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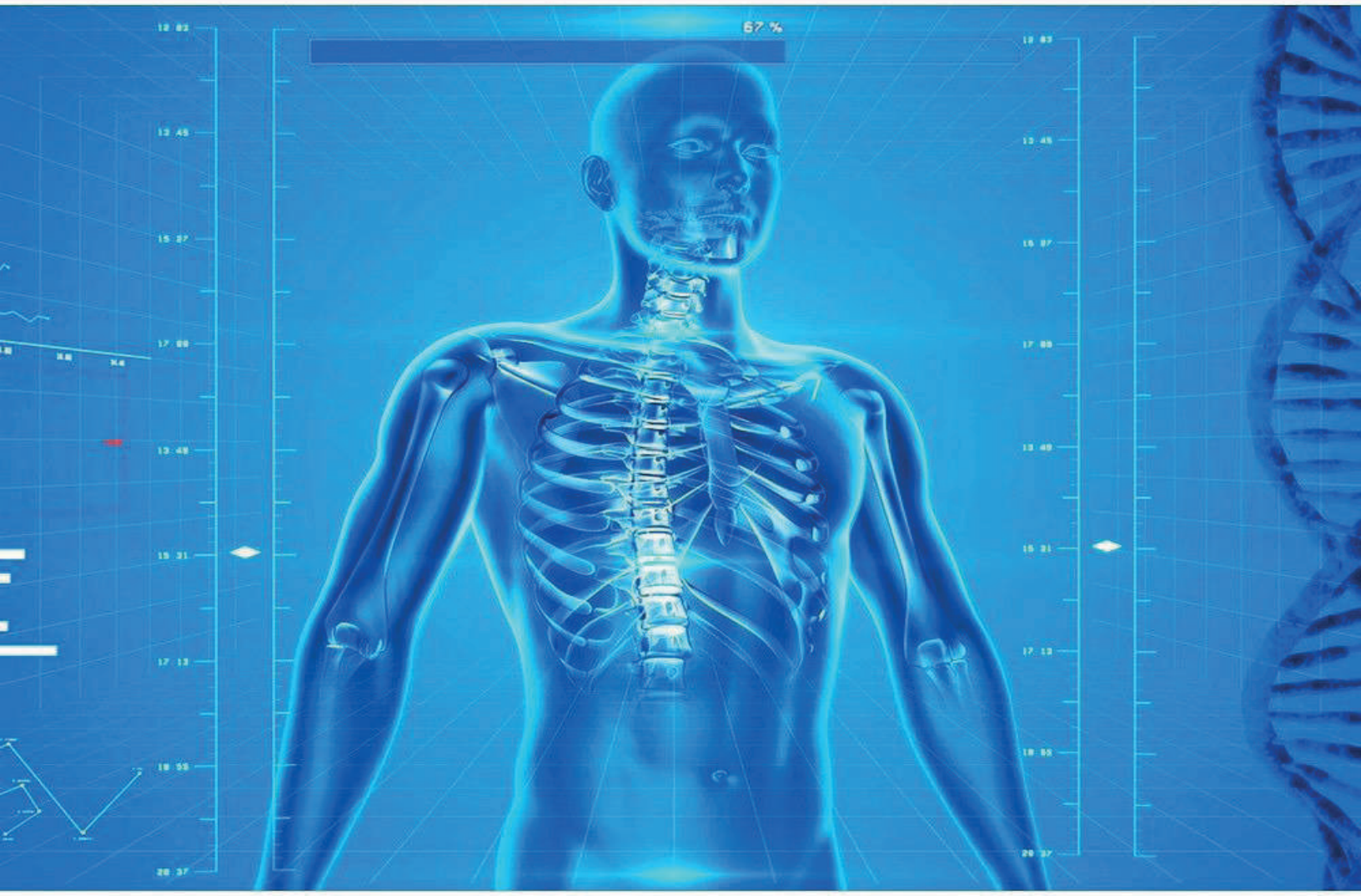

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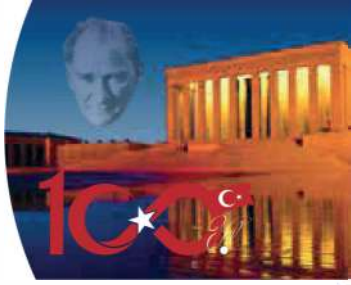

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ORAL PRESENTATIONS

OP-1

The relationship between transcription factor LEF1 and INSL5 in acute T-cell leukemia

Zeliha Emrence

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Objective: The Lymphoid Enhancer Factor 1 (LEF1) belongs to the LEF1/TCF (T-cell factor) transcription factor family. LEF1 functions as the main transcription factor of the WNT signaling pathway. Additionally, LEF1 has been shown to play a role in β-catenin-independent pathways such as TGF-β3 and Notch signaling. In our previous studies, we performed Chromatin Immunoprecipitation sequencing (ChIP-seq) using LEF1 antibody in Jurkat (Acute lymphoblastic leukemia) cells to identify binding regions of LEF1 on the genome. In this study, our aim was to demonstrate the relationship between LEF1 and INSL5.

Methods: Changes in INSL5 expression were examined using quantitative RT-PCR in Jurkat cells in which LEF1 was knockdown using siRNA and confirmed by western blot. Cells transfected with non-targetting siRNA (negative control siRNA) and cells grown under the same conditions without transfection were used as controls.

Results: The LEF1 expression was analyzed by quantitative RT-PCR in twenty-four hours after siRNA transfection. Cells treated with LEF1 siRNA showed a 76% (4.1-fold) suppression of LEF1 expression compared to LEF1 non-targeting siRNA. Changes in INSL5 expression were tested by quantitative RT-PCR when LEF1 was suppressed. Cells with LEF1 knockdown using siRNA showed a 73% reduction in INSL5 expression compared to the siRNA negative control.

Conclusion: The results of this study indicate that INSL5 could be a target of LEF1. Our study will contribute to a better understanding of LEF1 regulated pathways and the transcriptional regulatory network.

Keywords: Acute leukemia, INSL5, LEF1, jurkat cell line, siRNA.

Acknowledgement: This study was supported by Istanbul University Scientific Research Projects Unit. Project Code: 3092

OP-2

Neuroprotective activities of the two xanthones isolated from the roots of Polygala Azizsancarii

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Objective: The genus *Polygala* L. (Polygalaceae) is one of the diverse genera in the Polygalaceae, comprising more than 500 species occurring throughout the world. *P. Azizsancarii* is one of the endemic specie of the Polygala that is grown in Türkiye. Many of the *Polygala* species are used in folkloric medicine for different purposes such as anesthetics, anti-inflammatory agents, as well as for the treatment of central nervous system problems. The aim of this study was to determine the neuroprotective activities of two xanthones (1,3,6-trihydroxy-2,5,7-trimethoxyxanthone and 3-O-β-D-glucopyranosyloxy-1,6-dihydroxy-2,5,7-trimethoxyxanthone) that were isolated from the roots of *P. Azizsancariiin in vitro* cellular model of Alzheimer's disease (AD).

