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This addendum corrects errors in the Speed Talks and Poster sessions of the Supplement S1. The following abstracts were omitted by mistake from the original line-up. In addition, the abstracts P-02.03.3-002 and P-09.04.4-104 were truncated in the original document and are reproduced in full here.

OMITTED POSTER SESSIONS

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 Late-breaking abstracts and abstracts withdrawn after Congress cancellation are not included in this issue.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Abstracts submitted to the Congress are **not peer-reviewed**.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

** Each abstract has been given a unique number beginning with either the letters P or ST; the next part relates to the session in which the speed talk or poster will be presented.

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OMITTED POSTER SESSIONS

Tuesday 6 September 12:30–14:30

Autophagy: Regulation mechanisms

P-02.03.3-002
Apoptotic and necrotic effects of low dose bisphenol A in SHSY5Y neuroblastoma cells

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Bisphenol A (BPA) is a commonly used chemical in industry to make plastics. "Low-dose" term has been expressed for the first time in studies with BPA in 2001. The value of low dose was received as <1 μM for BPA in *in vitro* studies.

Nowadays majority of the population as a result of today's lifestyle exposured to low doses BPA chronically, thus importance of low-dose toxicity studies is revealed. In this study we aimed to examine cytotoxicity composed by low dose BPA in SHSY5Y cells in terms of appoptotic and necrotic effects.SH-SY5Y cells was seeded at 300.000 cells per well in 6-well plates and cultured in DMEM at 37°C with 5% CO2.SH-SY5Y cells were treated with low dose(1 pM, 1 nM) of BPA. Plate was incubated for 24 and 48 hours. After the incubation period, samples were pooled then washed with PBS in two times and 100 μl aliquot of cells from each sample was centrifuged at 12000 g, +4 °C for 2 minutes and resuspended in 100 μl annexin binding buffer added to 5 μl Annexin V and incubated at room temperature in the dark for 20 minutes. Then, samples were centrifuged and resuspended again in 100 μl of the same buffer and added with 1 μl PI at room temperature for 1–4 minutes and analysed at Tali® Image-Based Cytometer.

Reducing cell viability of low dose BPA in SHSY5Y neuroblastoma cells is revealed by MTT by our group in our previous studies. Cytotoxicity studies are conducted for 1 pm and 1 nm of BPA in 48 hours by taking into account this result. It is obtained that 1pM reduced cell viability to % 63 and 1 nM BPA decreased cell viability to %78. It is clearly ocuured via examinate of necrotic effects that group treated with 1 pM and 1 nM BPA was significiantly different from control group. It has observed group treated with 1 pM and 1 nM BPA has importantly difference compared control group.

The findings obtained from this study explain the cytotoxicity of BPA in SHSY5Y cells through necrotic and late apoptotic pathways.

P-02.03.3-005
Lack of Atg5 expression diminished apoptotic potential of cdk inhibitors due to increased Bcl-2 expression in MEF cells

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Autophagy is an intracellular degradation mechanism that is responsible for the delivery of unwanted cytoplasmic constituents such as misfolded proteins, damaged organelles or intracellular pathogens to the lysosomes, where the contents are degraded and recycled. Thus it is usually thought of as a survival mechanism activated upon conditions like nutrient deprivation and to be a delaying factor in chemotherapy. Purvalanol and roscovitine are strong apoptotic inducers which inhibit cyclin-dependent kinases (cdks) and lead to cell cycle arrest. In this study, moderate cytotoxic concentrations of these drugs (20 μM purvalanol and 30 μM roscovitine) induced autophagy regardless of Atg5 expression in mouse embryonic fibroblast (MEF) cells. Exposure of wild type and $Atg5^{-/-}$ MEF cells to each drug in a time-dependent manner resulted in a significant decrease in p62 protein levels but we did not observe the expected beclin-1 upregulation due to autophagy induction. In the interplay between apoptosis and autophagy, Atg5 and Bcl-2 are thought to be crucial players. While Atg5 is cleaved by calpains upon death stimuli and this truncated form of the protein triggers the intrinsic mechanism of apoptosis, Bcl-2 binds to Beclin-1 and inhibits its interaction with proteins necessary for autophagosome formation. We found that anti-apoptotic Bcl-2 was upregulated as an early response within 3 hours following cdk inhibitor treatment in $Atg5^{-/-}$ MEF cells, but not in wild types. Moreover, annexin V-PI staining data from flow cytometry have shown a higher survival rate of $Atg5^{-/-}$ cells following drug treatment compared to increased death population ratio in wild type cells, which correlates with the anti-apoptotic function of Bcl-2 in $Atg5^{-/-}$ cells. Although Bcl-2 upregulation decreased apoptotic efficiency of drugs in lack of Atg5, it did not alter autophagy response in these cells. Therefore Bcl-2 supported cell survival function of autophagy related to Atg5 in non-canonical pathway.

P-02.03.3-006
EBR induced macroautophagy regardless of Atg5 expression

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Epibrassinolide (EBR) is a member of brassinosteroids, plant hormones with a structural similarity to mammalian steroids that play an important role in cell proliferation. However our previous data suggested that EBR induces ER stress and activates various pathways that leads to programmed cell death (PCD) and autophagy in cancer cell lines. Autophagy is a process of self-degradation that delivers cytoplasmic constituents to the lysosome. In this process Beclin-1, LC3 and ATGs (autophagy related genes) are key molecules in formation of autophagosome membrane, which are triggered by ULK-1 signalling. Autophagy consists of the sequestration, transportation to lysosomes and degradation of many cellular substance. We found that EBR treatment (30 mM) triggered autophagy and apoptosis in various cancer cell lines. We observed the same results in $Atg5^{-/-}$ MEF cells, which are known to be autophagy deficient. Therefore, EBR induces autophagic response regardless of ATG5 protein, which is known to be one of the key proteins to form autophagosomes. The upregulation of autophagy inducing proteins and LC3 following EBR treatment was also observed. We found that EBR treatment (30 mM) caused less than 30% cell viability loss in a time dependent manner. We continued with the same dose (30 mM) of EBR treatment to compare the results with cancer cell lines. Hyperactivation of autophagy mediates SAPK/JNK

protein levels. We suggested that EBR induces autophagic cell death via activation of SAPK/JNK in colon carcinoma cell lines. Therefore, autophagy and death-asssociated protein kinase pathway should be discussed in the future work.

P-02.03.3-007
Fine tuning of selective toxicity of anti-tumorigenic compounds

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In abnormal cells with defects in apoptosis, autophagy allows prolonged survival. Recent studies of cytostatic effects of model compound, trioxohydroxytetrafluorotriborate (BoF), selective cytotoxicitywas observed in relation to the availability of cellular calcium ions. In order to assess the mode of action of BoF gene expression array results were analysed using IPA. The large number of genes affected by BoF are in specific autophagy induced apoptotic pathway. SALSA RT-MLPA kit for apoptosis revealed overexpression of genes involved in mitochondrial mediation of apoptosis at concentration of 0.25 mg/ml.

In this presentation the role of each specific gene observed as deregulated after treatment with BoF is evaluated within the light of recent autophagy research findings that suggest inhibiting the transfer of calcium ions to mitochondria is toxic to cancer cells, which "suggests that calcium addiction by mitochondria is a novel feature of cancer cells". Cancer cell proliferation and apoptosis depend on the intracellular Ca^{2+} concentration, and the expression of numerous ion channels with the ability to control intracellular Ca^{2+} concentrations has been correlated with cancer. In the experiment with controlled depletion of ER- to-mitochondria Ca^{2+} transfer in autophagy driven environment, autophagy itself is not sufficient for survival in tumor cells as oppose to non-tumor cells. These findings suggest that tumorigenic cell dependance on constitutive transfer of Ca^{2+} to mitochondria could be a promising new target for antitumor treatments.

