex 2 formation and apoptosis.

Computational biology

ST-03.02.2-005
Design of novel uracil derivatives as inhibitors of carbonic anhydrases I & II, acetylcholinesterase, butyrylcholinesterase, and glutathione reductase using *in silico*, synthesis and *in vitro* studies

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The classical high-throughput screening methods have proven to be very costly and have had limited success in finding new drug leads. The industry appears to be suffering from innovation costs and would benefit from new, more rational approaches to drug design which exploit the recent advances made in molecular and structural biology and computational chemistry. Computational chemistry provides a range of simulation tools for description of protein-ligand binding, statistical methods for analysis of the binding data that help to predict the optimal ligands, and molecular modeling tools that enable construction of novel ligands. Crystal structures of many enzymes have been determined during the last decade. This has opened a broad avenue of experimental and computational studies that aim to obtain a better understanding of the structure-function relations in these enzymes. On the computational side, rapid developments in high performance computing and computational methodologies have enabled accurate molecular-level simulations of biomolecules and their interactions with ligands. This is particularly important for the design of drugs targeting these enzymes because computational studies can help to relate the functional data on binding of specific drugs to the molecular structure of a target protein. Finding such ligands that bind with high affinity could lead to therapeutic interventions that modulate the function of an enzyme as desired. The aim of this inter-disciplinary study is, thus, to investigate the molecular mechanisms and energetics of novel uracil derivatives as inhibitors of different enzymes (Carbonic Anhydrase I, II, Acetylcholinesterase, Butyrylcholinesterase, and Glutathione Reductase Enzymes) using computational methods (i.e., molecular docking and molecular dynamics (MD) simulations). Proposed uracil derivatives by computational methods are synthesized and *in vitro* tests were then performed for validation of predictions of molecular simulations.

ST-03.02.2-006
Design of an efficient device for measurements of iron binding kinetics to *Haemophilus influenzea* ferric binding protein

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Iron is an important metabolite for all the organisms. Its ability to carry oxygen makes it crucial for mammalian cells, whereas in pathogenic bacteria, ferric ions are fundamental for growth and virulence. Humans can receive iron from nutrients, while bacteria have evolved special mechanisms to sequester iron from the environment. Haemophilus influenzae have developed a distinctive mechanism to hijack iron from human transferrin that involves using the Ferric Binding Protein (hFBP) in shuttling iron from the outer to the inner membrane in the periplasmic space.

In this study, hFBP was expressed and purified by recombinant DNA technology. Apo and holo hFBP were characterized by circular dichroism, small angle x-ray scattering and dynamic light scattering. A continuous-flow microfluidic device was built to monitor iron binding dynamics of hFBP that occurs on the time scale of milliseconds. Several microfluidic chips housing an effective mixing component were designed and fabricated by using either PMMA or PDMS as the material. The reaction is monitored in the micro channels with a high-resolution camera and analyzed by a program plotting color intensities at different points along the channel. To determine binding kinetic constants, the color change of the reaction was related with absorbance values and single exponential decay curves were obtained from which characteristic times were derived.

The different devices are assessed in terms of efficiency of mixing and precision of the measurements. A best design is proposed to use in determining the differences in the iron binding kinetics to hFBP at various ionic strength and/or pH values. While previous measurements using a stopped-flow device were made at a single pH/ionic strength value, the new set-up paves the way for fast and reliable relative affinity prediction under different conditions for a protein such as hFBP that survives a range of environmental conditions.

ST-03.02.2-007
Investigating the role of alternative polyadenylation in lung squamous cell carcinoma

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Polyadenylation is an RNA processing step that involves the cleavage of pre-mRNA at a poly(A) site and addition of the poly (A) tail. Approximately 70% of human genes contain multiple poly(A) sites and can undergo alternative polyadenylation (APA). APA could lead to the production of mRNA isoforms with variable lengths of 3'UTRs. Gain or loss of cis-regulatory regions in 3'UTR isoforms can alter mRNA stability and translation dramatically. In particular, a strong association has been found between proliferation and 3'UTR shortening through the use of proximal polyA sites. Previous studies that explored the effects of 3'UTR shortening have mainly focused on the regulatory effects of microRNAs (miRNAs); however, it is well known that 3'UTRs also harbor sites for several RNA-binding proteins (RBPs). In this study, we developed a computational model that incorporates APA-related changes in both miRNA and RBP sites. To map miRNA sites, we utilized TargetScan predictions as well as Ago-CLIP-derived peaks. For RBP sites, we scanned the 3'UTRs with RNAcompete motifs, and also included CLIP-derived peaks. Then we used a recently proposed approach called DaPARS to infer proximal and distal APA sites from raw RNA-seq datasets for matched tumor-normal samples in lung squamous cell carcinoma. We identified the RBP and miRNA sites that are lost or gained due to APA and developed a regression model that links these alterations with gene expression changes. Our analysis revealed a strong association between the loss of binding sites and downregulation for a number of RBPs. One of these RBPs is ELAVL1, a well-characterized stabilizing factor. Altogether, these results indicate that future studies of APA must incorporate the regulatory effects of RBPs in addition to miRNAs.

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Plant biochemistry and molecular biology

ST-02.08.5-008
Anti-breast cancer activities of some secondary metabolites isolated from lichen species

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Lichens are known as sophisticated symbiotic organisms with a great association between fungus, algae and/or cyanobacteria. Due to the tight metabolic relationship between these partners, they can produce more than 1000 unique secondary metabolites which potentially have comprehensive biological and pharmacological activities. We, therefore, isolated an anthraquinone (parietin), a fatty acid (caperatic acid) and a depside derivative (barbatolic acid) from the lichen species; *Xanthoria parietina*, *Flavoparmelia caperata* and *Bryoria capilaris*, respectively. Chemical structures of the isolated compounds were identified by performing FTIR, ^{1}H-NMR and melting point analyses and the substances were tested on the breast cancer cell lines; T47D and HCC1428, and healthy human vein endothelial cell line; HUVEC by using alamarBlue cell viability, LDH cellular membrane degradation activity and PicoGreen dsDNA quantitation assays. The performed *in vitro* assays were found in a correlation with each other, and barbatolic acid, as a depside derivative, was found more active than caperatic acid and parietin on the all cell lines. Parietin showed no significant activity. Consequently, the findings suggest that the substances showed no fortissimo anticancer activity on breast cancer cell lines or healthy vein endothelial cell line. However, they might be active on angiogenesis as previously reported or they might be employed for the prolong tumor control due to their low cytotoxic features [1–3].

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ST-02.08.5-009
Determination of the amount of nutshell taxanes for anticancer drug grown in Turkey with the most suitable optimization

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Taxane class compounds are known as the most important anticancer agents. It was first obtained from the bark of the Shell of the Taxus brevifolia Nutt in 1971 in USA. It has two important components as paclitaxel and docetaxel.This material is found in the extract of the barks, and obtaining this material has emerged as a problem due to the rarity of the tree, the growing process of trees is very time consuming and the need to the much shell. In this study, we tried to isolate the taxane components in nuts commonly found in our country with the most suitable method. For this purpose, we evaluated the green and brown shell and leaves of the nut in the acetone, methanol, and ethanol solvents, and determined the ratio of the taxane amounts with the most appropriate method in the most appropriate solvent. In addition to the paclitaxel in the extracts of the nutshells baccatin III, Cephalomannine, Erythromycin, 10-deacetylbaccatin were observed.

ST-02.08.5-010
Biochemical characterization of an essential housekeeping homolog of ClpB/Hsp100

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Plant Hsp101, yeast Hsp104, and *E. coli* ClpB are molecular chaperones that are non-essential, but heat-induced to confer thermotolerance in these organisms. They co-operate with the Hsp70 (DnaK) chaperone system to rescue proteins from an aggregated state. Apart from the cytoplasmic Hsp101, homologues are localized to chloroplasts and mitochondria in plants. It is known that the chloroplast ClpB is 'essential' to chloroplast development. But, the non-cytosolic ClpBs are poorly understood. Cyanobacteria that are thought to be the ancestors of chloroplasts are unusual from heterotrophic bacteria because they have two ClpB paralogs, ClpB1 and ClpB2. ClpB1 is induced and plays an important role under temperature stress. In contrast to ClpB1, ClpB2 is expressed constitutively, and an essential protein. Here, we report that the housekeeping ClpB2 from the cyanobacterium *Synechococcus elongatus* shows distinct biochemical properties from ClpB1.

His-tagged ClpB1, ClpB2, DnaK, DnaJ, and GrpE from *S. elongatus* were over-expressed in *E. coli* and affinity-purified. An NADH-ATP-coupled assay was employed for measuring ATPase activity. Aggregation of heat-denatured MDH/LDH was analyzed by monitoring the increase in the apparent absorbance at 360 nm.

It is known that ClpB/Hsp104 forms a hexamer that is a functional form for the chaperone. ClpB1 formed a hexamer/heptamer, whereas ClpB2 did not. The ATPase activity of ClpB2 was 10 times as low as that of ClpB1. ClpB1 showed the chaperone activity to solubilize protein aggregates in collaboration with the DnaK/DnaJ/GrpE chaperone system, whereas ClpB2 did not and rather inhibited the disaggregation reaction. In contrast to *E. coli* ClpB/Hsp104, both cyanobacterial ClpBs showed the activity to prevent aggregation of heat-denatured proteins.

The different biochemical characteristics of ClpB1 and ClpB2 may underlie the distinct cellular functions of these two chaperones and may give us some hints to reveal functions of non-cytosolic ClpBs.

ST-02.08.5-011
Investigating role of miR319 genes on chilling stress during maize leaf growth

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Maize (*Zea mays* L.) is a chilling sensitive crop plant that requires relatively high temperature for optimum growth and yield. MicroRNAs (miRNAs), a 19–25 nucleotide long class of noncoding endogenous small RNAs, have been proved to play versatile roles in plants in processes such as growth, development

and stress tolerance. Although chilling-responsive and growth-regulating miRNAs have been detected in several plant species, little is known about their roles in maize. miR319 is a conserved miRNA that targets TCP transcription factors regulating several developmental processes. Maize leaves are a useful model for growth studies thanks to their spatial gradient of growth zones, which are meristem, elongation and mature zones (from leaf base to tip). This study aims to identify the role of miR319 on the chilling response and growth regulation in maize.

Maize seedlings were grown under low night and optimum temperatures for stress and control conditions respectively. To observe stress effects on maize seedlings, the analyses were conducted at morphological, cellular and transcriptional levels on the fourth leaves of seedlings. At the morphological level, the leaf length was measured every day and leaf elongation rate was calculated. The leaf area was determined by an image analyzer when the leaf had fully matured. To observe the chilling effect at the cellular level, samples from the mature zone of leaves were collected and cell length measured under the microscope. To determine whether cell proliferation was influenced by chilling, cell production was calculated by a kinematic approach.

We identified four members of miR319 in maize which are miR319a, miR319b, miR319c and miR319d sharing identical maiRNA sequence and targeting TCP5 gene and their expression analysis is ongoing across different growth zones at transcriptional level.

Finally, this is the first study investigating the role of miR319 and TCP5 genes on the chilling and growth responses of maize.

ST-02.08.5-012
Bioinformatic analysis and molecular characterization of ethylene-responsive transcription factor *RAP2-12* from olive

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Ethylene-responsive transcription factor *RAP2-12* is associated with ethylene-response, activation of hypoxic gene and hypoxia tolerance of plants. Under normal aerobic conditions it localizes to the plasma membrane but under hypoxia conditions it accumulates in the nucleus. It expresses in leaves, seedlings, seeds and flowers. By far, there is no report describing *RAP2-12* gene in olive (*OeRAP2-12*). In this study, we aimed to gather information about ethylene-responsive transcription factor Oe*RAP2-12*.