Methods: The xanthones were isolated for the first time from the roots of *P. Azizsancarii* and were established by spectroscopic methods, including 1D-NMR (^{1}H NMR, ^{13}C NMR, DEPT-135), 2D-NMR (COSY, NOESY, HSQC, HMBC). $A\beta_{25-35}$ peptideswere used to create an Alzheimer's disease model in human neuroblastoma (SKNAS) cells. The cytotoxicity of xanthones was evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. β-amyloid, α-synuclein, tau, JAK2, STAT3, caspase 3 and BMP-2 protein distribution was investigated using indirect immunoperoxidase staining in cells.

Results: We found that 100 µM concentrations of both xanthones were more effective for neuroprotection at 48 h in both SKNAS and in vitro AD model cells *In vitro* AD model cells, both xanthones decreased distributions of JAK, STAT3 and BMP2 proteins that play a role in the protective effect on neurons in neurodegenerative disease. Additionally, xanthones decreased α-synuclein and tau aggregation in AD model cells.

Conclusion: Our results suggested that both xanthones that are isolated from *P. Azizsancarii* can be potentially used as neuroprotective agents. Both components showed protective effects against Alzheimer's disease *in vitro* AD model cells.

Keywords: Alzheimer's disease, endemic, neuroprotective effect, *polygala azizsancarii*.

Acknowledgement: This work was supported by TÜBİTAK, Türkiye (Project Number: 118Z708).

OP-3

Down-regulation of glycerol-3-phosphate dehydrogenase and monoacylglycerol lipase in lymph node metastasis of triple-negative breast cancer: potential biomarkers for breast cancer metastasis

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Objective: Breast cancer (BC) remains a significant global health concern, with metastases to lymph nodes or distant organs being responsible for the majority of breast cancer-related deaths. Early detection of breast cancer metastasis is crucial for effective disease management. Despite advancements in medical sciences and technologies, the incidence and mortality rates of BC continue to rise. Previous research conducted by our group revealed that glycerol-3-phosphate dehydrogenase (GPD1) and monoacylglycerol lipase (MAGL) were significantly down-regulated in tissue and serum samples of triple-negative breast cancer (TNBC) patients compared to other BC subtypes and controls. Considering the aggressiveness of TNBC, we sought to investigate the potential association of GPD1 and MAGL levels with lymph node metastasis.

Methods: To accomplish this, we assessed alterations in GPD1 and MAGL levels in lymph node protein extracts from metastasized BC subtypes (Luminal A, Luminal B/Her+, Luminal B/Her2-, Her2 OE, TNBC) using western blotting. A comparative analysis was performed to identify statistically significant expression differences among the groups.

Results: Our findings demonstrated that GPD1 and MAGL were substantially down-regulated in lymph node metastasis of TNBC subtype, more so than in other BC subtypes and controls.

Conclusion: These results highlight the potential significance of GPD1 and MAGL as promising biomarkers for TNBC onset and metastasis. Further investigations into the functional roles of these proteins could offer valuable insights into the development of targeted therapies for aggressive breast cancer subtypes.

Keywords: Breast neoplasms, glycerol-3-phosphate dehydrogenase, lymphatic metastasis, monoacylglycerol lipases.

Acknowledgement: This study was supported by Kocaeli University Scientific Research Projects Coordination Unit under grant number 2014/057.

OP-4

Reduced expression levels of STAT1 and STAT3 upon thymoquinone treatment on breast cancer cells

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Objective: Thymoquinone (TQ), a natural compound, has demonstrated anti-cancer properties in breast cancer (BC) by exhibiting anti-proliferative, anti-metastatic and immunomodulatory effects. Signal transducers and activators of transcription (STAT) 1 promotes tumor growth by suppressing tumor immune surveillance and inducing invasiveness. Likewise, STAT3 increase tumor aggressiveness by promoting metastasis and angiogenesis. Aberrant activation of them detected in diverse cancer types including BC. Nevertheless, despite their tumorigenic role, they paradoxically exhibit tumor suppressor potential. In this study, we examined the effects of TQ on the expression of STAT1 and STAT3 proteins in BC cells, aiming to gain insights into their complex and multifaceted roles.

Methods: MCF7 cells were treated with TQ at 15 µM concentration for 48 hours at 37 °C, as previously optimized. Isolated proteins from the TQ-treated and non-TQ-treated (Control) MCF7 cells were validated by 1D SDS-PAGE analysis. STAT1 and STAT3 protein expressions were measured by Western Blot. Protein bands were quantified by ImageJ and the significance of the results was analyzed using GraphPad Prism (10.0.1) through an unpaired t-test.

Results: High-quality total protein was acquired. TQ-treated MCF7 cells exhibited significantly reduced STAT1 and STAT3 expressions in ~23 and ~ 290 fold, respectively ($p<0.05$).

Conclusion: TQ inhibited MCF7 cell proliferation and conspicuously decreased STAT1 and STAT3 expressions implying that TQ suppresses tumorigenic characteristics of these genes. Given that TQ has the potential to affect the STAT-related pathways, our findings highlights the promising use of TQ in the prevention and treatment of BC. Extensive research may clarify underlying molecular pathways of TQ and contribute to the identification of novel anticancer approaches.

Keywords: Breast neoplasms, STAT1, STAT3, thymoquinone.

OP-5

MicroRNA-target gene analysis as a possible immune response in COVID-19 patients

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Objective: Although the COVID-19 pandemic has lost its effectiveness, we still do not have enough information to explain the underlying genetic mechanisms and transcriptional communication between genes. MicroRNAs, which are known as one of the epigenetic factors affecting transcriptional products, have an effect on many cellular mechanisms including inflomatory responses in the formation of many diseases such as cancer and diabetes. Based on these interactions, bioinformatics analysis of array microRNA raw data of COVID-19 patients set on GEO (Gene Expression Omnibus) is to shed light on the understanding of target gene interactions on the inflammatory response-mRNA mechanism.

Methods: Gene-protein interactions were performed by identifying target genes and pathways with KEGG using miRNA samples of patients with COVID-19 infection set as GSE 182183, GSE 236017, GSE 182152, GSE 166160 from GEO database using DIANA TarBase v.8, TargetScan, miRTarBase, and miRDB.

Results: Bioinformatic analysis of GSE miRNA data showed that down-regulation of hsa-miR-15a-3p, hsa-miR-27a-3p and hsa-miR-146a-5p may be effective on the activation of pro-inflammatory cytokines-cytokine interactions (EGFR, IL-7, IL-15, IL-17RB, IL-6ST), while up-regulation of hsa-miR-192-5p, hsa-miR-150-3p, hsa-miR-34c and hsa-miR-150-5p altered the regulation in many samples.

Conclusion: MicroRNAs are expected to contribute to the understanding of the molecular targets of mRNA mechanisms, as well as possible vaccine studies, in addition to being a prognosis to be considered as an inflammatory response to the COVID-19 disease genetic interaction mechanism.

Keywords: COVID, cytokine interaction, mikroRNA, post-transcriptional regulation.

OP-6

Non-myelotoxic agents can be used as conditioning before hematopoietic stem cell gene therapy

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Objective: Here, we aimed to develop a non-myelotoxic regimen that can be used as preparatory conditioning before transplantation of genetically corrected HSCs. G-CSF, VLA-4I and AMD3100 are known as HSC mobilizers or affect BM permeability and may support the homing of intravenously infused (transduced) HSCs back to the BM niche, without inducing any harm.