P-02.03.3-008
c-Jun has critical role in CDK inhibitors-induced reactive oxygen species-dependent autophagy and apoptosis in prostate cancer cells

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Purvalanol and roscovitine are cyclin dependent kinase (CDK) inhibitors and roscovitine is currently being used in phase I clinical trials in the treatment of cystic fibrosis. A number of reports-showed anti-tumor activities of purvalanol and roscovitine in several types of cancer is mainly attributable to their capacity to induce the apoptotic cell death of tumor cells. In this study, we showed that both CDK inhibitors have different response on MAPK signal mechanism in the induction of autophagy and apoptosis in LNCaP, DU145 and PC3 prostate cancer cells. Although purvalanol downregulated c-Raf, p38, Erk1/2, Sapk/Jnk, roscovitine did not show the same effect. The downstream targets of JNK include the transcription factor c-Jun, which was upregulated with CDK inhibitors is related with activation of p62 and AMPKα to induce autophagy in prostate cancer cells. Both purvalanol and roscovitine induced autophagy as well as apoptosis through reactive oxygen species (ROS) generation, which is mediated by JNK activation and AKT/mTOR inactivation in prostate cancer cells. Furthermore, induction of autophagy by co-treatment of rapamycin with CDK inhibitors had inhibitory effect on MAPK signaling pathway as well as could decrease production of ROS. Interestingly, although co-treatment of rapamycin with purvalanol downregulated expression of c-Jun, roscovitine and rapamycin treatment upregulated. To determine the relationship of MAPK activation and ROS generation in purvalanol and roscovitine-induced autophagy, cells were co-treated with U0126, MEK inhibitor, and N-Acetyl cysteine (NAC). After that LC3-II expression was analyzed by westernblotting. Inhibition of MAPK signals pathway downregulated purvalanol and roscovitine-induced c-jun as well as ROS generation. However, the rate of late apoptosis was increased after co-treatment of MEK inhibitor with CDK inhibitors. Therefore, these results indicated that purvalanol and roscovitine induced autophagy and apoptosis through distinct mechanism in the way of c-Jun expression and ROS generation.

P-02.03.3-009
A study on the role of autophagy in differentiated PC12 cells

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Autophagy is an essential cellular pathway for degradation of macromolecules and organelles. Whether active autophagy has beneficial or unfavorable consequences remains a controversial issue in neurodegenerative disorders. Cytidine-5'-diphosphocholine (CDP-Ch), an intermediate in the biosynthesis of membrane phospholipids, is known to have neuroprotective effects. In this study, we aimed to investigate the effect of CDP-Ch treatment on the autophagic machinery in nerve growth factor (NGF) -differentiated PC12 cells. We analyzed several autophagic markers including LC3II/I, p62, BECN1, ATG5-ATG12, ATG7 by Western blot analysis and confocal microscopy in the presence and absence of CDP-Ch. We also used the autophagy activator Rapamycin (mTOR inhibitor) and the autophagy inhibitor 3-methyladenine (3-MA). An immediate alteration in p62 expression was observed in the initial 6 hours (h) of treatment with NGF. We observed changes in the level of LC3-II following 72 h of CDP-Ch treatment. Furthermore, we examined changes in cell viability (MTT test) and Lactate dehydrogenase (LDH) levels due to CDP-Ch treatment of PC12 cells. We are currently studying the effects of CDP-Ch treatment on mitochondrial membrane potential, mitochondrial oxidative phosphorylation and mitochondrial morphology. This study will help to explain the mechanism of action of neuronal autophagy and thereby contribute to the design of new therapeutic strategies for treatment of neurodegenerative disorders.

This work is being supported by The Scientific And Technological Research Council Of Turkey (Grant number:114Z494).

P-02.03.3-010
Effects of tyrosine kinase inhibitor Imatinib on autophagy in BCR-ABL positive leukemia cells

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Better understanding of programmed cell death mechanisms is important in determining treatment strategies and designing drugs for malignant diseases. Chronic myeloid leukemia(CML) is a clonal myeloproliferative disorder arising from the neoplastic transformation of the hematopoietic stem cell. The chimeric BCR-ABL gene resulting from the t(9;22)(q34;q11) translocation is considered the primary genetic defect in CML and confers the malignant cells resistant to programmed cell death (PCD). Research on the molecular mechanisms that tyrosine kinase inhibitors(TKIs) activate in CML cells mostly focus on apoptosis as most well known programmed cell death type. The roles of other PCD pathways that effect cellular response to TKIs have been less illuminated

Aim of this study is to investigate the role of autophagy in CML biology and in resistance to therapy. Imatinib was used as being the most commonly used TKI in CML therapy. By applying increasing concentrations of Imatinib to the CML cell line K562, we generated an Imatinib-resistant K562 subclone (K562-Ir). Autophagy was evaluated in sensitive and resistant K562 cells after treatment with Imatinib. Cells were transfected with LC3B-RFP containing Bac vector, after which the fluorescence intensity of the autophagic marker LC3B protein was measured by flow cytometry. Additionaly, autophagic marker protein expression levels were compared between sensitive (K562) and resistant (K562-Ir) cells *via* Western blot experiments.

Cell percentages of measured fluorescence intensity was 1.15% in K562 control cells; 1.73% in 1 mM Imatinib treated K562 control cells; and 9.63% in 10 mM Imatinib treated K562-Ir cells. When compared with control groups, it is evident that Imatinib resistant K562 cells have higher autophagy rates. These results may indicate that autophagy is an important player in resistance against TKIs used in CML. Our results imply that resistant cells exploit a survival pathway mediated *via* autophagy.

P-02.03.3-011
Crosstalk between BAG-1, Bcl-2 and Beclin 1 can be a determinant for autophagy involvement in the apoptosis mechanism

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BAG-1 as an anti-apoptotic protein belongs to BAG family and has a significant role in important regulatory cellular pathways with its alternatively translated isoforms. These isoforms, which localize in different parts of the cell, interact with several molecular targets to modulate vital metabolic pathways including transcription, apoptosis, cell proliferation, hormone action and cell migration. One of the partner proteins of BAG-1 is an anti-apoptotic Bcl-2, that can interact with multiple survival and apoptosis related partner proteins, like the major authophagic protein Beclin 1. Expression levels of Beclin 1 and Bcl-2 are key aspects in the switch between autophagy and apoptosis in the cell. Studies in recent years revealed that understanding the relation between autophagy and apoptosis is necessary for the improvement of cancer therapeutics. In this line, we aimed to investigate BAG-1 effect in the Beclin 1-Bcl-2 crosstalk and also understand the details of this mechanism in cell survival/ death in breast cancer cells. We observed that c-Myc, c-Raf and Akt proteins, which are responsible for cell survival, are upregulated in BAG-1 overexpressed cells. On the other hand, overexpression of BAG-1 leads to increased Beclin 1 phosphorylation and upregulation of autophagic proteins like Atg7, Atg16 and Atg5 in a time dependent manner, suggesting autophagy presence under cellular survival conditions. In addition, immunoprecipitation studies showed that BAG-1 and Beclin 1 can interact in MCF-7 and MDA-MB-231 breast cancer cells. As a conclusion, we think that BAG-1 interacts with Beclin-1 and influences the decision mechanism of cell survival or death via regulating the autophagy/apoptosis switch through the interactions with Bcl-2.