Nucleotide BLAST (to find the similarity of genes in different organisms), protein BLAST (BLASTp and BLASTx to find the similarity of proteins in different organisms), BioEdit (to build the predicted protein, to prepare a hydropathy graph, to prepare graphs of amino acid composition and nucleotide composition), Phylogenetic analysis (to make phylogenetic trees from the nucleotide sequence and from the predicted amino acid sequence), ExPASY software (to find isoelectric point and molecular mass of the predicted protein), SWISS-MODEL (to build predicted three-dimensional protein), STRING (to find protein-protein interactions and protein similarities) and Primer3 (to design primers) program were utilized to conduct the analyses.

BLAST analyses revealed *OeRAP2-12* had a diverse sequence among other plants. Amino acid composition analysis revealed OeRAP2-12 has 392 amino acids. Its calculated molecular mass is 43.1 kDa and calculated theoretical isoelectric point (pI) is 4.69. OeRAP2-12 protein is rich in serine and asparagines and therefore presents a hydrophilic character. Primers to amplify the full length *OeRAP2-12* were designed using Primer3 software. Phylogenetic analyses based on nucleotide and amino acid sequences revealed genetic trees that grouped plants based on *OeRAP2-12*. *OeRAP2-12* sequences from different olive cultivars are being obtained to determine its polymorphism among olive cultivars. Analyses and studies about gene's functions are still continuing.

ST-02.08.5-013
Determination of genetic characterization and bioethanol yield of selected maize lines and cultivars

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In this study, the morphological and genetic charecteristics of 10 inbred maize lines and 10 maize cultivars were anlayzed and their bioethanol production were investigated. After harvesting, biomass, weight without corn cob, leaves and stems and height of plants were recorded in the field experiment. Leaves of seedlings were harvested for DNA isolation and PCR (Polymerase chain Reaction) analyses. After maturity, the leaves and stems of harvested corn plants were ground and ethanol yield was determined out from these ground materials through fermentation and enzymatic hydrolysis by using GC-MS. The correlation analysis was performed between the morphological data and ethanol productivity of plants. As a result, a positive correlation between ethanol productivity and plant height and leaf weight were found ($P<0.05$). The cell wall components (glucose, xylose, arabinose, galactose, mannose) affecting the ethanol yield were analysed by using SSR specific genes by polymerase chain reaction (PCR). PCR analyses were determined as a result of the diversity between varieties and lines. The variation was observed 10% among populations, but the variation was 90% within population and this was found to be statistically significant ($P < 0.05$). As a result of this study, it can be said that Kermess plant yielded much higher ethanol production when compared to other corn genotypes. In conclusion, it can be suggested as Kermess plant would be recommended in order to obtain higher ethanol yield.

ST-02.08.5-014
The epithelizan effect of the *Morus nigra* extracts on rats

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Introduction: Several studies have indicated that Morus species show antioxidant, anti-inlammatory, anticancer, antimicrobial and antidiabetic properties Darker Morus fruits, such as Morus nigra, is richer source of phenolic compounds, including

flavonoids, anthocyanins and carotenoids. Morus species are used in the treatment of several illnesses and disfunctions in traditional medicine due to the above mentioned compounds. In this study we aimed at investigating the epithelizan effect of the extract of *Morus nigra* fruits on skin by using rats.

Material and Methods: Ethanolic *Morus nigra* extract analyzed with spectrophotometric and HPLC methods to detect its antioxidant capacity and phenolic compounds content. Three study groups were designed that contain six rats in each group. Surgical applications carried out with bilateral graft method on rat skin under anesthesia. Grafts added on same rats by patching. Wounds in control group were treated with only serum physiologic (SF) while in other groups were treated by including 1% *Morus nigra* extract + Furacin pomade combination and only Furacin pomade (commercially available ephitelizan). Study groups were compared in several days according to various statuses such as vascularization, rate of wound healing and range of scars.

Results: Well known eight polyphenolic compounds were determined in HPLC and TPC was found 36.87 ± 0.36 (mgGAE·g^{-1} powder). We have also found that, 1% *Morus nigra* extract + Furacin pomade combination was most effective on wound healing of donor in rats then other combinations in the 13th day ($P < 0.001$). This combination also had highest wound healing rate among all over donors and patchings in 10th and 13th days.

Conclusion: In this study we suggest that ployphenolic compounds in Morus nigra extract, may stimulate the epidermal regeneration, angiojenezis by using growth factors such as epidermal growth factor and their antioxidant and antimicrobial properties.

Miscellaneous

ST-Mis-011
Construction of a fructose inducible expression vector for a non-pathogenic photosynthetic bacterium *Rhodobacter sphaeroides* to generate high value added products

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Purple non sulfur (PNS) bacteria belonging to the group of photosynthetic bacteria can produce a large number of high value added products like 5-amionolevulinic acid (5-ALA), vitamin B12, coenzyme Q10, poly-β-butyric acid and carotenoids. Contrary to *E. coli* and most of the gram negative bacteria, *R. sphaeroides*' being non-immunogenic increased its usage in many biotechnological processes. Like other bacteria, specifically designed expression vectors are needed to produce high value added products with PNS bacteria. In this context, a fructose inducible expression vector for Rhodobacter sphaeroides was constructed to generate high value added products. Firstly, carefully selected vector parts were combined in silico and then synthesized to form a synthetic construct. The construct includes promoter of fruBKA operon, 6-Histidine tag, stop codons in three reading frame and a strong transcription terminator (BBa_J95029). Then, the construct was ligated to the broad host range vector frame pBBR1MCS2 forming the final construct. There is an NruI (TCG/CGA) recognition site just proceeding ribosome binding site and where the gene of interest could be cloned and expressed. As a proof of concept, the red florescent protein gene was cloned and its expression was tested in *R. sphaeroides*. As a conclusion, a molecular tool towards the development of *Rhodobacter sphaeroides* as a robust and sustainable cell factory was constructed in the context of synthetic biology.

ST-Mis-012
cAMP activated protein kinase (PKA) plays role in regulation of TNF-induced tumor necrosis factor receptor type I (TNFR1) signaling

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Tumor necrosis factor alpha (TNFα) is an inflammatory cytokine regulating proliferation/apoptosis in a wide variety of cells. TNF elicits its biological activities by binding to its Type I (TNFR1) and Type 2 (TNFR2) receptors.Pathways activated by TNFR1 are extensively studied, however, pathways regulating TNFR1 activity remain elusive.Regulation of TNFR1 signaling by cAMP has been previously reported, but the mechanism of this regulation is not known.In the death domain of TNFR1, there are 2 threonine residues (T411 and T417) which fit to PKA consensus motif (RxxT/S).Thus, we reasoned that TNFR1 could be phosphorylated by PKA, regulating its signaling capacity.

After demonstrating PKA-TNFR1 interaction with reciprocal co-IP, we performed in vitro kinase reaction.Then, we site directionally mutated T411 and T417 to alanine (A) and aspartic acid (D), to inhibit and mimic phosphorylation.All the experiments were done in 293T cells transiently transfected with wild type or mutated TNFR1 plasmids.p38, ERK, CREB, and Stat3 activations were determined by western blot.Luciferase assay was used for examining NF-kB activation and EMSA was performed to determine Stat3 DNA binding. TNFR1 shedding was analyzed by ELISA and apoptosis induction was determined by caspase3/8 activation assays.

According to our data, both T411/417A or T411/417D mutants negatively affected TNF-induced p38 activation, while the same mutants increased TNF-induced ERK activation.Although T411 and T417A mutants positively stimulated TNF-induced CREB phosphorylation, T411/417D mutants did not have the same effect.T411A/417A mutants suppressed activation of STAT3 by TNF, but T411D/417D double mutant positively stimulated TNF-induced STAT3 activation.In contrast, T411/417A mutants positively and T411/417D mutants negatively affected TNF-induced NF-kB activation.We observed increased apoptosis in both T411A and T417A mutants.

In conclusion, TNFR1 is phosphorylated by PKA, and this phosphorylation differentially affects TNFR1 signaling.

ST-Mis-013
Cancer gene discovery using a single-copy Sleeping Beauty transposon mouse model

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Large-scale sequencing of whole-cancer genomes has revealed multiple and diverse genetic alterations underlying the carcinogenic process. However, understanding their biological contribution and the interplay between them still represents a major challenge. Forward insertional mutagenesis in mice has already

proven to be a valuable method to identify new genes and signalling pathway interactions in cancer. Here, we developed a novel whole-body transposon mutagenesis screen in mice, specifically designed for the discovery of *Pten*-cooperating tumor suppressor genes, in which the mobilization of a single-copy inactivating *Sleeping Beauty* (SB) transposon is coupled to *Pten* disruption in the same genome. The analysis of 127 transposition-induced prostate cancers identified 116 known and candidate tumor suppressor genes. Functional testing in immortalized human prostate cells validated the synergy of *ZBTB20*, *CELF2*, *PARD3*, *AKAP13* and *WAC* with *PTEN* for preventing invasion in vitro. Evaluation of large patient cohorts revealed that reduced expression of these genes is significantly associated with prostate cancer progression and shorter recurrence free survival, supporting the clinical relevance of these findings. Our approach recapitulates faithfully the sporadic nature of human tumorigenesis, where mutations in relevant cancer genes occur randomly in individual cells from any tissue that are surrounded by healthy cells. Analysis of other cancer types developed in these mice will enable to disentangle the tumour suppressor networks operative in PTEN-deficient cancers and hopefully will pave the way for new combinatorial therapeutic strategies.

Structural biology: Membrane complexes and supercomplexes

ST-03.03.3-003
Lipid-protein interactions in the regulated betaine symporter BetP studied by FTIR spectroscopy

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The secondary active betaine transporter BetP is the major defense system for the gram-positive soil bacterium *Corynebacterium glutamicum* to counteract hyperosmotic stress. To survive, *C. glutamicum* counteracts the high external osmolarity by the activation of osmolyte uptake systems as BetP. BetP imports exclusively betaine into the cytoplasm by co-transport of two Na(+) ions. BetP senses the increasing cytoplasmic K(+) concentration as a measure of hyperosmotic stress *via* the C-terminal domain and regulates transport activity by a yet unknown interaction network with the N-terminal domains and cytoplasmic loops, but also lipids.

In the present study, the lipid-protein interactions of BetP upon K(+) activation were investigated on a molecular level by using FTIR spectroscopy equipped with an ATR accessory to identify specific changes under activating conditions. IR spectroscopy is a perfect tool to selectively probe the perturbations on the hydrophobic lipid tails, the interfacial region or on the polar head groups of lipids by a membrane protein. To investigate BetP in a native-like environment we have reconstituted the transporter in two-dimensional crystals formed from native *C. glutamicum* lipids (1:1:1 PG:PI:Cardiolipin).

K(+)-triggered transport regulation in BetP depends on the presence of anionic lipids. FTIR revealed that activating K(+) ions destabilize protein-lipids interactions in BetP. We observed that under activating conditions phospholipid head groups undergo K(+)-dependent changes in their electrostatic and H-bonding properties favouring weak H-bonds or unbonded head groups while the hydrophobic tails become more mobile and even disordered upon K(+)-induced activation of BetP. Modulation of lipid binding with functionally important domains in BetP is a key player in the regulation mechanism of BetP. With this work, we contribute a dynamic view on regulatory lipid-protein interactions in a secondary transporter.