Methods: Mice were pre-treated with BU, G-CSF, VLA-4I or AMD3100. c-kit+ RAG2-/- BM cells from male mice were transduced with a lentiviral vector carrying hRAG2co and transplanted into female RAG2 mice. Expression of CD45, CD3, CD19, CD11b, CD45R and NK1.1 in peripheral blood (PB) was measured at 1 and 3 months after transplantation. Spleen and BM cellularity were assessed at 6 months post-transplant.

Results: PB cell counts increased significantly in the groups treated with G-CSF, VLA-4I and AMD3100, but not in the BU-treated group. Reconstitution of CD3+ and CD19+ PB cells after transplantation of mice was similar in all groups, although BU-treated mice showed accellerated CD19+ reconstitution. At 6 months after transplantation, all mice displayed full immune reconstitution, despite different methods of conditioning. Reconstituted spleen lymphocytes responded to activation signals. Survival of mice treated with BU was significantly lower than survival of mice in any of the other groups, with the highest survival in the AMD3100-treated group followed by G-CSF and VLA-4I, although the overall immune reconstitution was best in mice treated with the latter groups.

Conclusion: Here, we show that the non-myelotoxic chemicals G-CSF, VLA-4I, and AMD3100 are highly effective as conditioning regimen before HSC gene therapy and can be used instead of BU. The most robust immune reconstitution was observed after treatment with G-CSF and VLA-4I.

Keywords: G-CSF, gene therapy, hematopoietic stem cell gene therapy.

OP-7

A new perspective on the potential role of plectin and its isoforms in cancer development

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Objective: The expression of *PLEC*, which encodes plectin protein, varies in different cancer types. It acts as a multifunctional linker that organizes cytoskeletal networks and a scaffolding protein in signal transduction. In this study, *PLEC* and its unique eight isoforms (*PLEC1, PLEC1a, PLEC1b, PLEC1c, PLEC1d, PLEC1e, PLEC1f, PLEC1g*) were analyzed to determine whether they can be diagnostic/prognostic biomarkers in the diagnosis and progression of cancer with bioinformatics-based studies.

Methods: RNA-seq data collected from TCGA (The Cancer Genome Atlas) were analyzed to determine the expression level of *PLEC* and its eight transcript isoforms for 12 cancers (BRCA-breast invasive carcinoma, COAD-colon adenocarcinoma, HNSC-head and neck squamous cell carcinoma, KIRC-kidney renal clear cell carcinoma, KIRP-kidney renal papillary cell carcinoma, LIHC-liver hepatocellular carcinoma, LUAD-lung adenocarcinoma, LUSC-lung squamous cell carcinoma, PRAD-prostate adenocarcinoma, STAD-stomach adenocarcinoma, THCA-thyroid carcinoma, UCEC-uterine corpus endometrial carcinoma) and four cancer stages. Prognostic and diagnostic performances of *PLEC* and its isoforms with Kaplan–Meier (KM) and Receiver Operating Characteristics (ROC) curve, respectively, were evaluated for 12 cancer types and four stages.

Results: Each *PLEC* isoform had differential expression profiles in different cancer types and stages, whereas *PLEC* expression did not change significantly with the specified criteria. Taking into consideration the expression of transcript isoforms, KM, and ROC curve analyses, it was noteworthy that *PLEC1d* might be a potential prognostic and diagnostic biomarker candidate for KIRC metastatic stages, and *PLEC1f* might have a particularly prognostic effect for LUSC.

Conclusion: The data show that only gene-level-based analysis may produce false negative/positive results in biomarker determination studies. Thus, isoform level analysis is essential. Additionally, some plectin isoforms may play an important role in cancer development and progression, especially in the process of invasion and metastasis, and they may be potential prognostic/diagnostic biomarkers specific to the cancer stage. However, further functional studies are needed to support these findings.

Keywords: Cancer, diagnostic biomarker, *PLEC*, plectin isoforms, prognostic biomarker.

OP-8

Analysis of ferroptosis-related gene expression variations in the HEPG2 cell line

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Objective: A recently identified type of iron-dependent cell death triggered on by lipid peroxides is known as ferroptosis. For regulating ferroptosis, numerous pathways and associated genes have been found. In this study, the ferroptosis inducer and its inhibitor were used to produce in vitro ferroptosis in the HEPG2 cell line to research how the expression of genes involved in ferroptosis pathway is affected.

Methods: After optimization 35μM Erastin was used to create ferroptosis in HEPG2 cell line. Ferrostatin-1, a ferroptosis inhibitor, was used to inhibit ferroptosis. Ferroptosis was verified using flow cytometry and cell counts utilizing a hemocytometer. A quantitative RT-PCR (qPCR) analysis was performed on the *SLC7A11, GPX4, BNIP3L*, *RAD21* and *HMOX-1* genes in the HEPG2 cell line after cell culture optimization.

Results: In the condition of 35 μM Erastin addition alone, viability was 56.27% compared to the control, while it was 82.15% in the condition of 35 μM Erastin+2.5 μM Ferrostatin-1. Lipid peroxidation was 46.93% higher in the 35 μM Erastin condition compared to control, but only 14.15% higher in the 35 μM Erastin+2.5 μM Ferrostatin-1 condition. While *RAD21* and *GPX4* gene expression decreased in the presence of erastin and ferrostatin-1, *SLC7A11*, *BNIP3L*, and *HMOX-1* gene expression increased in comparison to the control according to qPCR results.

Conclusion: As a result, ferroptosis was successfully induced in cell culture with erastin and ferrostatin-1. In the HEPG2 cell line, it was found that the expression of the genes *RAD21, SLC7A11*, *BNIP3L, HMOX-1* and *GPX4* changed in the same way when erastin and ferrostatin-1 were used in combination. These findings demonstrate that erastin, but not ferrostatin-1, influences the expression of these genes. It has been suggested that ferrostatin-1 may influence gene expression indirectly by lowering lipid peroxidation.

Keywords: Ferroptosis, HEPG2, SLC7A11.

OP-9

RAB27A gene therapy and assessment of possible tumorigenic effects

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Objective: The small GTPase protein RAB27A is involved in intracellular membrane traffic, vesicle formation, and exocytosis. *RAB27A* mutations cause the immunodeficiency Griscelli Syndrome type II (GS-2). In this study, we used gene addition using a lentiviral vector (LV) and gene editing using homologous recombination with CRISPR-Cas9 for correction of GS-2 mesenchymal stem cells (MSC) and induced pluripotent stem cells (iPSC). At the same time, we performed a risk assessment to assess the possible tumorigenic effects of RAB27A overexpression on hematopoietic stem cells (HSCs) and MSCs using LV.

Methods: CRISPR elements (gRNA and donor DNA) were designed for RAB27A exon 3 delAGinsC and exon7 delCAAGC CRISPR/Cas9 editing. LV-PGK-RAB27Aco, SF-RAB27Aco-GFP, and UCOE-RAB27Aco were used for lentiviral transduction, and LV-PGK-GFP was used as control. CRISPR/Cas9 was used for correction of GS-2 MSCs and iPSCs, followed by sequencing of the mutation sites of the corrected iPSCs. After lentiviral transfer, RAB27A gene and protein expression were examined using qRT-PCR, flow cytometry, and Western Blot. For tumorigenic risk assessments, RAB27A was overexpressed in healthy donor MSCs and HSCs using LV-SF-RAB27Aco-GFP and transplanted into $Rag2^{-/-}$ mice.