Tuesday 6 September 12:30–14:30

Extracellular matrix and metalloproteinases

P-02.07.5-001
Adaptor protein Ruk/CIN85 induces EMT, migration and invasiveness of mice breast adenocarcinoma 4T1 cells

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Introduction: Adaptor protein Ruk/CIN85 was demonstrated to be involved in essential cellular processes such as intracellular signaling, cell death, proliferation, motility, adhesion and invasion. The aim of this study was to examine Ruk/CIN85 role in 4T1 cells migration and Matrigel invasion and found possible molecules – EMT markers, dependent on Ruk/CIN85.
Methods: As a model to study the role of Ruk/CIN85 in 4T1 cells EMT, migration and invasion we used previously obtained 4T1 sublines with stable Ruk/CIN85 overexpression and downregulation. To monitor 4T1 cells migration and Matrigel invasion in real time we used the xCELLigence Real-Time Cell Analyser (RTCA) DP Instrument equipped with a CIM-plate 16. Expression levels of EMT markers were evaluated by Western-blot and/or real-time PCR. Visualization of EMT markers was performed by fluorescent microscopy.
Results: Ruk/CIN85 was demonstrated to intensify 4T1 cells real-time migration and Matrigel invasion. After analysis of EMT markers expression we identified strong interdependence of Ruk/CIN85 and vimentin and E-Cadherin: Ruk/CIN85 overexpression is accompanied with increased level of mesenchymal marker vimentin and decreased level of epithelial marker E-cadherin. Obtained data cooresponds to immunofluorescent imaging results.
Conclusions: Our data suggest that Ruk/CIN85 may control 4T1 breast adenocarcinoma cells migration and invasion as well as EMT.

This study was supported by SCOPES project funded by Swiss National Science Foundation (IZ73ZO_152361).

P-02.07.5-002
Determination of the substrate repertoire of ADAMTS2, 3, and 14 reveals their integrated functions in extracellular matrix organization and TGF-beta signaling

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ADAMTS2, 3 and 14 are collectively named procollagen N-proteinases (pNPs) because of their specific ability to cleave the aminopropeptides of fibrillar procollagens. Several reports also indicate that they could be involved in other biological processes, such as blood coagulation, development and male fertility but the potential substrates associated with these activities remain unknown. This work describes the application of cutting edge MS/MS mass spectrometry (N-TAILS technology) in the search for new substrates for ADAMTS2, 3 and 14. We have identified several novel substrates, specific or common to the three enzymes, highlighting their role in extracellular matrix organization and blood vessel development. Some of these substrates (fibronectin, C-propeptide of type III collagen, LTBP1, DKK3 and betaglycan) have been biochemically validated in various models *in vitro* and *in vivo*. The precise cleavage sites have been determined by Edman sequencing or by Amine Terminal Mass Spectrometry (ATOMS). N-terminomics and validation data have led to the identification of preferential cleavage sites for the three ADAMTSs. These results completely modify the current paradigm about ADAMTS2, 3 and 14 as our data clearly show that they can cleave many other components of the extracellular matrix as well as several regulators of the Wnt and TGF-beta pathways, with corresponding functional consequences in the response to TGF-beta stimulation in human dermal fibroblasts.

This work shows that ADAMTS2, 3 and 14 should henceforth be considered as proteases having an integrative function in many aspects of extracellular matrix deposition and remodeling. This original study based on high-throughput screening of substrates by proteomics opens the path to a better understanding of the pathophysiological functions of the overall ADAMTS family.

P-02.07.5-003
MMP 2 and MMP 9 expression levels in adipose tissues of obese patients

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The Matrix Metalloproteinase (MMP) is a neutral endopeptidase family that plays an important role in the development of obesity. In cardiovascular diseases, the levels of the MMPs in circulation appear as a potential biomarker. The MMP expression and activity are regulated by various factors such as insulin resistance and obesity. Regulating the adipose tissue and extracellular matrix (ECM) again is accepted as a part of the obesity pathophysiology. It is considered that the MMPs play a role in the remodeling of the ECM; and the MMP-2 and 9 may also have an abnormal relation with the ECM metabolism. Studies still continue in order to learn the other roles of the MMPs. The MMP-2 and MMP-9 are the important genes that are studied to examine the mechanism of obesity.

The aim of this study is to determine the expression of MMP-2 and MMP-9 gene expressions and protein levels in adipose tissue of the obese group and the healthy control group of Turkish population.

In this study, 30 obese patients and 30 healty controls were used. Gene expression levels were determined by real time quantitative polymerase chain reaction (RT-qPCR), protein levels were measured by ELISA method.

The body mass index (BMI) fasting insulin, total cholesterol, triglyceride and LDL cholesterol levels were found to be significantly higher in obese patients than in control ($p < 0.001$). The MMP-2 and MMP-9 gene expressions were found to be decreased in obese group according to controls ($p = 0.004$, $p = 0.045$) respectively. There was a significant relationship in MMP-2 and MMP-9 protein levels between the adipose tissues of obese patients and controls ($p = 0.001$, $p = 0.003$) respectively.

As a result we suggest that MMP-2 and MMP-9 genes and protein levels may be associated with obesity however further studies including larger number of subjects should confirm our findings.

P-02.07.5-004
Predictive value of tissue inhibitor of metalloproteinases-1 in response to radioiodine therapy

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Thyroidectomy followed by radioiodine (I-131) ablation of the residual thyroid tissue is considered the ideal treatment for patients with papillary thyroid cancer (PTC) or papillary thyroid cancer associated with Hashimoto's thyroiditis (PTC+HT). Despite some progress in recent years, relatively little is known about the radiation-induced proteins expression in vivo.

In this study, matrix metalloproteinases (MMPs) together with their tissue inhibitors (TIMPs), involved in tissue remodeling after I-131 therapy, have been examined in 51 patients (8M/46F) with PTC and 38 (3M/38F) with PTC+HT. Peripheral blood samples were collected just before and, subsequently, at 4 days after I-131 administration (3.7 GBq). PTC+HT patients had positive titers of anti-thyroglobulin autoantibodies (TgAb). The serum levels of TgAb, MMP-2, MMP-9, TIMP-1 and TIMP-2 were measured by ELISA.

There were no significant changes in serum concentrations of MMP-2, TIMP-2 and MMP-2/TIMP-2 ratio after I-131 in the two groups. In PTC patients, I-131 administration resulted in an increase with 26% in TIMP-1 level ($p = 0.005$) and a reduction with 44% in MMP-9/TIMP-1 ratio ($p = 0.003$). In PTC+HT patients it has been observed an increase with 18% in TgAb level ($p = 0.001$), 5% in MMP-9/TIMP-1 ratio ($p = 0.003$) and unchanged TIMP-1 serum concentration. TgAb titers were positively correlated with MMP-9/TIMP-1 ratio ($r = 0.51$, $p < 0.001$).

Our data suggest that radioiodine therapy for PTC patients, but not for PTC+HT, modulates the balance of MMP-9/TIMP-1 for anti-invasion and anti-migration by augmenting TIMP-1.

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Wednesday 7 September 12:30–14:30

Mechanisms and regulation of protein translocation

P-02.04.4-001
The role of canonical and non-canonical NF-κB pathway in growth hormone-mediated resistance mechanism against curcumin induced apoptosis in MDA-MB-231 breast cancer cells

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Curcumin (diferuloylmethane), an antioxidant polyphenol that is isolated from the plant turmeric (*Curcuma longa*) has been shown to trigger apoptosis in various cancers such as breast, lung, leukemia, prostate cancer. The molecular machinary of curcumin-induced apoptotic cell death via inhibition of NF-κB and its downstream gene products including c-myc, Bcl-2, COX-2, CyclinD1, Bcl-x_L and MMP-9. Thus, in this study, our aim is to clarify the role of NF-κB signaling in growth hormone (GH)-induced resistance profile against curcumin induced apoptosis in MDA-MB-231 breast cancer cells. In order to generate GH over-expressed MDA-MB-231 breast cancer cells, GH inserted PC3.1 plasmid was transfected via liposomal transfection and neomycin selection. Curcumin decreased cell viability in dose- and time-dependent manner in both cell lines. 25 μM curcumin inhibit cell growth and colony formation and trigger apoptotic cell death. In addition, curcumin (25 μM) downregulated Bcl-2 and Bcl-x_L expression and upregulation of PUMA and Bax protein in both cell line. Moreover, curcumin induced dephosphorylation of IKKa/β and IKBa and downregulate NF-κB and IKKa in MDA-MD-231 wt and GH+ breast cancer cells. Thus, although GH induced metastatic profile in MDA-MB-231 breast cancer cells, GH-induced resistant profile overwhelmed by curcumin treatment *via* inhibiting nuclear NF-KB migration.