ST-03.03.3-004
Membrane compartmentalization at the presynaptic active zone of Xenapses – pure presynaptic boutons induced on micropatterned host substrate

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Maintaining of synaptic transmission requires segregation of exo- and endocytic zones. In order to visualize functionally distinct nanodomains by high resolution microscopy we grew hippocampal neurons on microstructured glass coverslips, functionalized with synaptic cell adhesion proteins. Formation of purely presynaptic sites is triggered on this microstructured host substrate, which we call "Xenapses". We found that Xenapses contain several active zones facing the coverslip and hudreds of synaptic vesicles. Our conditions facilitate growth of both vGlut- and vGAT-positive synapses. Experiments with calcium sensors, FM dyes and endogenously expressed pHluorin constracts have shown, that Xenapses respond to stimulation and are functionally normal. Xenapses allow to reconstruct separate nanodomains with high localization percision using single molecule localizational microscopy. TIRF-dSTORM of various CAZ (cytomatrix of active zone) proteins allows to investigate distribution and interaction of proteins during presynapse formation and function. Xenapses form active zones, positive for Bassoon, whereas clathrin predominantly localyzes in periactive zones. Syntaxin clusters are associated with active zones, but not co-localized with Bassoon. We suggest a new model of the presynaptic membrane compartmentalization: Bassoon forms the core of the active zone surrounded by synaptic vesicle fusion machineries; active zones are separatd by endocytic zones.

Monday 5 September 17:30–19:30, Hall D

Host–pathogen interactions

ST-04.01.1-005
Imaging the interactions between oral pathogens and probiotic lactic acid bacteria on the adhesion to human gingival fibroblast cells by confocal and fluorescein microscopy

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Oral pathogens may engage in the formation of dental and periodontal diseases by interacting with one another. Besides,

following the adhesion to the gingival cells, chronic inflammation and tissue destruction may occur. *Porphyromonas gingivalis* (*P. gingivalis*) and *Streptococcus mutans* (*S. mutans*) have been accused of being major pathogens in the formation of periodontitis and caries formation respectively. Recent studies revealed that probiotic lactic acid bacteria could be a candidate in the course of the competitive inhibition of the adhesion of pathogenic bacteria to host cells. The aim of this study is, therefore, to show the competitive inhibitory effect of probiotic *Lactobacillus rhamnosus* (*L. rhamnosus*) ATCC#9595 on the adhesion of *S. mutans* ATCC#25175, and *P. gingivalis* ATCC#33277 to human gingival fibroblast (hGF) cells by fluorescein and confocal microscopy. In this in-vitro study, bacteria were cultured in their specific media and hGF cells were grown on cover glasses in the 24-well cell culture plates under cell culture conditions. Subsequently, the hGF cells were co-cultured with different combinations of *S. mutans, P. gingivalis,* and *L. rhamnosus*. The adhesion capacities of the pathogens were then evaluated by confocal (Syto 24 and nile red stains) and fluorescein microscopy (multiplex staining: Hoechst 33342, propidium iodide, calcein-AM) at different times. The confocal imaging revealed the internalization of *P. gingivalis* into hGF cells at 90 min. Furthermore, the adhesion and internalization of *P. gingivalis* decreased when *L. rhamnosus* was co-cultured with *P. gingivalis*. On the contrary, no significant inhibition effect of *L. rhamnosus* on the adhesion capacity of *S. mutans* was observed. It was concluded that *L. rhamnosus* can be used as an adjunct to clinical therapy of periodontitis.

ST-04.01.1-006
Alteration of cell membrane constituents by dengue virus for successive evasion of immune response

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The increase in MHC class I molecules on cell membrane has been well established in dengue virus infection. Unlike Hepatitis C virus infection, the study on cell membrane co-stimulatory signalling molecules in dengue infection is not well documented. Due to the pivotal role of co-stimulatory signalling for effective activation and expansion of $CD8^+$ T lymphocytes, this study aims to elucidate changes in the abundance of the co-inhibitor PD-L1 signalling protein that may play a role in host-pathogen interactions in DV infection.

Liver HepG2 cells were cultured and infected with whole dengue virus. Western blot and flow cytometry techniques were employed to investigate the cell membrane proteins. The expression of HLA molecules in both experimental sets were monitored as reference.

Results demonstrated simultaneous increase in abundance of PD-L1 protein and HLA molecules in dengue-infected cells. The increase of co-inhibitor PD-L1 protein on cell membrane inhibits the activation of T lymphocytes upon binding to the MHC class I molecules. Furthermore, this up regulation of PD-L1 expression may also outnumber the presence of other co-stimulator molecules. Despite the increase in cell surface antigen presentation, the inhibitory effects of PD-L1 causes T lymphocytes anergy, resulting in ineffective neutralization of dengue-infected cells by these patrolling immune cells.

This study has described the potential modulation of co-signalling molecules by dengue virus to evade the activation of T lymphocytes that are responsible for effective killing of infected cells. Up-regulation of PD-L1 by dengue virus may be one of the mechanisms for successful immune evasion. Additional functional studies on these and other signalling molecules would provide further insights into the host-immune cells interaction in dengue infection.

Mechanisms of pro-inflammatory diseases

ST-04.02.2-005
Serum Gas 6 and omentin levels and relationship with flow mediated dilatation, aortic distensibility, left ventricule mass index and disease severity in patients with psoriasis

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Background: *Growth arrest–specific gene 6 (*GAS6*) encodes a vitamin K–dependent protein regulates inflammation, insülin resistance, angiogenesis, and atherosclerotic plaque formation*. Changing in aortic distensibility (AoD) may reflect the features of the atherosclerotic process. Omentin, a novel adipokine, is considered associated with vascular, metabolic, and various chronic inflammatory diseases. Psoriasis is an autoimmune, inflammatory and chronic disease.
Objectives: This study aimed to determine the association between serum Gas6 and omentin levels with BMI, insulin resistance, flow mediated dilatation, Aortic distensibility and Left ventricule mass index (LVMI) in Patients with Psoriasis
Methods: The study was conducted on 30 healthy subjects and 52 patients with psoriasis. Circulating glucose, cholesterol, insülin, HbA1c and CRP levels analysed and insulin resistance were determined.Gas 6 and omentin were anaysed with ELISA method. Transthoracic echocardiography was used to measure the Beta index, aortic distensibility (AoD), left ventricular mass index. Flow mediated dilation was measured with high resolution ultrasound machine.
Results: Serum Gas6 and Insulin resistance (HOMA-IR) levels were higher ($P < 0.001$) in patient group whereas omentin were lower (p.0.001) comparing with control group. Aort distensibility and beta index were low in patient group (P: 0.01 and P: 0.02 respectively). Also serum Gas 6 levels were significantly correlated with HOMA-IR (P: 0.020, r: 283) and LVMI ($P < 0.01$, r: 689).Disease severity (PASI: psoriasis area and severity index) was not correlated with any parameter in the study.
Conclusions: Circulating Gas6 and omentin levels and aort distensibility and beta index are significantly different in two groups. Serum Gas 6 levels are strongly correlated with insülin resistance status and left ventricule function status. Gas 6 may play a role assessing insülin resistance and left ventricule function status in patients with psoriasis.

ST-04.02.2-006
Behçet's syndrome: the critical balance between the pro-inflammatory and the anti-inflammatory genes

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Initially described in 1937 by the Turkish dermatologist Prof. Hulusi BEHÇET, Behçet's syndrome (BS) is a chronic, relapsing, multisystemic inflammatory disorder. Its predilection for a

characteristic geographic region, the documentation of the significant association between the *HLA-B51/B5* allele and BS, and not infrequent familial occurrence of the syndrome raised the question of a genetic element in the etiopathogenesis of BS. Today, the strong and complex (polygenic) genetic background underlying BS is substantially well documented.

A comprehensive definition of the potential pathogenetic mechanism underlying BS states that "the syndrome results from a pro-inflammatory, innate immune system derived response sustained by acquired immune system responses against environmental and/or self-antigens". With the above definition in hand, the collectively documented and unexceptionally admitted theme in BS pathogenesis is its pro-inflammatory nature. On what grounds this pro-inflammatory condition/milieu develops is a subject of intense research and until recently, upregulation of the pro-inflammatory genes was the mainly emphasized mechanism. Among others, *TNF*, *IL-1*, *IL-6*, *IL-8*, *IL-10*, *IL-12*, *IL-17*, and *IL-18* are the prominent pro-inflammatory cytokine genes with documented associations with BS.

Previously, *MEFV* polymorphisms/mutations have been shown to associate the occurrence and to modify the clinical severity of BS. Recently, there is cumulating data pointing to a significant effect of the anti-inflammatory genes in the immunopathogenesis of BS. Among these, *CLEC12A*, *TNFAIP3*, and *CD69* seem to be of importance. Noteworthily, the heterozygous loss-of-function mutations in *TNFAIP3* gene lead to an early-onset autoinflammatory disease closely resembling BS. This presentation will cover the balance between the pro- and the anti-inflammatory genes in the context of BS and will discuss the potential role of the anti-inflammatory genes in the immunopathogenesis of BS.

Molecular mechanisms of inflammation

ST-04.04.4-004
Protective effect of alpha-linolenic acid on gentamicin induced ototoxicity

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Alpha-Linolenic acid is one of the fatty acids known as omega 3. Previous studies have shown the anti-oxidant and anti-inflammatory effects of this fatty acid. Alpha-Linolenic acid prevented cell damage by inhibiting apoptotic pathway. Also, it is known that gentamicin activates apoptotic mediators and causes necrosis in kidney. Due to this reason, a study is planned to examine the protective effects of alpha-Linolenic acid on gentamicin induced ototoxicity by evaluating inflammation and apoptotic mediators. For this purpose, 100 $mg{\cdot}kg^{-1}$ gentamicin and 200 $mg{\cdot}kg^{-1}$ alpha-Linolenic acid are administered to mice for 9 days. On 9th and 10th days, rotarod performance was assessed to evaluate the effects of gentamicin and alpha-Linolenic acid treatment on motor coordination of mice. On 10th day, cervical dislocation is applied to mice and inner ears are taken out. ELISA is used to evaluate protein expressions. Gentamicin treatment decreased fall latency of mice and gentamicin treatment together with alpha-Linolenic acid treatment increased fall latency of mice. Gentamicin treatment also increased expression of phospholipase A2 (plA2), cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS). Furthermore, it increased expression of proapoptotic proteins bax and kaspase-3 and decreased expression of antiapoptotic protein bcl-2. Gentamicin treatment together with alpha-linolenic acid treatment recovered the change of expression of these enzymes. In conclusion, this study showed that alpha-Linolenic acid could be usefull to prevent gentamicin induced ototoxicity by inhibiting apoptosis and inflammation.

ST-04.04.4-005
Immunosuppressive fibroblasts located around breast tumor tissues are exposed to higher levels of T-HELPER-2-like cytokines

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Tumor cells are not isolated elements; they rather reside in a rich contexture consisting of fibroblasts. Effector T cells play crucial roles in immune responses against tumors. Although effector T cells are known to recruit to the tumor, they are known to demonstrate weaker responses. Tissue fibroblasts have previously been shown to affect T-cell functions. In addition, fibroblasts residing close to tumor tissues may demonstrate immunosuppressive features. Our aim is to investigate the presence and effects of $T_{HELPER}2$-like cytokines on fibroblasts located around tumor tissues. N-Nitroso N-Methylurea (NMU) induced experimental mammary carcinogenesis model was utilized. Tumors were harvested surgically under sterile conditions for fibroblast cell isolation using enzymatic digestion. CD172a (SIRPα) can be used to demonstrate the nature of cytokines present during fibrocyte differentiation. For this reason, CD172a expression of fibroblasts in the tumor microenvrionment was analyzed compared to healthy tissue fibroblasts. In addition, genes associated with $T_{HELPER}2$-like cytokine signaling were analyzed with gene set enrichment analysis in a human microarray study from Gene Expression Omnibus. Flow cytometry experiments showed that fibroblasts around tumor tissues had decreased expressions of CD172a, compared to fibroblasts from healthy tissues ($P = 0.024$). This finding suggests the presence of higher levels of $T_{HELPER}2$-like cytokines in the environment of fibroblasts which are located around breast tumor tissues. In addition, genes associated with $T_{HELPER}2$-like cytokine signaling were upregulated in fibroblasts around tumors (in comparison to healthy tissue fibroblasts). Our results imply a higher $T_{HELPER}2$-like cytokine exposure for the fibroblasts around the tumor. $T_{HELPER}2$-like cytokines might also be responsible from the generation of immunosuppressive features of these fibroblasts. Thus, we plan to further investigate this possible mechanism. No conflicts of interest.