Results: Sequencing revealed correction of a fraction of the GS-2 iPSCs after CRISPR editing. Transduction with PGK-RAB27Aco (MOI:30) and UCOE-RAB27Aco (MOI:100) resulted in expression of RAB27A >50% of the MSCs. A 10.000-fold increase in RAB27A expression was observed after transduction of GS-2 iPSCs with PGK-RAB27Aco. However, overall, RAB27A expression was higher after use of PGK in comparison to UCOE. SF-RAB27Aco was used to examine the possible oncogenic effects of RAB27Aco overexpression, but no evidence of tumorigenicity was found in either PB or histological examinations of transplanted mice.

Conclusion: RAB27A expression was successfully achieved in both healthy and GS-2 cells using the LV-PGK-RAB27Aco, UCOE-RAB27Aco, SF-RAB27Aco constructs. RAB27A expression was higher with the PGK promoter than with the methylation-resistant UCOE promoter. CRISPR induced high levels of cell death. No oncogenic effects were observed in *in vivo* experiments as a direct result of overexpression of RAB27A in HSC and MSCs. LV-RAB27A gene therapy was effective without oncogenic side effects.

Keywords: CRISPR elements, gene therapy, RAB27A.

OP-10

4-hydroxytamoxifen-loaded exosomes derived from mesenchymal stem cells play a role in the inhibition of the PI3K/MAPK and hippo pathways in tamoxifen-resistant MCF 7-TAM1 breast cancer cells

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Objective: In estrogen receptor-positive breast cancer treatment, PI3K/MAPK and Hippo pathways are gaining attention for their role in tamoxifen resistance. The aim of the study is to determine the efficacy of exosomes released by mesenchymal stem cells (hMSCs) as drug delivery systems and to investigate the impact of 4-hydroxytamoxifen (4-OHT) loaded exosomes (4-OHT-Exo) applied to MCF7-TAM1 cells on the PI3K/MAPK-Hippo pathways.

Methods: In the study, exosomes from the culture medium of hMSCs were isolated using a kit reagent. Exosome characterization was performed using TEM, western blot, and nanoparticle tracking analysis (NTA). Drug loading was performed using electroporation The cytotoxic effect of 4-OHT on MCF7-TAM1 cells was determined by WST-1 analysis. Expression levels of genes in the PI3K/MAPK and Hippo pathways (Control-*ACTB, ESR1, PIK3CA, MAP2K1, SRC3, YAP1, TAZ, MST1, MST2, LATS1* and *LATS2*) in the analysis groups as were analyzed using an RT-PCR. Pathway activation analysis and cell-cycle analysis were performed.

Results: The exosomes, whose average size distribution and density were examined by NTA analysis, were found to have sizes ranging from approximately 50 to 200 nm with a concentration of 3.40e+10±6.20e+08 particles/ml. The viability rates of MCF7-TAM1 cells treated with 4-OHT and 4-OHT-Exo at 48 hours were determined to be 48.8% and 37.6%, respectively. In MCF7-TAM1 cells, PI3K activation rates were 49.1% for the control, 44.6% with 4-OHT alone, and 38.1% for the 4-OHT-Exo group. When examining statistically significant gene expression levels, a decrease of -2.68-fold in *ESR1* (p=0.006), -1.77-fold in *PIK3CA* (p=0.028), and -1.58-fold in the *TAZ* gene (p=0.037) was observed. Additionally, 4-OHT-Exo arrested the cell-cycle at the G0/G1 phase.

Conclusion: In the current study, it was determined that tamoxifen-loaded exosomes positively affected the mechanism of action of the drug. The utilization of exosomal natural vesicles as a drug-delivery approach contributes to the therapeutic efficacy of endocrine therapy.

Keywords: Breast cancer, drug delivery system, exosome, hippo signaling pathway, PI3K signaling pathway, tamoxifen.

Acknowledgement: This study was supported by the Scientific Research Projects Unit of Bursa Uludağ University under project number TGA-2021-198. The author Havva Tezcan Unlu is a 100/2000 Higher Education Board scholar in the field of Molecular Oncology and a 2211-C domestic priority areas doctoral fellow.

OP-11

The minor allele of angiopoietin-like protein-8 rs2278426 affects coronary artery disease and type 2 diabetes mellitus pathology mechanisms

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Objective: Coronary artery disease (CAD) is the main cause of death worldwide. Triglycerides, which are one of the causes of CAD when consumed excessively, are broken down into three fatty acids and one glycerol by lipoprotein lipase (LPL). Angiopoietin-like protein-8 (ANGPTL8) mainly function in the lipid mechanism by regulating LPL activity. In this study, we aimed to determine the associations between an *ANGPTL8* polymorphism-rs2278426, and severity and presence of CAD.

Methods: A total of 1367 unrelated Turkish individuals who underwent coronary angiography recruited for the study and individuals grouped in accordance with coronary angiography results as CAD (n=736) or non-CAD (n=549) and type 2 diabetes mellitus (T2DM) status. Subjects were genotyped for rs2278426 (C/T) by quantitative real-time PCR. The allele and genotype distribution of the rs2278426 polymorphism were examined. Moreover, associations between genotype distributions and selected biochemical parameters were evaluated.

Results: Among CAD patients, T allele carriage frequency was lower in T2DM group (p=0.046). Moreover, in the male non-CAD group, T allele carriage was more prevalent among T2DM patients compared to individuals without T2DM (p=0.033). In logistic regression analysis adjusted for obesity, which is an essential risk factor of T2DM, non-CAD T allele carrier men had increased risk for T2DM (OR= 2.244, 95% CI: 1.057-4.761, p=0.035). In addition, stenosis (p=0.002), and SYNTAX score (p=0.040), which are markers of CAD severity were lower in T allele carrier men compared to non-carriers in T2DM group.

Conclusion: *ANGPTL8* is a potential protein that could helped to elucidate the pathology mechanism of CAD and T2DM. We found that T allele carriage of *ANGPTL8* rs2278426 has a protective effect on CAD in T2DM patients. Further research should be executed to explore the nature of the association between *ANGPTL8* rs2778426 and CAD/T2DM.

Keywords: Atherosclerosis, angiopoietin like protein 8, coronary artery disease.

OP-12

Investigation of the level of mitochondrial protein phosphoenolpyruvate carboxykinase 2 in the cerebrospinal fluid of Alzheimer's disease

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Objective: Alzheimer's disease (AD) neuropathology includes amyloid plaques, neurofibrillary tangles, and neuronal loss. AD involves extracellular senile plaque formation. Increased amount of Aβ aggregations seen in the brain of AD cases. β-amyloid (Aβ) peptide can potentially translocate to the nucleus and affect the transcription of some genes in the nuclear genome. Given that, Aβ might act as a transcription factor (TF). Our investigations with the FpClass prediction tool indicated a direct interaction Aβ with three TFs (Jun, Fos, and RELA). These three TFs control the expression of mitochondrial Phosphoenolpyruvate Carboxykinase 2 (PCK2) protein. PCK2 has been shown to mediate gluconeogenesis. Neurons require high mitochondrial activity, and it has been suggested that PCK2 protein may play a critical role in neuronal function and neurodegeneration. In this study, we hypothesized that PCK2 mitochondrial protein alteration in AD cerebrospinal fluid (CSF) samples may be related to AD progression and have the potential to be monitored as a biomarker.