Acknowledgment: This study was supported by TUBITAK 1001 research project (Project No: 113Z791).

P-02.04.4-002
Involvement of sphingolipids in plasma membrane CFTR stabilization: a new possible therapeutic strategy for cystic fibrosis lung disease

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Cystic fibrosis transmembrane conductance regulator (CFTR) is a chloride channel, expressed at the apical surface of epithelial cells. Mutations in CFTR gene cause Cystic Fibrosis (CF), an autosomal recessive disease characterized by severe lung disease, due to the loss of CFTR at the cell plasma membrane (PM). Many pharmacological agents have been designed to increase the surface level of mutated CFTR (correctors), as well as its PM stability and activity (potentiators), even if their efficacy seems to be time-limited in particular for the most common CF-causing mutation F508del. Several factors contribute to the PM CFTR stability, including its compartmentalization in the sphingolipid (SL)-enriched lipid rafts and interaction with the scaffolding protein ezrin. Based on these findings, we investigate the effects of potentiators and correctors on CFTR PM microenvironment.

We analysed the SL composition and the phosphorylation state of ezrin in CF and non CF bronchial epithelial cell lines, treated or not with VX-809 (corrector) and VX-770 (potentiator). In addition, in both cell lines we evaluated the SL pattern of lipid rafts.

In both cell lines treated with VX-809 and VX-770, we observed a significant reduction of phosphorylated ezrin, which is a component of lipid rafts. Interestingly, even if the total cell membrane SL content did not change, in lipids rafts from both treated cells, we found a marked increase of all SL species, in particular ceramide, glucosylceramide and ganglioside GM3 which could be responsible for the ezrin dephosphorylation.

These preliminary results indicate that combined treatment with corrector/potentiator induces modification in lipid rafts organization in terms of proteins and lipids, that could limit the stability of mutated CFTR at PM level. These results could permit the development of new therapeutic strategies for CF treatment.

Supported by: Italian Cystic Fibrosis Research Foundation (grant FFC #09/2015 to AT and MA).

P-02.04.4-003
New approaches in cellular microbiology; endoplasmic reticulum stress and apoptosis in infections

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Endoplasmic Reticulum (ER) organelle in eukaryotic cells is the major organelle in the synthesis of protein and post-synthesis modifications. The protein production capacity increases in the ER organelle as a result of physiological events such as cell differentiation or compliance with certain environmental conditions. If this situation becomes chronic, the balance between the production and folding rates of proteins in ER organelle is destroyed, and the table called ER stress arises.

Cells activate the UPR (Unfolded Protein Response) mechanism to minimize the damage caused by unfolded or misfolded proteins arising as a result of ER stress. The response to be given by UPR to ER stress is most likely to drag the cell to the apoptosis. UPR becomes active through three ER transmembrane proteins, PKR-like ER protein kinase (PERK), inositol-requiring protein 1 (IRE1) and activating transcription factor 6 (ATF6).

The viral glycoproteins synthesized at quite high rates are shown as the primary candidate of ER stress formation in viral infections. Although ER stress in viral infections has been shown by strong evidences, some viruses trigger ER stress while some viruses suppress it.

In previous studies performed on bacteria, the relationship between ER stress and UPR was demonstrated especially in a group of Streptococcus infections. However, despite everything, the connection between bacterial pathogens and UPR has not been completely explored and enlightened. Different responses to ER stress arise in the infections of different species in bacterial infections as in viral infections.

There are new studies performed recently for the fact that parasite triggers ER stress in plants/animals which are infected by it

more than itself through chemical secretion in parasitic infections. For instance, about 40 different terpenoids develop after Nematocidal activity, and some of these are thought to be associated with ER stress.

P-02.04.4-004
The effect of dual PI3K/mTOR inhibitor (PI-103) on phospho-forkhead box protein O1 and O3 (p-FoxO1/3) and a disintegrin and metalloprotease 10 (ADAM10) protein expression in HER2 + breast cancer cells

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PI3K/Akt/mTOR signaling pathway may be reactivated through different mechanisms in breast cancer. Akt activation triggers FoxO phosphorylation and FoxO translocates to the cytosol while Akt inhibition allows FoxO retention in the nucleus where it activates receptor tyrosine kinase promoter. Up-regulation of ADAM10 expression in cancer cells may cause human epidermal growth factor receptor 2 (HER2) cleavage resulting in drug resistance. In this study, the effects of PI-103 on p-FoxO1/3 and ADAM10 protein expressions were investigated in HER2 + SKBR3 cells. The effects of PI-103 on HER2 and HER3 mRNA expression and protein activation were also tested.

SKBR3 cells were treated with 1 μM PI-103 for 8 and 24 hours and mRNA expression was determined by qPCR. Cells were treated with 1 μM PI-103 for 1-3-6-12-24 hours and protein levels were evaluated by Western blot.

HER2 and HER3 mRNA level did not change significantly due to dual inhibition. PI-103 treatment did not affect expression pattern of p-HER2 and p-HER3 in SKBR3 cells. ADAM10 protein expression was found to be stable in treated SKBR3 cells. Although p-FoxO1/3 protein expression decreased after PI-103 treatment for one hour, incubation for 24 hours resulted increase in p-FoxO1/3 protein expression.

We have shown previously that PI-103 treatment transiently blocked Akt phosphorylation. In accordance with transient Akt inhibition, mRNA expressions of HER2 and HER3 were not induced via nuclear retaining FoxO1/3. Reversible pathway inhibition did not lead to any change on p-HER2 and p-HER3 and ADAM10 protein levels. The steady state ADAM10 expression in cells may exclude the possibility of increased HER2 truncation after PI-103 treatment. Decrease of p-FoxO1/3 expression following one hour treatment reflects Akt inhibition and retention of FoxO1/3 in the nucleus. FoxO1/3 phosphorylation via Akt recovered in a time dependent manner. Reactivation mechanisms of PI3K/Akt pathway are critical on cell fate in breast cancer.

Wednesday 7 September 12:30–14:30

Intracellular organization

P-02.05.5-001
Nicotinic acetylcholine receptors in mitochondria: structure, origin, mode of functioning

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Nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels mediating fast synaptic transmission in neuromuscular junctions, autonomic ganglia, and different neuronal pathways. In addition, nAChRs are found in many non-excitable cells to regulate cell viability, adhesion or proliferation. Recently, the presence of α3β2/β4, α4β2 and α7(β2) nAChRs has been demonstrated in mitochondria where they regulate the early stages of mitochondria-driven apoptosis. However, the detailed structure, mode of functioning and biosynthesis of mitochondrial nAChRs remain unknown.

We performed lectin-ELISA studies to show that mitochondrial α7-containing nAChRs differ from those expressed in the plasma membrane by glycosylation profile: mitochondrial nAChRs contained more sialic acids and more fucose residues. We suggest that carbohydrate residues may be a targeting signal directing the new-synthesized nAChR molecules to mitochondria.

Previous studies demonstrated that nAChR signaling in mitochondria is ion channel-independent, and instead includes mitochondrial kinases and may be triggered by the binding of either agonists or antagonists. We performed a set of studies with positive allosteric modulators (PAMs) of α7 nAChRs and found that the binding of 3-furan-2-yl-N-p-tolylacrylamide (PAM-2) completely blocks the release of cytochrome c from mitochondria under the effect of 0.9 μM Ca^{2+}. These studies indicate that conformational changes induced by PAM-2 binding are sufficient to trigger intramitochondrial signaling of a7 nAChRs.