ST-04.04.4-006
The ASC speck as novel antigen carrier

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NLR proteins such as NLRP3, NLRC4 and AIM2 have been shown to form inflammasome complexes together with the adaptor ASC and pro-caspase 1, upon stimulation by their respective pathogen associated molecular pattern stimulants. The inflammasome is a supramolecular spherical structure with a diameter of 2- 5 micrometers and is usually only one per cell. The function of the inflammasome is to activate caspase 1, which then cleaves the pro- 1L beta and IL-18 into their mature and secretable forms. When overexpressed the ASC adaptor can form supramolecular speck structures of its own, called pyroptosomes, which can be isolated. We have shown that the model antigen ovalbumin or fluorescent proteins such as EGFP and mCherry get incorporated into the ASC speck and when purified fusion specks are injected into mice, they lead to persistence of the antigen loaded specks in the animal for more than 3 weeks. We propose that co-aggregation of antigens on the ASC specks or inflammasomes after intracellular infection, results in the loading of ASC specks, which when released can be taken up by the incoming macrophages, such that antigen presentation is more efficient.

Novel signaling pathways controlling the cardiac function

ST-06.01.2-001
Calpain II and calpastatin of living human heart during coronary aortal bypass graft operation

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Calpains play crucial role in cardiac remodelling but also in apoptosis. However, we did not find a hint on calpain or its endogenous inhibitor calpastatin participation in human myocardium during coronary aortal bypass graft operation (CABG).

We tried to elucidate which, if any, changes of both calpain and calpastatin take place during CABG with two cardioplegic fluids: mineral St. Thomas Hospital (SCF) and Bretschneider fluid (BCF).

Small fragments (0.1–0.3 g) of right atrium (SFRA) were taken from myocardium of 24 patients (12 with SCF and 12 with BCF cardioplegy) at three times: (1) just before aorta clamping (control C); (2) just after aorta declamping (maximum cardioplegic ischemia MCI); and (3) after 30 min of reperfusion with patients own blood (R).

Activity of calpain was assayed with iodinated [125I] human serum albumin (IA) as a substrate within samples of homogenate of SFRA with 5 mM $CaCl_2$ (for calpain II + I) or 25 μM $CaCl_2$ (for calpain I) or 1 mM sodium versenate (EDTA) (calcium independent protease CIP). Activity was expressed as cpm of acid soluble products of proteolysis being formed in 1 min with 1 mg of protein.

We found earlier that calpastatin is (for calpain II) high affinity inhibitor. This made possible calculation of concentration of active forms of calpain II and calpastatin separately: using the set of simple equations based on the fact the calpastatin is thermolabile whereas calpains are not.

We found that (both with BCF and SCF cardioplegy) concentration of calpain II did not change significantly from control C to R. Quite differently, calpastatin concentration diminishes during cardioplegic ischemia (up to 40–50% of C) but returns to the control level during reperfusion.

So it seems the conditions favourable for apoptosis exist during cardioplegic ischemia rather than during reperfusion. Calpastatin, but not calpain itself, seems to be the most important factor regulating cell proteolysis and thus the hopeful device to protect myocardium.

Single molecule techniques – Applications in biology

ST-03.04.4-001
Mutation induced allosteric regulation of Rac1-PAK1 binding affinity investigated via molecular simulations and AFM

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Rac1 (Ras-related C3 botulinum toxin substrate 1) is a small member of Rho family of GTPases. Switch I region of Rac1 is responsible for the interaction with the downstream effectors such as PAK1 (p21-activated kinase 1), which is a serine/threonine kinase. Mutational activation of PAK1 by Rac1 has oncogenic signaling effects in cell proliferation, survival, invasion and metastasis. Q61L on Switch II region of Rac1 that is responsible for the regulation of Rac1 activation through GTP/GDP binding at p-loop leads to a constitutively active protein, whereas dominant negative T17N mutation at p-loop presents either a nucleotide free or inactive protein. Y72C on Switch II is an oncogenic mutation with an unknown mechanism.

The intrinsic allosteric dynamic regulation of the wild-type, T17N, Q61L and Y72C mutant Rac1 are studied with respect to PAK1 interaction by the Gaussian Network Model (GNM) and conventional Molecular Dynamics (MD) simulations with the effects of the mutations on the free energy landscape of the PAK1 being further explored and verified via Atomic Force Microscopy (AFM) experiments.

MD simulations reveal that constitutively active Q61L and oncogenic Y72C mutations show similar behavior and Switch II (Q61L) and p-loop (T17N) regions affect Switch I region (PAK binding) allosterically. Association of the positions Q61, T17 and Y72 with the global hinge sites supports the allosteric coupling between the position of these mutations and latter functional regions. AFM results complement the computational observations; such that the unbinding behavior of Q61L and Y72C mutants are similar and make the complex more stable compared to wild-type. Negative mutant (T17N) shows almost 3-fold decrease in the adhesion probability and has approximately non-specific binding behavior indicating the reliability of the experimental setup.

To this end, the present study provides a dynamic perspective for the functional implications of Y72C in oncogenic signaling.

Proteins in action

ST-02.02.2-015
A mechanistic study of the anti-angiogenic properties of the lipid-metabolizing enzyme 15-lipoxygenase-1 in different cancer cell models

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15-lipoxygenase-1 (15-LOX-1) is an enzyme that converts linoleic acid to 13(S)-hydroxyoctadecadienoic acid (13(S)-HODE) and is down regulated in many cancers. Re-expression of the protein in colorectal cancer cells can have tumor suppressive properties including decreased proliferation, increased apoptosis, reduced motility and invasion.

Although much data support the tumor suppressive role of 15-LOX-1, current information regarding 15-LOX-1's role in angiogenesis is limited. In this study we show that re-expression of 15-LOX-1 in colon cancer cell lines HCT-116 and SW480 and prostate cancer cell line LNCaP resulted in reduced expression and secretion of Vascular Endothelial Growth Factor A (VEGF-A), which acts as a potent vascular permeability factor with critical roles during angiogenesis. Reduced VEGF-A expression was caused by decreased transcriptional activity of Hypoxia-Inducible Factor 1α (HIF-1α), under both normoxic and hypoxic conditions.

Human Umbilical Vein Endothelial cells (HUVECs) incubated with conditioned medium (CM) taken from HCT-116 colon cancer cells transfected with the 15-LOX-1 vector showed reduced motility and tube formation compared to controls, along with a

decrease in the expression of the mesenchymal marker vimentin. To investigate 15-LOX-1 mediated signaling pathways during angiogenesis, a "Proteome Profiler Array for Angiogenesis" was utilized using proteins isolated from HUVECs incubated with CM. The data were analyzed using a custom written code in Matlab. We found significantly higher expression of Thrombospondin-1 (TSP-1) in HUVECs incubated with CM from 15-LOX-1 expressing HCT-116 compared to the control cells.

TSP-1 is a matrix glycoprotein that has potent anti-angiogenic roles and potential therapeutic potential. Acting through its receptor CD47, this protein is known to reduce cellular motility. We propose that enhanced TSP-1 expression significantly contributes towards the anti-angiogenic functions of 15-LOX-1.

ST-02.02.2-016
The mystery role of p66Shc adaptor protein in MCF-7 and MDA-MB-231 human breast cancer cells

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Mitochondria play a pivotal role in proper functioning of all living organisms. They are implicated in many processes such as Reactive Oxygen Species (ROS) production as well as apoptosis which in tumor cells seem to be impaired. Interestingly, these processes involve also a small p66Shc adaptor protein which demonstrates the unique prooxidative properties comparing to other ShcA proteins (p46Shc and p52Shc). Phosphorylation of p66Shc protein in Ser36 residue has a substantial impact on mitochondrial metabolism and may cause an inhibition of cell growth propagation and apoptogenic signals. These facts make p66Shc pathway a potential target concerning the cancer proliferation, tumor progression or metabolic reprogramming. Hence, the main aim of our project is focused on studies of p66Shc signaling pathway and its impact on bioenergetics parameters in human breast cancer cell lines and corresponding controls.

To achieve our goal two human breast cancer cell lines (MDA-MB-231 and MCF-7) and corresponding normal control cell lines (AG11137, MCF-10A) have been used. The level of Shc isoforms (p46, p52 and p66) as well as the status of antioxidant defense system have been estimated with the use of Western Blot technique. Mitochondrial bioenergetic parameters and ROS production have been determined with the use of respective fluorescent probes and measured using Infinite M200 Tecan microplate reader.

We have found differences in the profile of p66Shc signaling pathway, ROS level and antioxidant defence system between human breast cancer cell lines and respective controls.

Our data indicate that p66shc protein may play the specific role depending on the cancer type. However, it remains still mysterious in which mechanism p66Shc exerts its impact on tumor cells and requires further investigations.

ST-02.02.2-017
The structure of an engineered LRRTM2 synaptic adhesion molecule – implications for neurexin binding

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Synaptic adhesion molecules are key components in development of the brain, and in formation of neuronal circuits, as they are central in the assembly and maturation of chemical synapses. Several families of neuronal adhesion molecules have been identified such as neurexins and neuroligins, and in particular recently several leucine rich repeat proteins, *e.g.* Netrin G-ligands, SLITRKs and LRRTMs. The LRRTMs form a family of four proteins. They have been implicated in excitatory glutamatergic synapse function, and were specifically characterized as ligands for neurexins in excitatory synapse formation and maintenance.

We report the crystal structure of a thermostabilized mouse LRRTM2, with a T_m 30°C higher than the wild type protein. We localized the neurexin binding site to the concave surface based on protein engineering, sequence conservation and prior information on the ligand interaction with neurexins, which allowed us to propose a tentative model for the LRRTM: neurexin interaction complex. We also determined affinities of the thermostabilized LRRTM2 and the wild type LRRTM1 and LRRTM2 for neurexin-beta1 with and without Ca^{2+}. Cell culture studies and binding experiments show that the engineered protein is functional and capable of forming synapse-like contacts. Our small angle X-ray scattering data suggests that the wild type protein can form transient dimers. The structural and functional data presented here provides the first structure of an LRRTM protein, and allows us to propose a model for molecular mechanism of LRRTM function in the synaptic adhesion.

ST-02.02.2-018
Klf5 acts as a differentiation switch from proliferation to fusion in myoblasts

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Kruppel-like factor 5 (Klf5) is a ubiquitously expressed zinc finger transcription factor and the function stringently depends on the context as well as cell and tissue type. Klf5 is shown to be involved in cellular processes like maintenance of undifferentiated state in blastocysts, cell cycle regulation, apoptosis, differentiation, and migration.

A base-line expression of Klf5 is also present in steady state skeletal muscle and proliferating myoblasts. This expression is induced along with acute injury regeneration in vivo and myoblast fusion in vitro. Overexpression of Klf5 in proliferating myoblasts induces cell cycle arrest and a dominant-negative Klf5 accelerates the cell cycle. Conditional silencing of Klf5 in proliferating myoblasts accelerates motility and lessens the attachment of dividing myoblasts confirming previous observations.