Methods: CSF samples of 19 Alzheimer's patients and 12 cognitively healthy individuals with SCI who were age-matched with these patients were used. PCK2 levels in the CSF samples were measured with ELISA. The raw data was analyzed with GraphPad Prism. $p<0.05$ was accepted as a statistically significant difference.

Results: The results showed that PCK2 levels were higher in the CSF of Alzheimer's patients (323.5±164.6 pg/ml) compared to SCI controls (249.5±88.8 pg/ml), but this difference was not significant.

Conclusion: A relative increase in PCK2 levels in AD patients indicates that PCK2 has the potential to be a biomarker that can be monitored in the CSF of patients with AD. The study will continue to increase the number of patients.

Keywords: Alzheimer's disease, neurodegeneration, phosphoenolpyruvate carboxykinase 2.

Acknowledgement: The present work was supported by the Research Fund of Istanbul University, (Project no: 37292).

OP-13

Development of cffDNA isolation method for prenatal diagnosis

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Objective: The biggest challenge in prenatal diagnosis is the inability to analyze the well-being of the fetus without risking pregnancy. Research on obtaining cff-DNA from the maternal circulation and the development of screening tests are among the most up-to-date approaches.The aim of the study was to develop a cff-DNA isolation protocol in order to obtain sufficient and sensitive fetal DNA to be used in prenatal diagnosis.

Methods: Maternal blood samples (plasma) belonging to three trimesters (I., II. and III.) were taken between the 10th and 30th weeks of 30 pregnant women who were determined by ultrasound to carry a male fetus. Expression of genes was evaluated with qPCR method to show the presence of fetal DNA and by using primary probes designed specifically for SRY and DYS14 (gene region expressing Y chromosome) by presence-absent analysis. The increased expression of SRY and DYS-14 genes in the study indicates the presence of fetal genetic material in pregnant women without considering the maternal genome. The amount and purity of cffDNA obtained with the QIAamp DSP Virus Kit, MagMAX and the new protocol we have developed were compared.

Results: cffDNA amounts with the developed protocol; it was determined as 3.542±0.89 ng/ul in the first trimester, 4.191±1.127 ng/ul in the second trimester and 6.273±1.641 ng/ul in the last trimester. The fact that the amount of cffDNA was found at similar rates with the commercial kits shows the success of the study. After SRY and DYS14 genotyping was performed, it was determined that 90% (27/30) of 30 plasma samples had increased SRY and 93.3% (28/30) DYS14 gene expression.It was confirmed by postnatal data that 96.4% (29/30) of the babies were male.

Conclusion: Considering that postnatal data were also evaluated in the study, it was observed that the sensitivity and specificity of the cffDNA isolation method developed were quite high.

Keywords: cffDNA isolation, new protocol, prenatal diagnosis.

Acknowledgement: (Tubitak 2218, Proje No: 121C387)

OP-14

Mesenchymal stem cell modification with titanium-based nanotopographic surfaces

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Objective: Nanotopography offers an effective approach for cell manipulation mediated by material-matrix interactions. These interactions with the extracellular matrix elicit various cellular responses such as cytoskeletal rearrangements and activation of signaling pathways. The aim of this study was to investigate the effect of modified titanium dioxide (TiO2) nanotube surface arrays on stromal cell responses.

Methods: Three different modified surfaces were obtained by two-step anodic oxidation and visualized by scanning electron microscopy to quantify nanotube diameters and porosity. Then, mesenchymal stem cells (MSCs) were cultured on untreated and modified titanium surfaces for 48 hours and transcriptomic profile was performed by high-throughput RNA-sequencing. The modification, that changes extracellular matrix (ECM) related genes most, was examined in detail with additional surface modifications. This study design was also repeated with dermal fibroblasts to test whether the cellular responses obtained were common for stromal cells.

Results: MSCs that cultured on different nanotopographically modified titanium surfaces showed distinct transcriptomic profiles. Changes in the energy metabolism of cells were detected on nanotopographically rougher surfaces, while surfaces containing flatter and deeper pores increased the expression of genes associated with ECM production. In some of these nanosurfaces, which were diversified with additional modifications, significant increases in the expression of genes that directly affect cellular therapy success such as CXCR4 and VEGFA were detected. These critical gene expression changes were also demonstrated in experiments with dermal fibroblast cells, suggesting that this effect on transcriptomic profile can be generalized to stromal cells.

Conclusion: This study showed that nanosurface modifications can increase the therapeutic potential of MSCs only with pre-incubation step. In addition, considering that the developed surface can be easily adapted to GMP conditions, it can be said that it has the potential to enable an important transformation in the fields of regenerative medicine/biomaterials.

Keywords: Cell-surface interactions, CXCR4, mesenchymal stem/stromal cell, modified titanium surface, nanotopography, VEGFA.

OP-15

Transcriptional and post-transcriptional epigenetic changes in male infertility

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Objective: Changes in spermatozoa, which carry genetic and epigenetic information, cause infertility and transmission of transgenerational diseases. Our study investigated the relationship between the expression of *MESTIT1, MEST, GNASAS, CEP41, H19*, and *IGF2AS* genes and DNA methylation in spermatozoa and infertility.

Methods: After semen analysis, DNA and RNA extraction from spermatozoa were performed in fertile (n=30), oligospermia (n=30), and normospermia (n=30) groups. Gene expressions were evaluated by the Livak method after RT-qPCR. DNA methylation was measured by the pyrosequencing. Hypermethylation rates were determined according to the cut-offs. We perform Kruskal Wallis, ANOVA, Bonferroni, and Tamhane for post hoc.

Results: In the fertile group, hypermethylation rates of the first (33.3%), third (33.3%), and last (40%) of the 5 CpG dinucleotides of *GNASAS* were significantly lower than those in the oligospermia group (66.7%, 73.3% and 73.3%) (p=0.028, p=0.005 and p=0.026). The hypermethylation rate of the third CpG dinucleotide of *CEP41* in the fertile group (16.7%) was significantly lower than in the normospermia (46.7%) (p=0.036). No statistically significant difference in age, body mass index, gene expressions, and methylation rates of other genes among the groups. Sperm concentration in the oligospermia was significantly lower. The rate of normal morphology in the fertile group was significantly higher.

Conclusion: *GNASAS* is a maternal imprint genehypomethylated in sperm cells. Hypermethylated *GNASAS* is associated with impaired sperm production. *CEP41* is a non-imprint gene adjacent to the maternal imprint MEST gene. *CEP41* hypermethylation was higher in the normospermia than in the fertile. *CEP41* hypermethylation has been shown as a new candidate in the etiology of infertility with normospermia. Revealing epigenetics of infertility may reduce the risk of transgenerational epigenetic diseases.

Keywords: DNA methylation, gene expression, genomic imprinting, spermatozoa.

Acknowledgement: The study was supported by the Gazi University Scientific Research Projects Coordination Unit with TDK-2021-7023.