Finally, we performed studies of mitochondria isolated from knockout mice lacking α3, α7, α7β2, α5 or β4 nAChR subunits and found that the most critical for mitochondria are α7 and β2 subunits, because their absence was compensated with significant increase of β4 subunits.

Overall, our data help better understand the ways of nAChR delivery to and functioning in intracellular organelles.

P-02.05.5-002
Novel benzothiazole acetamide derivatives induces apoptosis in A549 lung adenocarcinoma

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Cancer, which is characterized by a shift in the control mechanism of proliferation and differentiation in normal cells, has increased dramatically in the last decades. Chemotherapy is one of the most widely used treatment regimens in cancer. In this present study, we aimed to investigate the possible underlying apoptotic mechanism for the cytotoxicity of new 2-[(1/4-methyl-(imidazol/benzimiazol/triazol-2/3-yl)thio]-*N*-(6-substituted benzothiazol-2-yl)acetamide considering anticancer activity of

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benzothiazole ring moiety. A549 lung adenocarcinoma cell lines were used in the studies. The cytotoxic activities of the tested compounds were determined by cell proliferation analysis using standard (3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Detection of apoptosis was performed using Annexin V FITC/PI apoptosis detection kit and measured on FACS ARIA I cell sorter flow cytometer. The IC_{50} values of the compounds were determined for A549 cell lines. Compounds 4 and 5 bearing imidazole ring and chloro, floro substituents on benzothiazole ring and also compounds 15 and 18 bearing benzimidazole ring and methoxy, ethoxy substituents on benzothiazole ring had significant cytotoxic activity with IC_{50} values lower than 118 μg/ml. Compound 5 showed the highest cytotoxic activity with IC_{50} value of 73 μg/ml, whereas cisplatin IC_{50} value was 17.3 μg/ml against A549 cells. Cytotoxic activity of compounds 4, 15 and 18 were found with IC_{50} values 115, 118 and 87 μg/ml, respectively. Compound 8, 15 and 18 showed the highest population of early and late apoptotic cells as 11.4, 7.3, and 19.1% respectively compared to cisplatin (21.0%). It was concluded that synthesized compounds had considerable anticancer activity against A549 cell lines. However compounds 4, 5, 15 and 18 including chloro, floro, methoxy and ethoxy substituents were the most active compounds against the A549 cell line.

P-02.05.5-003
Some triazine-benzothiazole derivatives have cytotoxic and anticancer properties on A549 lung adenocarcinoma

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Triazine derivatives and benzothiazoles show antitumor activities. Certain azanucleosides, 6-azauracil and 6-azacytosine), structurally based on 1,2,4-triazine nucleus have displayed an impressive array of biological activities, among which antitumor, antiviral, antimicrobial, anti-inflammatory, antiplatelet, antimalarial, and antifungal properties. It's known that thiazoles have also different biological activities including anticancer effect. In this study, we aimed to investigate the cytotoxic, apoptotic and enzymatic properties of series of novel 1,2,4-triazine derivatives bearing benzothiazole moiety against lung adenocarcinoma cell line (A549 cells) which used in our studies. The cytotoxic activities of the tested compounds were determined by cell proliferation analysis using standard (3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Apoptosis analyses were performed using BD Pharmingen Annexin V-FITC/PI apoptosis detection kit, according to the manufacturer's instruction. All measurements were performed on BD FACS Aria (I) cell sorter flow cytometer. The IC50 values of the compounds were determined on A549 cells. Benzothiazole including compounds and 6-methylbenzothiazole moieties, had significant cytotoxic activities. Cytotoxic activity of compounds LN6, LN7 and LN8 were found with IC50 values 12, 154 and 393 μg/ml, respectively. First compound (LN2) showed the highest cytotoxic activity with a IC50 value of 12 μg/ml whereas Cisplatin IC50 value was 17.3 μg/ml for A549 cells. Also, apoptotic effects of LN6, LN7 and LN8 on A549 cells were determined using flow cytometer. Our study results demonstrated that synthesized compounds LN6, LN7 and LN8 had considerable anticancer activity against A549 cells compared to Cisplatin. And also their biological activities were evaluated on acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE).

P-02.05.5-004
Physical interaction of septin3 & p60-katanin and role of septin3 & Tau relation in this interaction

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Microtubule cytoskeleton is important for differentiation, stability and functioning of neurons. p60-katanin is a critical enzyme that severs microtubules to provide dynamicity for microtubules. Septin3 has been considered as the fourth cytoskeletal polymer and is expressed primarily in neurons. Although its function is not determined, by similarity it can form filaments and can regulate neuronal processes.

Here, we aimed to identify the physical interaction domains of p60-katanin and Septin3 based on our previous study in which their interaction was identified by Yeast Two Hybrid screening. Another neuron specific protein, tau may have regulatory function for p60-katanin and Septin3 interaction as in p60-katanin and microtubule interaction.

To identify the interacting domains, deletion constructs of each protein were prepared to use in co-IP experiments. Then, by immunostaining of primary neurons, localizations of p60-katanin and Septin3 were analyzed. Tau-Septin3 interaction was also analyzed by co-IP and immunostaining.

Findings indicated that GTPase domain of Septin3 protein is responsible for the interaction of Septin3 and p60-katanin. In addition, the microtubule interacting domain of p60-katanin mediates the interaction with Septin3. Immunostaining experiment showed that Septin3 and p60-katanin co-localize mainly in neuronal cell bodies, but Septin3 filaments were disrupted in regions where p60-katanin is concentrated. Co-immunoprecipitation and immunostaining results pointed out that Septin3 protein interacts with Tau intrinsically, whereas p60-katanin and Tau have no physical interactions.

These preliminary findings indicate that p60-katanin interacts with Septin3 like microtubules and it may sever the filamentous structures formed by Septin3. Furthermore, Tau may provide stability for Septin3 filaments and may regulate the interaction of p60-katanin and Septin3. Further investigations can elucidate unknowns in this dynamic interplay for neuronal processes.

P-02.05.5-005
Development of a new microbial biosensor based on conductive polymer / multiwalled carbon nanotube and its application to paracetamol determination

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In this study, a new microbial biosensor based on *Bacillus sp*. was developed for sensitive determination of paracetamol. The microbial biosensor was modified by using carboxylated multiwalled carbon nanotube (cMWCNT) and conductive polymer, polyaniline (PANI) in presence of glutaraldehyde on a gold working electrode. Paracetamol measurements by the microbial biosensor were carried out at 0.5 V applied potential with amperometric method. In the optimization studies of the microbial biosensor, the effect of (amount or concentration of) the bioactive layer components such as MWCNT, PANI, *Bacillus sp*. were carried out. In addition, working conditions of the microbial biosensor such as pH and temperature were also investigated.

In the characterization studies of the biosensor some parameters such as linearity, reproducibility, storage stability, substrate specificity and interference effect on the biosensor responses were determined. In the reproducibility experiments (n = 7), the average value, standard deviation, (SD) and coefficient of variation (CV %) were calculated to be 250.65 μM, ±1.55, 0.62% for 250 μM paracetamol concentration, consecutively. Detection limit was calculated as 2.9 μM by using visual evaluation method. In addition, paracetamol were carried out in drug samples by the developed biosensor.

P-02.05.5-006
Cell study of the conjugate of histone with photoactivated rhodamine

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Visualization of the intracellular transport of biopolymer conjugates and their distribution in cells is very important for biological applications. First, the method of cell staining by promising precursor PFD (tetramethylrhodamine derivative) of fluorescent dye Rho813 was developed: 1) 0.5 mg PFD-NHS was dissolved in 50 μl DMSO and added to the 200 ml of bicarbonate buffer (50 mM), containing 2 mg of Histone H1.3; 2) obtained mixture was stirred for 1.5 hour at 20°C; 3) mixture was neutralized by 0.1 N acetic acid and distilled water was added to 500 ml final volume. Succinimide group of PFD promotes formation of covalent bonds with primary amino groups of histone H1 to form conjugate histone H1.3 with PFD (HPFD). The conjugate "HistoneH1-PFD" was purified from mixture using Sephadex G-25 chromatography.