The silencing Klf5 strikingly inhibits differentiation confirmed by transfection of dominant negative constructs. Silencing inhibited fusion, and myotube maturation by inhibiting B-catenin, desmin, NCAM and M-Cad. But silencing following fusion did not exhibit any impact on myotube maturation pinpointing a role in fusion. Klf5 silenced myoblasts also failed to fuse with their non-silenced counterparts, verifying an essential role for the induction of fusion.

Klf5 silencing repressed myogenin, the terminal regulator of differentiation. This differentiation block could be counteracted by forced expression of MyoD. These observations pinpoint Klf5 downstream of MyoD and upstream of myogenin. This role, was verified by transdifferentiating 3T3- L1 preadipocytes by forced expression of MyoD. These cells exhibited an upregulation of Klf5 along with fusion in a dose and time dependent manner.

These results attribute a novel role for Klf5 as a functional switch for the cell cycle arrest and induction of differentiation in myoblasts. Our current efforts are concentrated to elucidate the partners and targets of Klf5 along this process.

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ST-02.02.2-019
Optogenetic control of signaling in mammalian cells by Drosophila cryptochrome

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In optogenetics, light is used to control signaling pathways via light sensitive proteins. Adoption of new light sensitive proteins into current optogenetic methods can improve upon the temporal tuning and sensitivity of these methods. Cryptochrome (CRY) proteins reset circadian rhythms in many organisms and have attracted attention as possible optogenetic tools. Drosophila and mammalian molecular circadian clocks are essentially the same; however the mammalian CRY proteins are not light sensitive, therefore introduction of Drosophila CRY into mammalian cells can render the cells sensitive to light.

We exploited the light sensitivity of Drosophila CRY to control signaling in human cell lines. For this purpose, we expressed Drosophila CRY and its light-activated partner Jetlag (JET) protein fused to different control elements, which could be transcription factors, enzymes and any other regulatory proteins. We fused the Retinoid × Receptor (R×R) protein without dimerization domains to CRY and JET. Light-induced interaction between CRY and JET is expected to cause dimerization of truncated RXR proteins and activate target gene expression.

We stably expressed both RXR-CRY and RXR-JET in Hek293T cells and exposed the cells to black light at 0.02 $mW{\cdot}cm^{-2}$ intensity up to 36 h. We then probed the gene targeted by RXR dimerization, p21, by qPCR and immunoblotting and found that endogenous p21 can be activated by light in our system.

We show that Drosophila CRY can be used as an optogenetic tool in mammalian cells. Although, we only tested transcriptional control in our system, any other cellular events coupled to our system can be theoretically targeted by light. In drosophila cells, the light-induced CRY and JET interaction initiates a signaling pathway within minutes. Therefore, time resolution in minutes for controlling cellular events can be achievable. Control of different events by special design from minutes to days would add to the spectrum of optogenetic tools.

ST-02.02.2-020
Do serum nestin levels have a diagnostic effect in patients with colorectal cancer?

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Background: The colon and the rectum are parts of the large intestine, which is the lower part of the body's digestive system. During digestion, food moves through the stomach and small intestine into the colon. Nestin is considered as a type VI intermediate filament protein, which was originally described as a neuronal stem cell marker during central nervous system development. Nestin expression levels are downregulated with cellular differentiation. Nestin has also been reported in various types of cell lines established from human solid tumors, such as colorectal cancer (CRC). The objective of this study was to evaluate the serum levels of nestin in regard to diagnostic, predictive and prognostic value in CRC patients.
Material and Methods: The serum samples of the 120 consecutive patients with CRC who referred to Istanbul University Institute of Oncology and Bakirkoy Dr. Sadi Konuk Training and Research Hospital from 2015 to 2016 the healthy control group ($n = 40$) were obtained. The nestin protein assay employs ELISA. SPSS 21.0 was employed for data analysis.
Results: Serum nestin protein levels were significantly higher in patients with colorectal cancer than the healthy controls ($P = 0.00$). However, known clinical variables including response to adjuvant chemotherapy were not found to be correlated with serum nestin concentrations ($P > 0.05$). A significant relationship between other clinicopathologic variables including localization of rectum ($P = 0.04$), presence of metastasis ($P = 0.01$), vascular invasion ($P = 0.02$), poor grade ($P = 0.02$), high serum levels of LDH ($P = 0.03$), CEA ($P < 0.001$), CA 19-9 ($P < 0.001$), low serum levels of albumin ($P = 0.04$).
Discussion: Our data indicate that nestin protein can be used as a diagnostic parameters for CRC. Nestin is important in colorectal cancer targeted therapy. We believe it will be useful marker for clinicians to help decide the diagnosis of CRC.

ST-02.02.2-021
Biodegradation of lipids in the Wadi Hanifah River in Saudi Arabia by using *S. aureus* ALA1 lipase enzyme free or immobilized onto $CaCO_3$

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The present study describes the immobilization by physical adsorption onto $CaCO_3$ of a thermostable and alkaline lipase from *S. aureus* strain, aiming at its application in biodegradation of oil from the oily Wadi Hanifah water. After optimization of immobilized conditions, recovered enzyme activity was 95%. The optimum pH (12) and temperature (60°C) were nearly same for aqueous and immobilized enzymes while the immobilized lipase exhibited much better thermostability and kept 70% initial activity after 60 min incubation at 80°C against only 10% for the free lipase. The immobilization seems to improve lipase stability up to 58% for 48 h in a pH ranging from 6 to 11. Furthermore, the reusability and storage stability of the immobilized *S. aureus* lipase were markedly enhanced, with almost 100% residual activity when repeatedly used for 4 cycles and 58% activity retention when stored at 25°C for 120 days. Among activators/inhibitors β-mercaptoethanol, SDS, EDTA, and Co^{2+} showed inhibitory effect while $CaCO_3$ adsorption enhanced ~ 40% tolerance capacity of lipase against inactivating agents.

The free lipase displayed the highest activity using olive oil emulsion as substrate while the immobilized one was more effective against corn seed oil. Bioremediation of lipid-rich Wadi Hanifah water was carried out by the immobilized and the free lipases via the analysis of the total organic chloride (TOC), chemical oxygen demand (COD) and lipid content. Overall, the obtained results showed the potential of immobilized *S. aureus* lipase as a step in biological wastewater treatment and open new prospects for the application of *S. aureus* ALA1 lipase for several industrial applications.

ST-02.02.2-022
Hacettepe University Zebrafish Research Laboratory: zebrafish disease modeling by genome editing tools

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Zebrafish has become one of the most popular model organisms of choice in the biomedical research area covering a broad range of arrays since they were first used in the research in the late 1960s. Approximately 70% of human genes have at least one zebrafish orthologue. High fecundity, ex-utero developing transparent embryos, and easy laboratory maintenance makes the zebrafish attractive and advantageous for biomedical research.

Newly emerging gene editing tools like transcription activator-like effector nucleases (TALENs) or clustered regularly interspaced short palindromic repeats (CRISPR) provides capabilities to efficiently achieve specific gene manipulation by inducing double-stranded break at a specific site in the genome.

Hacettepe University is a major reference center for rare disorders with high in-out patient capacity.We used these availability of diversified patient phenotypes as an advantage and conducted a TUBITAK project in order to establish a Zebrafish Research Laboratory to investigate effects of new mutations on zebrafish. The first step of the project is to model a desmin mutation related to muscular dystrophy (LGMD2R, MIM 615325) using CRISPR/Cas 9 and TALEN technologies and generate a mutant zebrafish line to study the effects in a comprehensive perspective. However *desma* and *desmb* known as duplicated genes in zebrafish. To reveal the differentiated expression profile we performed In Situ Hybridization experiments and created *desma* and *desmb* knockout models to see if they replace for each other. In the long run, we aim to investigate the role of different muscular dystrophy genes identified by our group and mutations in different genetic diseases. Furthermore we have planned to form a catalogue of mutant zebrafish stocks consist of specific mutations that we hope to contribute to future studies of drug, treatment and more.

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ST-02.02.2-023
Structural approach for characterization of small heat shock proteins function

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Small heat shock proteins (sHSP) are a specific subclass of molecular chaperones. They are aptly characterized by their structure and mechanism of action. There is an accepted consensus that they form dynamic oligomers and reversibly bind unfolded proteins. They are inhibitors or modulators of the aggregation process. However, the exact architecture of sHSP-substrate complex and mechanism of its formation remain unclear. Our investigation concerns sHSPs of a model organism, *Escherichia coli*. It has two genomic sHSPs – IbpA and IbpB, which seem to act in close cooperation.

While we were able to show sHSP function in substrate reactivation *in vitro*, as well as to separate probable roles of both of them, the structural basis of their function is still unknown. Oligomerization tendency of sHSPs and inherent irregularity of sHSP-substrate co-aggregates make obtaining of structural data very challenging. We have implemented a crosslinking based method to overcome these obstacles and obtain structural data. Instead of using an external crosslinker, we use an unnatural amino acid exhibiting photocrosslinking activity – p-benzoilophenylalanine (Bpa). There is an established methodology for site-specific incorporation of this amino acid, as well as examples of successful applications in structural biology. Despite being much more labour intensive, requiring purification of a substantial number of crosslinking protein variants, this method gives us an opportunity to precisely control the crosslinking process. It enables analysis of protein-protein interactions even in highly irregular complexes. Up to now, we have shown that we are able to visualize sHSP-substrate interaction *in vitro* and *in vivo*, as well as sHSP-sHSP interaction *in vitro*. Moreover, we were able to analyze sHSP-sHSP crosslinking products using mass spectrometry and precisely define the crosslink site.

ST-02.02.2-024
Enhancement of L-asparaginase production by *Sarcladium strictum* in submerged culture by optimization of medium composition through statistical experimental design

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L-asparaginase (EC: 3.5.1.1) catalyzes degradation of L-asparagine into L-aspartic acid and ammonium. Regarding to the importance of L-asparagine as an essential amino-acid for leukemic cells, it is used as a chemotrophic agent to treat acute lymphoblastic leukemia. Aim of this study was the optimization of media composition in submerged conditions by a native isolated and genetically identified yeast strain *Sarcladium strictum* as a novel source for L-asparaginase production.

After incubation the filtrated media was used as the crude enzyme then the activity of L-asparaginase was measured by nesslerization. First, optimum incubation period was separately studied and best period incubation time (144 h) was chosen for the rest of optimization processes. The study was preformed using a 2-level placket-berman design to identify and optimize the main effective factors for carbon and nitrogen source and pH.

The experimental enzyme activity of 3.29 $IU{\cdot}mL^{-1}$ was obtained at the optimum culture conditions. Based on our results D-glucose, yeast extract, glycerol, starch and L-asparagine have significant positive effect on L-asparaginase production while whey, ammonium sulfate and molasses have significant negative effect. The key medium components to produce L-asparaginase were 2.5 $g{\cdot}L^{-1}$ of yeast extract, 5 $g{\cdot}L^{-1}$ of glycerol and 10 $g{\cdot}L^{-1}$ of L-asparagine.

Our results indicated that *S. strictum* is a suitable source for L-asparaginase production. Immunological properties and anticancer assay of *S. strictum* L-asparaginase are under way.

ST-02.02.2-025
Intermedin is a potent angiogenic mediator *in vivo* and *in vitro*

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Intermedin, also named as adrenomedullin 2, is a peptide which belongs to calcitonin/calcitonin gene-related peptide family.

Intermedin is an important bioactive peptide for vascular homeostasis. The aim of this study was to investigate whether intermedin had angiogenic effects in vivo and in vitro.