OP-16

Examination of the effect of vitamin D on neurite length and extension rate during neuronal development

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Objective: Vitamin D plays a role in essential processes in the brain. Vitamin D2 is a potent neuromodulatory compound that increases axogenesis and axon diameter in animal models of nerve trauma. In another animal study in which high doses of vitamin D3 were administered, it was shown that the number of preserved or newly formed axons at the proximal end, axon diameter at the distal end, and neurite myelination at both the distal and proximal ends increased. Recently, vitamin D has been shown to increase the expression of microtubule-associated protein-2 and axonal growth-associated protein-43 in rat cortical neurons. Based on this knowledge, we investigated whether vitamin D administration during development influences neurite length and extension rate in primary cortical neuron culture.

Methods: 10-8 M 1,25-dihydroxyvitamin D3 was administered 24 hours after primary neuron culture from 16-day-old rat embryos. After treatment, neurite lengths were measured under a live cell imaging microscope at 20X magnification at 30-second intervals for 30 minutes and at half-hour intervals for 12 hours.

Results: Our study showed that neurite length and elongation rate increased significantly in the vitamin D group compared to those in the control group 30 minutes after vitamin D administration ($p=0.0029$ and $p=0.0310$, respectively). In addition, neurite elongation rate and length were significantly increased in the vitamin D group compared to those in the control group 12 hours after administration ($p=0.0275$ and $p=0.0398$, respectively).

Conclusion: Few studies have investigated the effect of vitamin D on neurite outgrowth. The results of our study indicated that vitamin D administration to primary cortical neurons during development is associated with increased neurite length and elongation rate in the first 12 hours.

Keywords: Neurite outgrowth, neuronal development, vitamin D.

Acknowledgement: This study was supported by the TÜBİTAK 1002-A (Project No:123S438).

OP-17

Establishment of toxin resistant human mesenchymal stem cells by mutant elongation factor-2 coding single stranded oligonucleotides

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Objective: Targeted cytotoxins have been employed as anticancer treatments; however, because of their off-target administration, and systemic toxicity, their successful clinical translation in solid tumors has been hindered. Stem cells have been of high interest to be engineered as therapeutic delivery vehicles and accordingly, number of studies were reported their therapeutic potential. Accordingly, we proposed to engineer toxin-resistant stem cells to further modify them for the production of bacterial toxin (pseudomonas exotoxin; PE) based therapeutic fusions. To avoid endogenic toxin toxicity, stem cells must be toxin-resistant. For this aim, we proposed to engineer toxin-resistant stem cells in this study.

Methods: Naturally, elongation factor-2 (EF-2) catalyzes the transfer of peptidyl tRNA from ribosome A site to P site during peptide chain elongation. *Pseudomonas aeruginosa* exotoxin A (PE), inactivates EF-2 by ADP-ribosylation and, therefore, blocks protein synthesis. We modified human mesenchymal stem cells (hMSCs) using mutant EF-2 (mEF-2) coding single-stranded oligonucleotides (ssODNs) to establish hMSCs that can secrete Pseudomonas exotoxin (PE) fused targeted cytotoxins. The G-to-A transition in the first nucleotide of codon 717 is known to confer toxin tolerance, therefore; ssODNs were created accordingly and transfected into hMSCs. The cells were then subsequently treated with increasing doses (10-1000 ng/ml) of purified toxin and then, toxin-resistant cells (hMSCs-mEF2) were selected.

Results: The findings showed that hMSCs modified for toxin resistancy and toxin-resistant subpopulations existed. The sensitive populations were eliminated by the toxin treatments. This indicates that mEF-2 ssODN was functional to inhibit PE cytotoxicity in stem cells.

Conclusion: hMSCs were successfully modified for toxin resistancy. Considering the clinical translation relevancy of hMSCs, further engineering the hMSCs-mEF2 to secrete functional targeted cytotoxins may open up new opportunities for a broad range of cancer treatments.

Keywords: Elongation factor-2, mesenchymal stem cells, pseudomonas exotoxin, targeted therapy.

Acknowledgment: This study was supported by TÜBİTAK with project number 117S421.

OP-18

The effect of amyloid beta 1-42 protein on the expression of transcription factors in HEK293T cell line

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Objective: Researchers have reported that Aβ1-42 can be found in the nucleus, interact with DNA, and modulate the expression of many genes. In addition, it has been shown in the literature that Aβ1-42 can be transported from the cytoplasm to the nucleus in response to different antibiotic doses. These findings suggest that Aβ1-42 has a considerable DNA binding capacity and may function as a transcription factor. Instead of directly regulating the expression of individual genes, Aβ1-42 may affect the expression of general transcription factors and thus contribute to a broader regulation of expression. Therefore, our study aims to investigate whether Aβ1-42 influences the expression of important proteins in intracellular pathways involved in its own production and in the genesis of Alzheimer's disease pathology.

Methods: Using the FpClass and TRRUST databases, toxic (10 μM) and non-toxic (0.09 μM) doses of Aβ1-42 peptide were applied to HEK293T cells and RNA isolations were performed at 24, 48, and 72 hours after treatment. Then, qRT-PCR was used to determine how the expression of possible TFs that may interact with Aβ1-42 peptide changed in response to Aβ1-42 treatment.

Results: At 24 hours, the expression level of JUN201 increased in the group treated with 0.09 uM Aβ1-42 compared to the control group. At 48 hours, the expression level of JUN201 increased in the group treated with 10uM Aβ1-42 compared to the 0.09 uM Aβ1-42 treated group, but decreased at 72 hours compared to both the group treated with 0.09 uM Aβ1-42 and the control group. At 24 hours, the expression levels of SP1 and FOS increased in the group treated with 0.09 uM Aβ1-42 compared to both the group treated with 10 uM Aβ1-42 and the control group. At 48 hours, the expression levels of SP1 and FOS increased in the group treated with 10 uM Aβ1-42 compared to the group treated with 0.09 uM Aβ1-42. At 48 hours, the expression level of SMAD3 increased in the group treated with 10 uM Aβ1-42 compared to both the group treated with 0.09 uM Aβ1-42 and the control group. At 24 hours, the expression level of STAT3 increased in the group treated with 0.09 uM Aβ1-42 compared to the group treated with 10 uM Aβ1-42 and decreased at 72 hours compared to the control group. At 48 hours, the expression level of STAT3 increased in the group treated with 10 uM Aβ1-42 compared to the group treated with 0.09 uM Aβ1-42.

Conclusion: Our findings showed that the expression levels of JUN201, SP1, FOS, SMAD3, and STAT3 transcription factors changed due to Aβ1-42 treatment and these changes also differed depending on the dose and duration of treatment.

Keywords: Amyloid beta 1-42, gene expression, transcription factor.

Acknowledgement: This study was supported by Istanbul University-Cerrahpaşa BAP Unit (Project No: 34211).

OP-19

CD73 prevents cell proliferation, EMT and chemotherapy resistance in colorectal cancer

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Objective: The suppression of purinergic signaling within the context of cancer has become an important area of research. A key player in this field is the ecto-enzyme CD73 which has a role in the conversion of extracellular ATP to adenosine. However, the intrinsic role of CD73 in colorectal cancer (CRC) cells has not been fully investigated. Therefore, we aimed to study the role of CD73 in tumor development via using CRC cell lines.