Optimal concentration of PFD solutions for cell staining was 5 μg/ml. High resolution images of various cell types were obtained after light-induced activation of PFD to Rho813 dye. The microphotographs of native and fixed cells in order to evaluate the intracellular distribution of the conjugate were obtained using the confocal microscope.

The conjugate HPFD, capable of bright fluorescence in the red region of the spectrum (after photoactivation) was synthesized by procedure described elsewhere. It is important that in amounts up to 0.25 mg/ml of histone or HPFD are not toxic to HeLa cells. The data on transportation and intracellular localization of the conjugate were obtained with cell lines: HEK293, A431, HeLa, HBL-100 and MDCK. The obtained HPFD is able to penetrate in all studied cells, interact with major organelles and stay as small aggregates. This work was supported by the Russian Scientific Foundation grant 14-16-00046. The authors anticipated that resulting conjugates can be used for further study of biopolymer and drug delivery systems into human and animal cells.

P-02.05.5-007
Transcytotic trafficking of sonic hedgehog in MDCK cells: role of the cytoskeleton

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Several polarized epithelial cells express and secrete the morphogen Sonic Hedgehog (Shh) during development and adult life. Evidence from whole invertebrate animal models suggests that Shh can be secreted both apically and basolaterally. The sorting mechanisms of Shh in polarized epithelial cells remain little understood, constituting the main objective of this study. We used MDCK cells permanently transfected or microinjected with Shh expression vectors. Cells were cultured in transwells chambers to obtain a fully polarized monolayer. Polarized traffic was assessed by apical/basolateral domain selective biotinylation-targeting assays, pulse chase targeting assays, immunofluorescence and live cell imaging. We found Shh mainly distributed and secreted apically. However, pulse chase targeting assays revealed that Shh is first sorted to the basolateral plasma membrane and then to the apical domain, from where it is released to the medium. Time course analysis showed Shh transcytosis through the Rab-11 apical recycling endosome (ARE). Following the fate of basolateral antibody-tagged Shh we found that latrunculin-A decreases its basolateral localization. Instead, nocodazole abrogates the appearance of basolateral antibody-tagged Shh at the apical cell surface. However in nocodazole treated cells, when Rab11-endosome is disrupted, basolateral biotinilated Shh is secreted apically. Combining biotinylation, immune-tagging and imaging assays we directly demonstrate transcytosis of Shh. Basolateral sorted Shh moves through Rab11 endosomes but apical secretion is independent of the endosome integrity. The effects of latrunculin A and nocodazole revealed a differential involvement of microfilaments and microtubules in this Shh transcytotic trafficking. All these results show a transcytotic route of Shh to the apical cell surface involving Rab11-ARE and a differential role of microfilaments and microtubules.

P-02.05.5-009
A PtdIns(4,5)P2 signaling network involved in the regulation of focal adhesion disassembly

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Cell migration and invasion require the regulated turnover of integrin-dependent focal adhesions, but the underlying mechanisms remain incompletely understood. We previously showed that the disassembly of focal adhesion complexes occurs through clathrin- and dynamin-dependent endocytosis of ligand-activated beta1 integrins from adhesion sites. Interfering with beta1 integrin endocytosis blocked adhesion turnover, increased focal adhesion size, and decreased the rate of cell migration.

To further define the molecular machinery involved in focal adhesion disassembly, we used a focused RNA interference sceen in combination with dominant negative approaches to target components involved in clathrin-mediated endocytosis.

Here, we report on the identification of several cytoskeletal components, signaling molecules, and Bin-Amphiphysin-Rvs (BAR) domain proteins necessary for focal adhesion turnover. The targeting of these proteins to focal adhesion sites is orchestrated, either directly or indirectly, by the spatially restricted

synthesis of phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P_2) mediated by the Type I phosphatidylinositol phosphate kinase beta (PIP5K1b).

Together, our findings identify new endocytic and signaling components required for focal adhesion disassembly and provide evidence for a fundamental role of PIP5K1b in the regulation of this process. Future studies are aimed at determining how integrin endocytosis for focal adhesion disassembly is spatially and temporally regulated during cell migration and how this impinges on PIP5K1b function and its effectors.

Wednesday 7 September 12:30–14:30

Human microbiome (microbiota)

P-02.06.4-001
Detection of biofilm formation in ESBL producing *Klebsiella pneumoniae* by three different methods

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Klebsiella pneumoniae is an important opportunistic pathogen frequently causing UTIs (urinary tract infections), septicemia and pneumonia in immune-compromised individuals. These strains also responsible for nosocomial epidemics are usually multiresistant to antibiotics, and most of them produce extended-spectrum β-lactamases (ESBLs). Biofilm formation in *Klebsiella pneumoniae* is a major virulence factor used to colonize the human host. This is a dynamic process and different mechanisms are involved in attachment and biofilm maturation where they are kept together by a self-produced biopolymer matrix. The aim of this study was to investigate the biofilm-forming ability of ESBL producing clinical strains.

A total of 100 non-repetitive clinical isolates of *Klebsiella pneumoniae* were collected from various clinics of Samsun Education and Research Hospital. Biofilm formation was detected by three different methods, Tissue Culture Plate (TCP), Tube method (TM) and Congo red agar method (CRA) which could be used in a routine clinical laboratory.

Biofilm formation of the clinical *Klebsiella pneumoniae* isolates was examined by three different methods. Among 100 isolates tested by TCP method, 1 (1%) were negative biofilm producer, 16 (%16) as weak, 17 (17%) as moderate and 66 (66%) as strong biofilm producers. By tube method, 24 (24%) were negative, 67 (67%) as weak, 6 (6%) as moderate and 3(%3) as strong biofilm producers. By CRA method, 52 (52%) were negative, 28 (28%) as weak and 20 (20%) as strong biofilm producers.

These results suggested that different methods used for detecting biofilm formation may show difference. But TCP method counted as a gold standart for biofilm detection. Also ESBL producing bacteria that make them even more able to form biofilms. Biofilm formation together with resistance mechanims like ESBL may pose a public health problem for the persons.

P-02.06.4-002
The effect of vaginal isolated *Lactococcus lactis* on oncomirs (miR-21 and miR-200b) and cell signaling in ovarian cancer cell line

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Ovarian cancer is the leading cause of death among gynecological malignancies. Numerous data suggests that oncomiRs and AKT/NF-κB signaling pathways are fundamental players in many steps of tumorigenesis, progression and aggressiveness of ovarian cancer. The use of probiotics has received great attention as an alternative, inexpensive, and natural remedy to restore and maintain health. Promising data from clinical studies on the effect of probiotics in cancer therapy have been reported; nevertheless, the molecular mechanism involved in the interaction of probiotics and host cells are still poorly understood. In the present study we aimed to evaluate the beneficial effects of vaginal isolated bacterium (*L. lactis*) on regulating AKT/NF-κB signaling pathways and expression of oncomiRs in CAOV-4 cell line. The vaginal isolated bacteria were cultured on MRS agar. 16s rDNA gene sequencing was identified L. lactis. Further investigations on probiotic potential of isolates were applied according the guidelines recommended by FAO/WHO. Flowcytometry assay using AnnexinV-PI staining was performed to distinguish apoptosis and necrosis which was further confirmed by DAPI staining. To study the effect of *L. lactis* on CAOV-4 cell motility, we used scratch assay. Real time RT-PCR was carried out for detection of the expression of oncomiRs, and some key mRNAs in CAOV-4 cell line co-cultured with *L. lactis*. *L. lactis* was able to induce apoptosis in CAOV-4 cells that were confirmed by DAPI staining and flowcytometry assay. Furthermore, inhibition of cell migration was validated by scratch assay. Over expression of some key members of AKT/NF-κB signaling pathway and oncomiRs were found to be significantly associated with tumorigenesis in ovarian cancer. Probiotic therapy by *L. lactis* that targets important signaling pathways involved in initiation and progression of ovarian cancer may provide promising supplements and valuable strategy in the treatment of ovarian cancer.