Proangiogenic effect of intermedin was examined in vivo chick chorioallantoic membrane (CAM) model and in vitro tube formation assay. Its effect on endothelial cell proliferation was determined using the cell viability assay [MTT assay: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay].

CAM assay results showed that intermedin significantly and dose-dependently increased vessel density with respect to the control group after 24 h incubation of CAMs ($P = 0.037$). Intermedin treated endothelial cells had significantly higher tube length/area ratio compared to the control group in tube formation assay ($P < 0.05$). Proliferation of endothelial cells was also significantly higher in intermedin treated wells than control wells ($P < 0.05$) in MTT assay after 24 and 48 h incubations. 100 pM of intermedin was the efficient concentration in vitro models, while 200 and 500 nM doses were found to increase the vessel density on CAM in vivo. Budding, extravasation and sprouting of new vessels were seen clearly on CAM area at these concentrations.

Intermedin was found to enhance endothelial cell proliferation, migration and tube formation. We found that intermedin promoted angiogenesis in vivo and in vitro in a dose-dependent manner. These results provide evidence that intermedin is a potent angiogenic mediator.

Proangiogenic effect of intermedin may be useful in treatment modalities of antities related to angiogenesis. For example; intermedin may be a target in tumor angiogenesis or it may be useful in peripheral artery disease.

ST-02.02.2-026
Antibacteriophage action of *Bacillus altitudinis* extracellular ribonuclease

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Guanyl-preffering ribonucleases (RNases) which are secreted by Bacillus species were shown to exhibit antiviral effect on influenza, murrain, rabbies viruses in vitro and in vivo. However, their potential in defense of bacterial population against bacteriophage infection has not yet been studied.

Using microbiological and virological methods we have isolated Bacillus altitudinis B-388 bacteriophage from soil, purified it and studied its interaction with bacterial cells on different growth stages at multiplicity of infection (moi) 10 and 1e-6. Cell number, phage titer and RNase activity were monitored. Visualization was performed using scanning and transmission electron microscopy. Bacterial transcripts were analyzed by RNA-Seq.

It was found that *B. altitudinis* bacteriophage is DNA-containing (~23 kb) tailed phage with icosaedral head ~60 nm in diameter. After latent period of 15 min phages begin to release from cells and completely lyse them after 50 min. Cells became more susceptible to phage infection if culture fluid was removed before infection. When grown under starvation conditions but not in rich media *B. altitudinis* was able to reduce phage titer with increased synthesis of extracellular RNase. No RNase transcripts were found in the beginning of phages release. The less phages were added, the greater antiphage effect of RNase was observed. RNase exogenously added to washed bacterial cells decreased phage titer in dose-dependent manner.

RNase III and MazF are known to participate in antiphage defense. Secreted RNases of Bacillus are another example of antiphage agents which can interfere with phage adsorption or cause abortive infection. The action of Bacillus RNases is more significant under starvation, natural conditions of soil-borne bacteria.

ST-02.02.2-027
A novel signaling pathway that governs tumor metastasis: ceramide regulates direct crosstalk between TGF-ß and sonic hedgehog signaling

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Bioactive sphingolipid ceramide is a second messenger in cell membrane in response to inflammation and stress. Recent studies indicate that ceramide species play diverse biological functions in various cellular processes. Migration and cell mobility, a part of these processes, also are effected by ceramide metabolism. However, the molecular mechanism of CerS/ceramide involved is unknown. Here, we investigated the effect of CerS/ceramide on migration and its related signal pathways *in situ* and *in vivo* model. Interestingly, our data show that among CerS only CerS4/ceramide is involved to cell migration and tumor metastasis. Here, we also have generated CerS4$^{-/-}$ mice for *in vivo* studies. Interestingly, we observed that genetically loss of CerS4 resulted in irreversible alopecia, which was associated with hyperproliferation and migration of keratinocytes. Mechanistically, we show here that genetic loss or shRNA-mediated knockdown of CerS4 enhances cell migration by which ligand-independent signaling of TGF-beta receptors I and II in various cell types, including keratinocytes, mouse embryonic fibroblasts and cancer cells. Moreover, we found that ceramide directly interact with Smad7 and this interaction was decreased by shRNA-mediated knockdown of CerS4. Thus, ceramide-Smad7 binding modulates plasma membrane association of TGF-ßR1 at primary basal cilia, and inhibits its signaling through Sonic-Hedgehog (Shh) for migration. Furthermore, Ceramide accumulation at the primary basal cilia was decreased by knockdown of CerS4, and this was associated with direct interaction of TGF-ßR1 and SMO receptors in cilia. In fact, inhibition of TGF-ßR/Shh signaling or cilia formation using molecular or pharmacologic inhibitors almost completely prevented cell migration in response to CerS4 knockdown. These data revealed that CerS4/ceramide signaling plays key roles in the regulation of cell migration and metastasis via controlling the TGF-ßR and Shh axis at primary basal cilia.

Monday 5 September 17:30–19:30, Hall E

DNA repair and cancer

ST-05.01.1-009
Effects of *Ganoderma lucidum* on the expression of Nrf2, NQO1 and HO-1 levels in hepatocarcinoma cells

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Background: For centuries in Asia, *Ganoderma lucidum* (*G.lucidum*) has been used for health promotion. *G. lucidum*, anti-cancer effects have been revealed in both *in vitro* and *in vivo* studies. It has been observed to have numerous pharmacological effects including immuno-modulating, anti-inflammatory, anti-cancer, anti-diabetic, anti-oxidative and radical scavenging, and anti-

aging effects. Several modes of action by which carcinogens activate cancer have been diagnosed, containing overproduction of reactive oxygen species (ROS). Oxidative damage to cellular macromolecules can arise through overproduction of ROS and faulty antioxidant and/or DNA repair mechanisms. In addition, ROS can stimulate signal transduction pathways and lead to activation of key transcription factors such as nuclear factor erythroid-2 (Nrf2) and NF-κB.

Aims: In this study we investigated the antioxidant effects of *Ganoderma lucidum* in liver cancer cells. To counteract oxidative stress, cells have complicated mechanisms of defense against cancer, one of the most important mechanism involves the activation of Kelch-like ECH-associated protein-1 (Keap1)/Nrf2/ARE pathway, which leads to the expression of cytoprotective enzymes, such as NAD(P)H: quinone oxidoreductase 1 (NQO1) and heme oxygenase 1 (HO-1) etc.

Results and Discussion: We have investigated the effects of *G. lucidum* on mRNA and protein expressions of Nrf2, NQO1 and HO-1 level in hepatocarcinoma cells by using realtime PCR and Western blot analysis, respectively. The expression of NRF2/ARE related genes significantly changed following *G. lucidum* treatment. Similarly, the alterations in NQ-1 and HO-1 protein levels have been found in our study model. Nrf2 is the central transcriptional regulator in ARE-driven gene expression in response to oxidative stress. Therefore, Nrf2-mediated antioxidant response plays a vital role in preservation of intracellular redox homeostasis.

ST-05.01.1-010
The combination of temozolomide and vismodegib may induce necroptosis in glioblastoma cells

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Glioblastoma (GBM), which is the most common and aggressive primary brain tumor, is categorized as grade IV according to the classification of WHO. The treatment strategy of GBM consists of surgery, radiotherapy and chemotherapy with temozolomide (TMZ). TMZ is a DNA alkylating agent that is approved for GBM treatment by FDA. The recurrence of gliomas seems to be derived of the existence of cancer stem cells, thereby current therapeutic approach for GBM might not overcome the drug resistance. Moreover, the Hedgehog (Hh) signalling pathway has a significant role for survival of glioma stem cells. Vismodegib is an inhibitor of Hh pathway that is approved by FDA.

Currently, necroptosis is considered to be regulated, therefore it is also called as programmed necrosis. When apoptosis is deficient, RIP1 kinase activity is needed for the activation of necroptosis. In addition, the RIP1 kinase activity is crucial for necrosome formation. RIP1 can also manage downstream of PARP1 to achieve necroptosis. RIP3 interacts with and activates several metabolic enzymes including GLUD1 to trigger ROS production and necroptosis. On the other hand, overexpression of NOX4 leads to increased ROS production, thereby it may associate with initiation of necroptosis.

In our study, we aimed to determine expression levels of genes involved in necroptosis mechanism. Reverse transcription procedure was performed for cDNA synthesis and gene expressions were detected by RT-qPCR.

The expression levels of GLUD1, NOX4, PARP1, RIPK1, PPID genes which are involved in necroptosis were upregulated 2.33, 4.12, 2.01, 3.22, 2.38 folds in U-87MG cells treated with dose of the combination, compared to the controls, respectively.

Our findings suggest that the combination of these agents may be a novel therapeutic approach in GBM treatment by inducing necroptosis.

ST-05.01.1-011
Caspases activities in TNF-α applied HepG2 human hepatocellular carcinoma cell

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Tumor necrosis factor (TNF) is a cytokine that play a key role in cellular events such as cell survival, proliferation, differentiation, inflammation, immunity, and apoptosis. Although named Tumor necrosis factor for its tumor cytotoxicity, TNF has been implicated in a wide spectrum of other diseases.

In this study, the hepatocellular carcinoma cell line HepG2 was used and the cells were cultured in the absence (control) or presence of TNF-α for 24 h. The effect of TNF-α on caspase 3, caspase 9, and caspase 1 enzyme activities in hepatocarcinoma cells were examined in TNF-α treated and control cells using colorimetric assay kits.

There were significant increases in caspases 1 and 3 levels in TNF-α treated HepG2 cells compare to control cells.

TNF-α is a pro-inflammatory cytokine, secreted by inflammatory cells. This mechanism may be involved in inflammation-associated carcinogenesis. TNF could act both as tumor promoter, and cancer killer. Presented findings suggest that caspases-dependent cell death occurs in TNF-α applied HepG2 cells.

ST-05.01.1-012
The human cytomegalovirus DNA is not found in the genomic DNA of human glioma tumor samples

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Gliomas are widely met and very rapid progressed, malignant central nervous system tumors. Like all cancers they are developed as a result of activation of oncogenes and inactivation of tumor suppressor genes.

In recent years, studies have indicated that human cytomegalovirus (HCMV) has a role in glioma development. In previous studies HCMV DNA has been detected in the tumor biopsies of the many glioma cases. In our study we aimed to test whether HCMV genome is incorporated into genomic DNA of glioma cells and if it is incorporated, has any role in the any oncogene activation or any tumor suppressor inactivation.

We extracted the genomic DNA of tumor biopsies from 10 patients and measured the existence of HCMV genome by using real-time quantitative PCR (QPCR). Beside the patient samples, positive control DNAs were measured. A 105 bp region of the CMV genome specific amplicon was aimed to detect in fluorescence channel cycling green.

As a result, HCMV genome specific amplicon was not detected in the genomic DNA of any patient's tumor samples while it was detected in positive controls.

In conclusion our hypothesis couldn't be supported. We hypothesize that insertion of HCMV DNA, especially its promotor regions, into the genomic DNA causes to oncogene activation (e.g. EGFR over expression) or insertion of HCMV DNA into the tumor suppressor gene regions leads to their inactivation (e.g. p53

or PTEN). But any evidence couldn't be obtained to support this hypothesis because of no HCMV genome specific amplicon was detected from the genomic DNA of tumor samples. The reason of this may be explained like that; instead of whole virus genome insertion, small parts of it may be incorporated by homolog recombination into genomic DNA of glial cells. To investigate this new QPCR experiments are being planned by using various primers which are specific to virus promotors and other regions.