Methods: Stable CD73-depleted CRC cell lines were generated by CRISPR/Cas9 to be used in in vitro experiments. Moreover, gefitinib (EGFR inhibitor) resistant CRC cell lines were generated by repeated treatment of cells with gefitinib. Epitelial to mesenchymal transition (EMT) marker expressions were investigated by immunoblot and qPCR assays. Lastly, publicly available CRC patient single-cell RNA sequencing and methylation data were analyzed in silico.

Results: CD73 depletion in CRC led to larger xenograft tumors in mice and cell proliferation and motility was found to be increased. Moreover, overexpression of CD73 in gefitinib resistant CRC cell lines sensitized the cells to gefitinib and resulted in slower cell migration and increased apoptosis. Moreover, CD73-overexpressed cells formed smaller tumors in mice. Furthermore, our bioinformatics analyses, in line with our in vivo and in vitro findings, supports the tumor suppressor role of CD73 depending on stromal content as well as infiltrating immune cells found in tumors.

Conclusion: Our findings strongly suggest that CD73 has a cell-intrinsic tumor suppressive function in the CRC cells regardless of its role in tumor microenvironment. Therefore, these findings suggest that inhibition of CD73 may not result in the expected outcome of CRC patient with poor immune cell infiltration.

Keywords: CD73, chemotherapy resistance, colorectal cancer, EMT.

OP-20

In vitro investigation of the neuroprotective potential of boric acid in a rotenone-induced parkinson

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Objective: Parkinson's disease (PD) represents a multifaceted neurodegenerative disorder characterised by the progressive degeneration of dopaminergic neurons, ultimately resulting in neurodegeneration. The hallmark pathological features of PD encompass the reduction of dopaminergic neurons in the substantia nigra pars compacta and the accumulation of misfolded α-synuclein within cytoplasmic inclusions known as Lewy bodies. Rotenone, a naturally occurring toxin produced by tropical plants, possesses the capacity to penetrate the blood-brain barrier. Upon neuronal entry, it impedes proteasome activation, instigating α-synuclein phosphorylation, aggregation, Lewy pathology formation, and subsequent degeneration of nigrostriatal dopaminergic neurons. Boric acid (BA), representing the primary form of boron in human tissues and bodily fluids, assumes a pivotal role. A deficiency of BA in the human body is associated with diminished motor and cognitive functions. The aim of our study is to examine the possible protective effect of BA within a Rotenone-induced Parkinson's disease model established in the SH-SY5Y human neuroblastoma cell line, serving as an in vitro experimental paradigm.

Methods: The cytotoxic effect of BA was determined by MTT experiment as a result of the combined application of 200 µM, 100 µM, 50 µM, 20 µM BA and 50 µM Rotenone concentrations to the SH-SY5Y cell. Gene expression levels of signalling pathways involved in the cytotoxicity of SH-SY5Y cells of the Rotenone, BA, and Rotenone + BA groups were investigated by quantitative reverse transcriptase polymerase chain reaction (RT-qPCR) and changes in protein expression determined by the Western Blot method.

Results: The MTT experiment revealed that the viability of the PH model generated with 50 µM Rotenone was 64.58%, whereas the viability of the PH model generated with 200 µM BA was 116.36%, and a significant proliferation was observed. This specific dosage was selectively used for RT-qPCR and Western blot analyses. Notably, the co-administration of 50 µM Rotenone with 200 µM BA exhibited a marked attenuation of apoptotic processes, as evidenced by a notable reduction in the BAX/BCL-2 pro-apoptotic mRNA ratio, displaying a 0.54-fold change relative to the Rotenone-only group.

Conclusion: Consequently, these findings lead to the inference that boric acid (BA) may exert a neuroprotective effect.

Keywords: Apoptosis, boric acid, neuroprotective, Parkinson's disease, rotenone.

OP-21

Different aneuploidy mosaicism in peripheral blood sample with Inv5(q)(q22q33.3) in a girl patient who presented with short stature

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Objective: Paracentric inversions occur when two breaks in the chromosome arm result in a rejoin after the cut piece is reversed. The interchromosomal effect has been used to describe a change in the chromosome exchange distribution in the presence of an inversion. The possibility of balanced inversion carriers forming unbalanced gametes due to the interchromosomal effect is known. However, the possibility of mitotic errors has not been evaluated in the literature.

Methods: Here, we present the case of a 9-year-old female patient who visited our hospital because of her short stature. On examination, she also showed abnormalities in the skeletal and urinary systems. Karyotype analyses were performed on peripheral blood lymphocytes.

Results: Different chromosome aneuploidies monosomy, trisomy, and tetrasomy were observed to form in 15% of the 100 analyzed metaphase cells. The karyotype analysis of the patient who came to us for follow-up was repeated 10 years later, aneuploidies weren't observed and the patient had a karyotype of 46, XX, inv5(q)(q22q33.3).

Conclusion: We believe that balanced inversion carriage may have caused mitotic division errors in the blood cells of this patient. There is no information that paracentric inversion can cause segregation errors in somatic cells. In this case, it was shown for the first time that chromosome separation errors can occur in the somatic cells of balanced inversion carriers. Findings should be supported by further research.

Keywords: Chromosomal instability, chromosome inversion, interchromosomal effect, nondisjunction, short stature.

OP-22

Investigation the effect of the gamma-tubulin gene on neural development in the zebrafish crispant model

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Objective: Abnormalities of the microtubules are associated with various neurodevelopmental disorders, including lissencephaly, polymicrogyria, and autism spectrum disorders. Microtubule-targeted therapeutic agents for the treatment of neurodevelopmental diseases have been studied both in in vitro and in vivo neurodevelopmental model systems. In addition, mutations in the tubulin gene family and tubulin-related genes are associated with malformations. Gamma-tubulin (TUBG1) functions as a microtubule nucleating factor and is localized in the microtubule organizing center. *Tubg1* mutations are observed in patients with lissencephaly and microcephaly. TUBG1 localization for microtubule nucleation has been shown to be regulated through interactions with Axin, a component of the ß-catenin destruction complex. It is shown that Axin forms complexes with TUBG1 in centrosomes and silencing of Axin leads to disruption of centrosomal nucleation. However, the effect of the interaction between TUBG1 and Axin on the activation of the Wnt/ß-catenin signaling pathway and its role in the neurodevelopmental process are unknown. In this study, we aimed to investigate whether the proposed interactions between TUBG1 and canonical Wnt proteins could affect Wnt signaling activity.

Methods: The effect of TUBG1 examined in F0 *Tubg1* loss of function model (crispants) in zebrafish larvae. Wnt signaling activity determined by qPCR at the transcript level in the *Tubg1* crispants. Interaction between Axin-TUBG1 analysed using immunoprecipitation in Hek293T cells.

Results: The F0 *Tubg1* loss of function model (crispants) in zebrafish larvae recapitulates the microcephaly phenotype (small eye and head phenotype) and has a decreased level of Wnt/b-catenin signaling activity. The Axin1 interacts with TUBG1.

Conclusion: The TUBG1 regulates microcephaly phenotype through Wnt/b-catenin signaling in the zebrafish *Tubg1* crispants.

Keywords: Neural development, tubulin gamma 1, tubulinopathies, Wnt/b-catenin signaling, zebrafish larvae, crispants.