P-02.06.4-003
Influence of kefir on heat stress resistance and life span in *Caenorhabditis elegans*

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Microbiota affects human health. Kefir is fermented probiotic beverage. It contains very rich metabolites and a lot of microorganisms (lactic, acetic acid bacteria and yeast). Kefir may be produced traditionally from kefir grains and from lyophilised starter culture. There are studies about types, contents, qualities and health benefits of it. *C.elegans* is a wonderful model organism having with many aspects of experimental usefulness. Kefir grains were obtained from Akdeniz Univ. Engineering Faculty Dept. of Food Eng., and starter kefir were bought from a commercial company. Whole kefir microorganisms were used as the same way of *E. Coli* lawn on the Nematode Growth Medium. But kazein was precipitated and then the supernatant containing microorganisms were used. Fermentation of both kefir types were stopped when their pH ~4.6 that is optimal to consumption.

Microorganisms of kefir samples were calculated by colony forming units on specific agars. Life span of *C. elegans* under heat stress and normal life span were measured to find any heat stress response and longevity. There were statistically significant results in the both test groups of whole kefir samples produced with the grain and the starter culture under the heat stress. The average and maximum life spans under heat stress were higher, that is there was a thermotolerance. But under 20°C, life spans were not statistically significant. Probiotics are important provider of good microbiome. Our study showed an *in vivo* result supporting the health benefit of it. Two types of preparation procedures have showed the same results. But this study must be replicated, especially with each group of microorganisms in kefir and with each kind of strains in each group. However, kefir is a very complex microbiologic and metabolic medium and it is hard to study with it. So, there is more need to study kefir, especially in big volume, with different partnerships, in short time and in order to get more precise results.

P-02.06.4-005
Mobile phones as a reservoir of microorganisms

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Mobile phones are an indispensable form of communication used in our social life and in our professional careers. Our dependency on mobile phones together with its convenient size and mobile property makes it an object which comes into contact with not only the body but with various other surfaces. The objective of this study was to investigate the potential of mobile phones as a reservoir for microorganisms, and to determine whether alcohol wipes are sufficient at eliminating or reducing the number of microorganisms found on the surface of mobile phones. The mobile phones of 80 students were used in this investigation. To detect the presence of microorganisms, a sterile cotton swab was used to swab the front, back and sides of mobile phones and transferred onto Horse Blood Agar (HBA). The mobile phones were then cleaned using a commercial wipe (CHLORO SWAB) containing 70% isopropyl alcohol and 2% chlorhexidine. The mobile phones were then again swabbed in the same manner and transferred onto HBA. All agar plates were incubated at 37°C for 12-14 hours. Growth was assessed by counting the number of colonies present on HBA prior to and after the application of the wipe. By comparing the number of colonies on HBA prior to and after the application of the wipe, 98% of the mobile phones studied displayed some level of reduction in colony count. An 80-100% reduction in colony count was observed in 66% of the mobile phones studied while the remainder had a colony count reduction between 6 and 77%. The mobile phones tested harbored microorganism of various types (based on morphological assessment). This observation highlights the potential of mobile phones to be a major transmission source of microorganism to hands and other surfaces. The use of alcohol wipes serves as an efficient method to control the transmission of microorganisms from mobile phones to other surfaces. The outcome of the current study encourages the regular cleaning of mobile phones to be implemented.

Wednesday 7 September 12:30–14:30

Chemical and biochemical aspects of oxidative stress

P-09.04.4-104
Protective effect of borax and boric acid on total sialic acid and lipid-bound sialic acid levels against 3-methylcholanthrene and benzo(a)pyrene induced oxidative stress in rats

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The present study was performed to investigate total sialic acid (TSA) and lipid bound sialic acid (LSA) levels as possible in vivo chemoprotective effect of borax (BX) and boric acid (BA) against3-Methylcholanthrene (3-MC) and benzo(a)pyrene (B(a)P) induced oxidative stress in rats. The rats were divided into nine groups of six rats each. Group I: Control, untreated animals were given % 0.9 NaCl, Group II: The B(a)P were administered 25 mg/kg via ip. four times. Group III: The 3-MC-treated animals were administered 25 mg/kg via ip. four times, Group IV: BA was given 300 mg/l/day with water. Group V: BX was given 300 mg/l/day with water. Group VI: B(a)P 25 mg/kg via ip four times + BA 300 mg/l/day dosage with water. Group VII: 3-MC 25 mg/kg via ip four times + BA 300 mg/l/day with water. Group VIII: B(a)P 25 mg/kg via ip four times + BX 300 mg/l/day dosage with water. Group IX: 3-MC 25 mg/kg via ip four times + BX 300 mg/l/day with water. The experimental period was continued for 150 days. Statistical analysis showed that the 3-MC + BA group was significantly higher than the control group with regards to TSA and LSA levels $p < 0.001$, $p < 0.05$, 3-MC and B(a)P groups were also significantly higher than the control group regarding LSA level $p < 0.001$, $p < 0.01$. B(a)P group had increased level of TSA according to BA,BX groups $p < 0.01$, $p < 0.01$, and LSA $p < 0.05$. 3-MC group had increased level of TSA according to BA, BX groups $p < 0.001$, $p < 0.001$, and LSA $p < 0.01$, $p < 0.01$, 3-MC group had increased level of TSA according to B(a)P + BA, B(a)P + BX groups $p < 0.05$, $p < 0.001$, and LSA ($p < 0.05$), regarding B(a)P + BX and level of of TSA according to 3-MC + BX group ($p < 0.01$). Whereas, BA and BX groups had significantly lower level of TSA than B (a)P + BA and MC + BA groups $p < 0.05$, $p < 0.001$. It was determined that borax is more effective than boric acid in damage caused by 3-Methylcholanthrene and benzo(a)pyrene in rats.

OMITTED SPEED TALKS

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

RNA biology, biogenesis and processing

ST-01.02.2-001
Clinical significance of serum cmet and hgf protein and mRNA levels in lung cancer

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Lung cancer, also known as carcinoma of the lung or pulmonary carcinoma, is a malignant lung tumor characterized by uncontrolled cell growth in tissues of the lung. The main primary types are SCLC, and NSCLC. Hepatocyte growth factor (HGF) is a paracrine cellular growth, motility and morphogenic factor. Hepatocyte growth factor regulates cell growth, cell motility, and morphogenesis by activating a tyrosine kinase signaling cascade after binding to the proto-oncogenic c-Met receptor.c-met protein possesses tyrosine kinase activity. Indeed either the overexpression or the amplification of cMET, as well as the overexpression of the HGF, have been reported in lung cancer patients.In the current study, we aim to determine the serum levels of cmet/HGF by ELISA and mrna levels of this parameters by PCR verified lung cancer, healthy controls.

The serum samples of the 60 consecutive patients with lung cancer who referred to Istanbul University Institute of Oncology from 2015 to 2016 were obtained.The healthy control group consisted of 20. The cmet and hgf protein assay employs ELISA. The colored reaction product was measured using an automated ELISA microplate reader at 450 nm.For analyzing cmet and hgf specific mRNA in sera of the patients, cmet and hgf expression in RT PCR was performed in the LightCycler 480 Instrument.

Serum cmet ($P = 0.002$) and hgf ($P = 0.000$) protein levels and serum cmet ($P = 0.000$) and hgf ($P = 0.000$) gen expression were significantly higher in patients with lung cancer than the healthy controls.The correlation between protein and mRNA levels of cmet and hgf was statistically significant.