ST-05.01.1-015
Novel ellipticine derivatives: synthesis and effects on DNA cleavage mediated human topoisomerase II

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A number of compounds of either natural or synthetic origin target DNA topoisomerases, ubiquitous enzymes with important roles in replication, transcription, recombination, repair and other cellular processes. Several mechanisms for topoisomerase-targeting by the compounds have been reported. Drugs such as etoposide and amsacrine inhibit the religation of the broken DNA strands while others such as genistein were reported to affect by stimulating the formation of enzyme-DNA complexes. Some compounds with intercalative properties bind both to protein and its DNA substrate. Ellipticine, a well-known compound effective on topoisomerases, is not a widely-accepted drug due to its poor water-solubility, specificity, and potential to lead secondary malignancy and drug resistance. Therefore, over the last years, the generation of new ellipticine derivatives has gained a significant importance for developing new anti-cancer drugs. We previously reported the synthesis and biological activity of two novel derivatives. In the current study, we built upon our previous work by synthesizing four new derivatives via a novel pathway by our team and coupled the synthesis with kinetic analyses.

We generated new salt derivatives of N-methyl 5-demethyl-ellipticine by reacting N-methyl 5-demethyl-ellipticine with several alkyl halides in dimethylformamide. Characterization of new compounds carried out *via* spectroscopic analysis including IR, 1H-NMR and 13C-NMR. We analyzed compounds by determining their effects on topoisomerase II-mediated DNA cleavage and their ability to intercalate into DNA.

Ellipticine derivatives did not stimulate the formation of cleavage complexes. Results indicate that the derivatives are potent catalytic inhibitors of human topoisomerase II.

We anticipate our research to yield significant contributions to anti-cancer drug development studies.

Miscellaneous

ST-Mis-018
Chemotherapeutic effect of Everolimus and ABT-737 combination on renal cell carcinoma

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The renal cell carcinoma (RCC) comprises 2–3% of malignant tumors with a high mortality rate. The loss of von Hippel-Lindau (VHL) tumor suppressor gene observed in metastatic RCC patients is the cause of the elevated levels of PI3K/Akt/mTOR survival pathway. In this regard, drugs such as Everolimus are used in clinic to block the activity of the mTOR serine/threonine kinase albeit late onset drug resistance. The elevated level of anti-apoptotic Bcl-2 protein is one of the feedback loops for Everolimus resistance. In light of this information, we aimed to combine Everolimus with a Bcl-2 inhibitor, ABT-737 to overcome the Everolimus-dependent resistance in RCC tumors and to induce cell death.

Everolimus resistant RCC cell lines A-498 and Caki-1 were used in our study to investigate the effect of combinatorial treatment of Everolimus and ABT-737 on cell viability. The Annexin-V-PI staining was performed to check cell death, which was confirmed by the cleavage of caspase 9 and caspase 3 via Western Blotting. At molecular level, the protein levels for the members of Akt/mTOR pathway and Bcl-2 family were investigated by Western Blotting. The determination of the influence on tumor growth was analyzed *in vivo* in RenCa cell model and balb-c mice.

The combination of Everolimus and ABT-737 decreased the cell viability about 80% in A-498 and 70% in Caki-1 compared to the untreated cells in time-dependent manner. The decrease in cell viability was a result of apoptotic cell death confirmed by Annexin-V-PI staining and the cleavage of caspase 9 and caspase 3. The decrease in protein levels of components of mTOR pathway and Bcl-2 family was evident upon the combination treatment with Everolimus and ABT-737.

Taken together, our *in vitro* and *in vivo* data suggests that the combination treatment of Everolimus and ABT-737 is effective in the eradication of drug resistant advanced RCC. The results from this study can potentiate new clinical trials for RCC treatment.

ST-Mis-019
Does MW radiation affect gene expression, apoptotic level and cell cycle progression of human SHSY-5Y neuroblastoma cells?

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Neuroblastoma (NB) is a cancer that occurs in sympathetic nervous system arising from neuroblasts and nerve tissue of the adrenal gland, neck, chest, or spinal cord. It is an embryonal malignancy and affects infants and children. In this study, we investigated the effects of Microwave (MW) radiation on apoptotic activity, cell viability and cell cycle progression in human SH-SY5Y NB cells which can give information about MW radiation effects on neural cells covering the period from the embryonic stages to infants.

SH-SY5Y NB cells were exposed to 2.1 GHz W-CDMA modulated MW radiation for 24 h at a Specific Absorption Rate (SAR) of 0.491 $W \cdot kg^{-1}$. Control samples were in the same conditions with MW exposed samples but they were not exposed to MW radiation. The apoptotic activity of cells was measured by Annexin-V-FITC and propidium Iodide (PI) staining. Moreover, mRNA levels of proliferative and cell cycle proteins were determined by real time RT-PCR. The change in cell cycle progression was observed by using CycleTest-Plus DNA reagent.

No significant change was observed in apoptotic activity of MW exposed cells compared to control cells. The mRNA levels of *c-myc* and *cyclin D1* were significantly reduced in MW group ($P < 0.05$). The percentage of MW exposed cells in G1 phase was

significantly higher than the percentage of control cells in G1 phase. MW radiation caused cell cycle arrest in G1 phase. These results showed that 2.1 GHz W-CDMA modulated MW radiation did not cause apoptotic cell death but changed cell cycle progression.

Epigenetics and cancer

ST-05.02.2-006
Major mechanisms leading to deregulation of non-coding RNAs in early-onset prostate cancer

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Increasing evidence is showing that most epigenetic mechanisms of gene expression control include regulation by non-coding RNAs (ncRNAs). These molecules are very important in various epigenetic modification mechanisms such as transposon activity and silencing, position effect variegation, X-chromosome inactivation and paramutation and frequently deregulated in cancer. Mechanisms for ncRNA deregulation in prostate cancer (PCA) have been worked out for selected candidates and include genetic or epigenetic alterations. Newly generated cancer profiling data collected in large consortia allows now an integrative analysis of deep sequencing data. In the German ICGC project on about 90 early-onset PCA, genetic alterations (deletions) accounted for deregulation of up to 150 ncRNAs but did not affect all deregulated ncRNAs. Promoter hyper- and hypomethylation were observed for up to 20% deregulated ncRNAs. The large overlap of differentially expressed and aberrantly methylated ncRNAs was supported by 51 independent PCA. Differential promoter methylation and its (inverse) correlation to ncRNA expression were confirmed by MassARRAY. Based on our data we were able to identify 3 major mechanisms of ncRNA deregulation: non-coding mutation in promoter/regulatory region of ncRNA, promoter methylation of ncRNAs and loss/gain of alleles, carrying ncRNA gene. Further investigations are needed to unravel it with increased sample cohort and improved technique, that can allow distinguish allele specific events.

ST-05.02.2-007
Determination the anticancer effects of doxycycline (DC) application

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Introduction: Detecting a new effective and hypotoxic anticancer drug is an emerging new strategy in cancer chemotherapy. Doxycycline (DC) is an antibiotic that also inhibits tumorigenesis.
Methods: An *in vitro* approach was used to investigate the effect of pure DC on 3 types of tumor cell lines HeLa, RD & AMGM-5 glioblastoma in different concentrations & at different exposure times by MTT assay.
Results: DC exerted significant cytotoxic effects with all concentrations used (50–400 μg) on all cell lines examined.
Conclusions: Our data suggest that DC exerts its antitumor effect making the DC application as a valuable treatment option against the development of different human cancer types
Keywords: Doxycycline, Anticancer, Cell Line, Cervical Carcinoma RD, Glioblastoma.

ST-05.02.2-008
CXXC5 is an estrogen responsive gene

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17β-estradiol (E2), an estrogen hormone, mediates physiological and pathophysiological functions of breast tissue. E2 effects are mediated by estrogen receptor α (ERα), which is a ligand-dependent transcription factor. Upon binding of E2, ERα regulates target gene expressions through estrogen response element (ERE)-dependent and ERE-independent genomic pathways that result in alterations of cellular responses. We previously found using microarrays that *CXXC5* is an E2 responsive gene. Due to a zinc-finger-CXXC domain (ZF-CXXC), CXXC5 is considered to be a member of ZF-CXXC family which binds to non-methylated CpG dinuclotides of CpG islands and mediates transcriptions. Studies on signaling cascades responsible for the regulation of *CXXC5* gene expression are limited. This study aims to uncover the mechanism by which E2 signaling alters the transcription of the *CXXC5* gene. Protein synthesis and subcellular localization were assessed by western blot and immunocytochemistry, respectively. Gene expression in response to E2 was carried out by RT-qPCR. Dragon ERE Finder 3 was used to predict the potential ERE sequences on regulatory regions of the *CXXC5* gene. A proximal promoter region of the *CXXC5* gene was cloned into a reporter vector that express the *firefly luciferase* as the reporter enzyme. Flag tagged-ERα protein and biotinylated-EREs were used for electrophoretic mobility shift assay (EMSA). Hormone treatment of cells was performed for chromatin immunoprecipitation (ChIP) and for subsequent RT-qPCR. Our results for the first time revealed that *CXXC5* is an E2-responsive gene mediated by the E2-ERα complex through a direct interaction with an ERE sequence present in the proximal *CXXC5* promoter. E2-ERα augmented *CXXC5* expression, which was reflected in an increase in the level of CXXC5 protein present in the nucleus. The expression of *CXXC5* is mediated by E2-ERα through the ERE-dependent signaling pathway.
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ST-05.02.2-009
Aberrant nuclear-cytoplasmic transport by leukemia-associated Nup214-fusion proteins

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Nuclear transport receptors (NTRs) mediate nuclear-cytoplasmic transport through nuclear pore complexes (NPCs). NPC is composed of about 30 different proteins, termed nucleoporins.

Previous studies demonstrated that aberrant nuclear-cytoplasmic transport caused by mutations or overexpression of NPC and NTR components is closely linked to different types of cancer. Nup214, a component of NPC, has been found as fusion proteins in leukemia. *SET-Nup214* and *DEK-Nup214* fusion genes encode proteins consisting of histone chaperones, SET and DEK, and a part of Nup214. SET-Nup214 and DEK-Nup214 were shown to be involved in leukemogenesis, but their oncogenic mechanism remains unclear.

In this study, we examined the function of the Nup214-fusion proteins by focusing on their effects on nuclear-cytoplasmic transport.

We found that SET-Nup214 and DEK-Nup214 interact mainly with CRM1/Xpo1, an export receptor of NES proteins, and NXF1/TAP, an mRNA export receptor. SET-Nup214 and DEK-Nup214 decreased XPO1-mediated nuclear export of NES proteins involved in the NF-κκB signaling pathway by tethering Xpo1 onto nuclear dots where Nup214-fusion proteins are localized. We also demonstrated that SET-Nup214 and DEK-Nup214 expression inhibited NF-κκB-mediated transcriptional activity.

These results implicate that SET-Nup214 and DEK-Nup214 disturb regulation of gene expression through alteration of the nuclear-cytoplasmic transport system.

Functional genomics and proteomics

ST-08.01.4-007
Identification of novel mutations in AVP-NPII gene

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Central Diabetes Insipidus results from insufficient production of antidiuretic hormone arginine vasopressin (ADH, AVP: Arginine Vasopressin), which is caused by mutations in arginine vasopressin-neurophysin II gene (*AVP-NPII*).

In this study, we present the molecular and clinical features of a male Turkish patient and also his family with autosomal dominant neurohypophyseal DI caused by a novel mutation (p.E108D and p.R122H). The prospective clinical data were collected for the proband patient and his family members. The patient had severe polyuria (10.9 $L \cdot day^{-1}$), polydipsia (12 $L \cdot day^{-1}$), fatique, and deep thirstiness from his infancy. While being performed water deprivation test, diagnosis of central diabetes insipidus was confirmed according to increase in urine osmolality from 139 $mOsm \cdot kg^{-1}$ to 431 $mOsm \cdot kg^{-1}$ after desmopressin acetate injection. Some of family members of this patient had severe polyuria, nocturia, polydipsia, fatigue as well. To analyze the sequence of *AVP-NPII* gene of the patient and his family as well, we were sequenced all exons and intron-exon boundaries of the gene.