Our data indicate that cmet and hgf protein and gen can be used as a diagnostic parameters for lung cancer. These molecules are important in lung cancer targeted therapy. We believe they will be useful markers for clinicians to help decide the diagnosis and disease follow-up. of lung cancer.

ST-01.02.2-003
Transcription beyond borders has downstream consequences

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Transcription termination is emerging as an important component of gene regulation necessary to partition the genome and minimize transcriptional interference. In genomes where the density of genes is high it is clearly important to efficiently terminate transcription to prevent read-through into adjacent genes. In two separate studies, we showed (1) Arabidopsis RNA-binding proteins FCA and FPA play important roles in the *A. thaliana* genome in RNA 3′ processing and transcription termination, thus limiting intergenic transcription. We used whole-genome tiling arrays to show that a wide spectrum of genes and transposable elements are misexpressed in the *fca-9 fpa-7* (*fcafpa*) double mutant. There was a significant bias for misregulated genomic segments mapping to the 3′ region of genes. In addition, the double mutant misexpressed a large number of previously unannotated genomic segments corresponding to intergenic regions. We characterized a subset of these misexpressed unannotated segments and established that they resulted from extensive transcriptional read-through, use of downstream polyadenylation sites, and alternative splicing. In some cases, the transcriptional read-through significantly reduced expression of the associated genes. FCA/FPA dependent changes in DNA methylation were found at several loci, supporting previous associations of FCA/FPA function with chromatin modifications. (2) Arabidopsis RNA silencing enzyme DICER-LIKE 4 (DCL4) has a role in transcription termination of the *FCA* gene. DCL4 directly associates with FCA chromatin in the 3′ region and promotes cleavage of the nascent transcript in a domain downstream of the canonical polyA site. In a *dcl4* mutant, the resulting transcriptional read-through triggers an RNA interference–mediated gene silencing of a transgene containing the same 3′ region. In conclusion, DCL4 promotes transcription termination of the FCA gene, reducing the amount of aberrant RNA produced from the locus.

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

RNA biology, biogenesis and processing

ST-01.02.2-004
The relationship of obesity with adiponectin level and ADIPOQ gene variants among young adult women

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Recent investigations have emphasized the important role of certain adipose-produced proteins that involved in the regulation of the energy metabolism of the whole body. Adiponectin is an important adipokine that plays a role in regulating body metabolism and immune response. Adiponectin is encoded by the *ADIPOQ* gene. Variation in the *ADIPOQ* gene may affect Adiponectin level/activity thus influencing obesity. In this study, single nucleotide polymorphisms (SNPs) in the *ADIPOQ* gene (I146T and G276T) were genotyped to assess their effects on body mass index (BMI) of young adult women. The women were divided into underweight, normal, overweight and obese according to BMI. The circulating levels of adiponectin were measured using commercially available ELISA kits. Genetic polymorphisms were genotyped using the PCR-RFLP method. G276T and I164T SNPs are common in the examined population as the frequency of G allele of 276 SNP was 54.8 % and for T allele of 164 SNP it was 41.7 %. Circulating adiponectin levels were related to BMI and were lowest in the obese versus overweight, normal weight and underweight groups ($P < 0.01$). However, *ADIPOQ* gene SNPs (I146T and G276T) showed no association with BMI groups. In conclusion, the results may suggest that adiponectin level, but not *ADIPOQ* gene SNPs, is a good indicator to BMI in young adult women.

ST-01.02.2-005

Assemblies of RNA polymerase II C-terminal domain with processing factors investigated using integrative structural biology

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RNA polymerase II (RNAPII) is a eukaryotic enzyme responsible for transcription of protein coding and non-coding genes. RNAPII uses its long and flexible C-terminal domain (CTD) to recruit specific protein/RNA-binding factors for regulation of transcription. The CTD consists of tandem repeats of the consensus YSPTSPS. The CTD sequence is post-translationally modified in a dynamic manner, yielding specific patterns that are recognized by appropriate factors in coordination with the transcription cycle events.

To follow the structural assemblies of CTD with different effector molecules involved in transcription, we have developed a model system that allows mimicking the full-length CTD with the specific phosphorylation pattern. By combining this system with advanced nuclear magnetic resonance spectroscopy (NMR), X-ray crystallography, small angle X-ray scattering (SAXS) and cryo-electron microscopy we follow changes in structural behavior the CTD and respective binding factor at the atomic level resolution.

Here, we report a hybrid structure of a long CTD fragment bound to multiple copies of processing factors that are involved in the 3'-end processing and transcription termination.

The reconstruction of multisubunit complex with the 3'-end processing and transcription termination factor shows that the CTD retains its highly flexible character upon binding, forming a beads-on-a-string topology. However, the 3'-end processing and transcription termination factor dimerizes using a previously unknown dimerization domain. This dimerization event creates topological and mobility restraints, which in turn tunes its affinity towards the CTD and governs exposure of the CTD sequence to other protein factors.

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

Chemical and biochemical aspects of oxidative stress

ST-09.04.4-025

Thiol/disulphide homeostasis in untreated schizophrenia

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Introduction: The aim of the study was to investigate dynamic thiol/disulphide (SH/SS) homeostasis in untreated schizophrenia.
Material and Methods: Blood thiol/disulphide homeostasis status, which reflects native thiol-disulphide exchanges, was investigated in 87 untreated patients (52 males, 35 females), and the obtained results were compared with 86 healthy controls. Blood serum native thiol and total thiol (ToSH) concentrations were measured in a paired test. The half value of the difference between native thiol and ToSH concentrations was calculated as the disulphide bond amount.
Results: SH and ToSH concentrations were found to be significantly lower ($P < 0.001$ for both) in patients with untreated schizophrenia compared with the control group, whereas disulphide levels were significantly higher ($P < 0.001$). Schizophrenia patients had significantly higher SS/ToSH and SS/SH ratios and a significantly lower SH/ToSH ratio compared to those of healthy individuals.
Discussion and Conclusions: SH and ToSH amounts were found to be insufficient in untreated schizophrenia patients. Additionally, according to the results of the study, thiol/disulphide homeostasis was also disturbed by a shift to the disulphide bond formation side.

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

Miscellaneous

ST-Mis-023

Serum ischemia modified albumin levels in non-obese young women with polycystic ovary syndrome

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Introduction: Polycystic ovary syndrome (PCOS) is the most common endocrine disorder of reproductive-age women. The exact underlying pathophysiologic mechanism still remains unclear. It is well known that metabolic changes seen in PCOS patients are important risk factors for future cardiovascular diseases. Ischemia-modified albumin (IMA) is a marker of chronic hypoxia and oxidative stress. The available literature is conflicting with regard to whether serum IMA levels are increased in PCOS patients. The present study aimed to evaluate the serum IMA levels in non-obese young women with PCOS and compared to healthy controls.
Materials and Methods: A total of 82 non-obese young women with PCOS were included in this controlled cross-sectional study. 41 patients were enrolled in each group. The two groups were matched in terms of age and body mass index (BMI). The diagnosis of PCOS was made according to the Rotterdam criteria.
Results: There were no significant differences between the two groups in terms of demographics, markers of glucose metabolism, lipid profiles, and inflammatory markers including C-reactive protein and neutrophil -lymphocyte ratio. The mean Ferriman–Gallwey score and dehydroepiandrosterone sulfate level were statistically significantly higher in the PCOS group ($P < 0.05$). There was a significant positive correlation between serum IMA and BMI in all groups ($P = 0.013$). The levels of IMA were significantly higher in PCOS patients when compared to the controls (0.44 ± 0.12 vs. 0.35 ± 0.10, $P = 0.001$).
Discussion and Conclusion: We found that serum IMA levels were higher in non-obese young women with PCOS than in the age-BMI matched healthy controls. Elevated serum IMA level which is indicator of oxidative stress may play an important role in the pathogenesis of PCOS. This elevation may contribute to the increased cardiovascular diseases risk in PCOS patients.

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