According to the sequencing results, we detected novel heterozygous missense mutations at codon 108 and 122. Codon 108 mutation is a substitution of Glu (GAG) by a Asp (GAT) and codon 122 mutation is a substitution of Arg (CGC) by a His (CAC) in exon 3. After we found E108D mutation which was the first mutation that we found via proband, we sequenced *AVP-NPII* gene of other family members. Then we found other new mutation, R122H, in distant relatives of the proband. Interesting parts of this study are that family has kin marriages and family members just have one of these mutations if they have mutant AVP-NPII. A three-dimensional protein structure predictions were shown for the mutant AVP-NPII protein and compared with the wild type. In our future studies we are planning to do functional analyses studies for understanding the function of the p.E108D and p.R122H mutations.

ST-08.01.4-008
Mitochondrial DNA-binding gene regulatory proteins: a leucine/isoleucine zipper motif in freshwater haplosclerid sponges

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DNA-binding proteins play important roles in the regulation of particular gene expression, DNA replication and recombination. *In vitro*, Leucine zipper proteins (L-ZIP) was shown to act as gene regulatory protein in the nucleus, while in the mitochondria no such activity has been reported. Here we report an *in silico* study about the presence of a leucine/isoleucine zipper motif in mtDNA of freshwater haplosclerid sponges.

Mitochondrial *nad4L* and *nad2* protein-coding genes were found to have a L-ZIP domain by a novel created software code for zipper identification. Protein modeling and protein-protein interactions were predicted by SWISS-MODEL and COTH servers, respectively. MILC and MELP algorithms were used for codon usage computing and gene expression prediction for 14 mt protein-coding genes.

A highly conserved bZIP domain of *nad4L* was detected among all diploblastic and triploblastic animals, while bZIP domain in *nad2* is well conserved only in freshwater Haplosclerida, not even in other demospongian species. The putative Leucine zipper motif L^{15}-L^{22}-I^{29}-L^{36} of *nad4L* and L^{15}-L^{22}-L^{29}-I^{36} of *nad2* is placed at the N-terminus.

Our findings suggest that *nad4L* has a conserved gene expression pattern among all selected proteins from haplosclerid taxa, showing plastic features in order to form a homodimer rather than heterodimer with the Nad2 protein. Moreover, our findings of over 10 palindromic conserved blocks, implicate their possible role in regulation of transcription due to possibility its serve as a target sequences for N*ad4L* homodimer and/or N*ad4L*/N*ad2* heterodimer DNA-binding site.

Preliminary *in silico* evidence suggests that mitochondrial proteins can also take part in gene regulation, acting as transcription factors. Further *in vitro* studies are necessary to establish the effect of these proteins in gene regulation process.

ST-08.01.4-009
(-)-Roemerine limits carbohydrate uptake which leads to alternative nutrients as acetyl-CoA sources

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The development of antibacterials with novel action mechanisms is a challenging task due to the rapid increase in the bacterial resistance mechanisms. In this sense, plants have drawn attention as natural sources for novel antibacterials. Alkaloid-containing natural compounds are candidates in this quest but the scarcity of information on their mode of action hampers their use. In the current study, the transcriptional changes in *Escherichia coli* TB1 following 1-hr treatment with (-)-Roemerine were investigated with microarray analysis. Genome-wide mRNA expression profiles were achieved using Agilent One-Color Microarray Based Exon Analysis. A set of 213 genes was found to be differentially expressed (P-value<0.05) and this gene set was further analyzed

using clustering and functional annotation tools. The presence of (-)-Roemerine significantly reduced the expression of outer-membrane channel proteins involved in carbohydrate up-take. This is consistent with previous reports, which claim that the starvation responses protect cells and maximize their chances for long-term survival. The down-regulation of the major nutrient influx gates forced cells to a reorganization for utilization of alternative sources. Here we got clues that (1) β-oxidation cycle of fatty acids and (2) degradation of phenyl acetic acid from amino acids serve as alternative sources of acetyl-CoA. Acetyl-CoA thus synthesized can further be metabolized through TCA cycle for generation of energy.

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ST-08.01.4-010
Sodium borate induced proteomic changes in hepatocellular carcinoma, Hep3B was evaluated by using nanoHPLC-captivespray-qTOF

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Sodium borate (NaB) was known to interact with NAD^+, an essential coenzyme for sirtuins which function as NAD^+-dependent deacetylase or ADP ribosyltransferase. They are involved in regulation of cell survival and longevity in response to the changes in cellular energy state upon stress condition like caloric restriction. Cellular NAD^+/NADH level is an indicator of energy homeostasis and driving factor for sirtuins to target proteins in a variety of biochemical pathways. In this study, serum starved hepatoma cell line, Hep3B was used in order to reveal the effect of NaB on its proteome.

Total cellular proteome (TCP) was harvested from Hep3B cells treated with 15 $\mu g \cdot mL^{-1}$ NaB for 72 h (optimized through cell viability assay) by using in-house formulated buffers. Mitochondrial proteome (MP) was prepared by following the protocol modified from *Cimen, H. et al.* (Biochemistry, 2010). TCP or MP TCP samples were analyzed with Thermo Dionextm UltiMatetm 3000 RSLCnano and with Bruker Compact mass spectrometry. Data were evaluated with ProteinScape 3. Immunoblotting studies with antibodies against the proteins identified from analyses were performed to confirm the changes of corresponding protein expression level in hepatoma resulted from NaB treatment.

We have identified an overall reduction in proteins related with mitochondrial functionality due to NaB treatment. Particularly, the reduction in the synthesis rate of respiratory chain complex subunits was confirmed with total OXPHOS cocktail. On the other hand, the interaction between NAD^+-NaB led to enhanced sirtuin activity leading to reduced acetylome through the changes in NAD^+/NADH level of Hep3B cells.

The NAD^+-NaB interaction results in proteomic changes, particularly mitochondrial biogenesis, in hepatocellular carcinoma, Hep3B unveiling activity of sirtuins to reprogram cellular energy state. Treatment of cells with NaB or further improved compounds is expected to become indispensable to manipulate cellular metabolism.

ST-08.01.4-011
Molecular shield for succesful anhydriobiosis: proteomics analysis of the cells of an anhydrobiosis insect

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Anhydrobiosis is an phenomenon of survival under complete dessication, found, apart from plants, in several groups of invertebrates. Among them, is the largest and most complex organism – the sleeping chironomid *Polypedilum vanderplanki (Chironomidae, Diptera, Insecta)*. Ability to withstand the water loss, in the larvae of this midge is based on the complex mechanism, involving coordinated activity of a groups of key genes, allowing to form a "molecular shield" combined by sugar trehalose and protein components. In order to get deeper insight on the contribution of the proteins to anhydrobiosis, we conducted a proteomics profiling of both larvae of the midge and the cell culture derived from embryonic mass of the insect. We have employed iTaq method to quantify distribution and dynamic of peptides changes in the course of desiccation-rehydration. We have succesfully obtained 2045 peptides corresponding to 347 proteins in the larvae and 1978 peptides corresponding to 129 proteins in the cells of the chironomid. The analysis confirmed our previous observations of the strong impact of the genes located in "Anhydrobiosis-Related islanDs" in the genome – special regions containing multiple paralogs specific for the sleeping chiornomid and not found in other insects. More than 40% of the proteins abound-ant in the dry larvae and cells were represented by ARID-derived ones. At the same time, numbe of the proteins increased in response to the desication in the cell culture was lower that that of the whole larvae, suggesting the existance of tissue-speciific protein profiles required for succesful anhydrobiosis.

ST-08.01.4-013
The proteomics profiling of molecular targets of tocotrienols in human breast cancer cells

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Tocotrienols are the subgroup of the vitamin E family that are known to hardwire many biological processes. The isoforms of tocotrienol had been proven to possess numerous health benefits besides its potent anti-tumorigenic activities.

In this study, the changes of protein abundance in untreated human breast cancer cell line (MDA-MB-231) relative to changes that occurred following tocotrienols (γ and δ) treatments, were measured using label-free quantification analysis. The protein samples were subjected to peptide generation workflow and LC-MS/MS on Orbitrap-based mass spectrometer. The data obtained were analysed using the Proteome Discoverer and Perseus software packages for filtering, clustering, statistical bioinformatics analysis and functional classification of proteins.

The differential expression of the proteins revealed tocotrienol treatment affected the expression of apoptosis-mediated proteins. Overall, the data imply a cell death mediated mechanism that triggers proteins associated with the cell death pathway suggesting a therapeutic potential for tocotrienols in cancer microenviroment. The mechanism appeared to be different for γ- and δ-tocotrienols. Previous studies had showed that γ-tocotrienol having pronounced effects when compared to δ-tocotrienol. Our investigation found that these isomers have their own unique effect of the proteome of cancer cells and interestingly, also mediate some exclusive set of proteins such as the PRKC pro-apoptopic protein which is capable of selectively inducing apoptosis in cancer cells and sensitizing the cells to diverse apoptotic stimuli. The other apoptosis mediators such as the apoptosis inhibitor, API5 and the apoptosis-inducing factor 1, AIFM1 were also seen regulated.

The findings of this study had provided insights into functions and the mechanisms of action of tocotrienols in breast cancer which could further offer a novel and effective strategy for the treatment of malignant cells.

Miscellaneous

ST-Mis-024
Colocalization analysis shows that PH domain of BCR may play role in proteasomal degradation and clathrin-mediated endocytosis through proteins CTTN and USP1 but unlikely to influence cell adhesion

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BCR/ABL fusion protein is a hallmark of myeloproliferative disorders such as chronic myelogenous leukemia and acute lymphoblastic leukemia. The difference between these phenotypes of disorders is determined by the presense or absence of DH and PH domains of BCR. Earlier research determined 23 potential interaction partners of PH domain of BCR in K562 cells by mass-spectrometry. Among them are cortactin (CTTN), collagen type 4 (COL4A1), ubiquitin-specific protease 1 (USP1). CTTN is involved in cytoskeleton reorganization and clathrin-mediated endocytosis. COL4A1 regulates cell adhesion and proliferation through interaction with β-integrin. USP1 is resoponsible for deubiquitination of proteins which prevents their proteasomal degradation.

To determine the localization of these proteins in relation to PH domain of BCR, vectors pmKate2C-PH, pmCitrine-C3-PH, pECFP-C3-CTTN, pECFP-C3-USP1, pEGFP-C3-PH were constructed. Vector pmCherry-clathrin was a gift by Skrypkina (IMBG, Kyiv). For detection of COL4A1 rabbit polyclonal antibodies were used with anti-rabbit Cy3 secondary antibodies. HEK293T and K562 cells were transfected with appropriate vectors, fixed in 4% paraformaldehyde 24 h after transfections and stained by DAPI. Cells were imaged under Carl Zeiss 510 Meta microscope. Images were analysed in Fiji software.

USP1 and PH domain partially colocalize in the nucleus. CTTN, PH domain and clathrin colocalize in perinuclear region and in some areas of cytoplasm. We have not detected considerable colocalization between PH domain and COL4A1 in K562 cells.

Our data indicate that PH domain of BCR may recruit USP1 possibly preventing proteasomal degradation of BCR/ABL by deubiquitination. Colocolization of PH domain with clathrin and CTTN shows that BCR/ABL may be involved in receptor-mediated endocytosis consequently deregulating downstream signalling pathways. Absence of colocalization between PH and COL4A1 may indicate their interaction may require specific conditions.

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