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OP-23

Evaluation of miRNAs as genetic biomarkers in genetic generalized epilepsy

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Objective: Genetic generalized epilepsy (GGE) is a subtype of epilepsy that involves the entire brain and has a genetic etiology. GGE accounts for 15-20% of all epilepsies. There is no specific genetic biomarker widely used that contributes to the diagnostic process of this disease. In the last decade, there has been a radical increase in the number of studies on the use of small non-coding RNAs found in the human genome as genetic biomarkers for various neurological diseases. MicroRNAs (miRNAs), the best-known small non-coding RNAs, play a critical role in regulating neuronal biological processes through the modulation of gene expression. Therefore, miRNAs have assumed an important role in biomarker research due to their stability in clinical samples. In our study, we examined the expression levels of miR-106b, miR-130a-3p, and miR-194-5p in GGE patients with the aim of evaluating their performance as diagnostic biomarkers.

Methods: 21 patients with isolated GGE and 18 healthy controls were recruited in this study and total RNA was extracted from blood samples of participants. cDNA synthesis was performed from the obtained RNAs. The expression levels of miR-106b, miR-130a-3p and miR-194-5p were analyzed via qRT-PCR, and receiver operating characteristic (ROC) curves were generated and area under the curve (AUC) was calculated to evaluate the diagnostic values.

Results: The expression level of miR-130a-3p has shown statistically significant increase in patients compared to controls ($p<0.05$). The AUC value determined in the ROC analysis is 0.725. However, the expression levels of miR-106b and miR-194-5p are not detected statistically significant. Their AUC values in the ROC analysis are 0.648 and 0.571, respectively.

Conclusion: The varying expression levels of miRNAs that we used in our study may be associated with the etiopathogenesis of GGE. Our findings suggest using miR-130a-3p as potential biomarker in the diagnosis and prognosis of GGE.

Keywords: Genetic biomarker, genetic generalized epilepsy, microRNA, ROC curve.

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OP-24

Evaluation of delta 9-tetrahydrocannabinol effect on hyperinsulinemic gastric tissue

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Objective: Hyperinsulinemia (HI) occurs with chronically increased insulin levels and is strongly associated with metabolic diseases. Previous research show that delta 9-tetrahydrocannabinol (THC) has medicinal benefits. This study aims to investigate the relationship of THC between oxidative stress and apoptosis in the gastric tissue of hyperinsulinemic rats, as well as its effect on gastrointestinal hormones.

Methods: Male Sprague Dawley rats were used in this study and they were randomly divided into four groups (n=8 in each group): Healthy control (CTRL), HI, THC administered control (THC), and THC administration to HI (HI+THC). The effect of THC on rat gastric tissues with HI was evaluated using immunohistochemical staining and biochemical analyses.

Results: The number of poly (ADP-ribose) polymerase-1 and proliferating cell nuclear antigen (PCNA) immunpositive cells in the HI group was significantly reduced compared to the CTRL group. The number of PCNA immunpositive cells was significantly increased in the HI+THC group compared to the HI group. The number of caspase-3 immunpositive cells in the HI group showed a significant reduction compared to the CTRL group. Accordig to the findings from caspase-3 and caspase-9 immunohistochemical staining revealed that THC increased apoptosis in gastric tissues to some extent. The number of obestatin and ghrelin immunpositive cells was significantly higher in the HI+THC group than in the HI group. According to biochemical analyses, glutathione and malondialdehyde levels were significantly higher in the HI+THC group than in the HI group.

Conclusion: Both immunohistochemical and biochemical analyses revealed that THC administration may affect the regulation of gastrointestinal hormones and regeneration in the fundus of rats with HI. The results show that THC may be a promising therapeutic agent for gastric tissue damaged by HI.

Keywords: Delta 9-tetrahydrocannabinol, gastrointestinal hormones, hyperinsulinemia, oxidative stress.

OP-25

Reductase 2 (DHRS2) can be the novel inflammatory response regulator in breast cancer cells

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Objective: Breast cancer has a high incidence in women and molecular heterogeneity. Until today, there was a belief that breast cancer was immunologically "cold" compared to other cancers. However, recent studies have shown that the tumor microenvironment regulates the immune response, which varies from patient to patient and is dynamic. Therefore, our study examined the effect of DHRS2 gene expression on selected genes in the pyroptosis pathway, inflammatory response, and cytokine release.

Methods: MCF10A, MCF7, T47D, and MDA MB 231 are four human breast cell lines used in this study. After that, DHRS2 expressions were manipulated with gene-specific siRNA and gene-expression lentiviral vectors with liposome-dependent transfection individually. The highest DHRS2 silencing and overexpression in cells were determined via the qRT-PCR method and used for further analysis. Selected pyroptosis gene expression levels were investigated in DHRS2-manipulated and non-transfected cells. Gene expression changings were calculated with ΔCT method, and correlation analysis was made with GraphPad Prism v.8.

Results: PYHIN1 (IFIX), MEFV, and CASP1 had the highest expression change ratio among all selected genes. IL1A and IL1B had no expression in Luminal A type cells besides triple-negative cells, and non-cancerous cells showed increased gene expression when DHRS2 level also increased in the cell. Other genes also tended to change according to the amount of DHRS2 transcript in the cell.

Conclusion: It is curious how DHRS2, which is known to be associated with MDM2, affects other regulators of MDM2, such as IFIX. IFIX is one of the crucial proteins known to play a role in pyroptosis-mediated cell death and inflammatory response. Moreover, IL18 has a proinflammatory activity for tumor progression associated with DHRS2. Our findings suggest that alterations in the DHRS2 gene directly impact the inflammatory response in breast cancer and may serve as a marker for further cancer studies.

Keywords: Breast cancer, DHRS2, inflammation, pyroptosis.

OP-26

Identification of FDA-approved drugs as potential IRE1α inhibitors using molecular docking and molecular dynamics simulations

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Objective: High IRE1α activity has been associated with poor prognosis and protumoral roles in various cancers, including prostate cancer, multiple myeloma, and glioblastoma. Inhibiting IRE1α activity has the potential to induce apoptosis, reduce angiogenesis, and sensitize cancer cells to chemotherapy and immunotherapy. While several IRE1α inhibitors are known in the literature, many have not progressed beyond the preclinical stage, limiting their therapeutic use. In this study, we explored the interaction of a total of 2048 FDA-approved drugs and active metabolites with IRE1α using computational biology methods.

Methods: We conducted molecular docking simulations to investigate the interactions between IRE1α and 2048 compounds, selecting the top 20 compounds based on their docking scores and three known IRE1α inhibitors for further analysis. The selected compounds underwent 10 ns molecular dynamics (MD) simulations and free energy calculations. Subsequently, the top 3 compounds and 3 inhibitors were subjected to 100 ns MD simulations with three independent replicates, and free energy calculations were performed using the MM/PBSA method.

Results: Virtual screening and MD simulations identified Zafirlukast, Irinotecan, and Dutasteride as the top compounds with the highest binding scores and binding free energies among the 2048 screened compounds. The known IRE1α inhibitors, Sulfonamide inhibitor, Compound 18, and G-6904, exhibited average binding free energies of -228, -144, and -129 kJ/mol, respectively, while Zafirlukast, Irinotecan, and Dutasteride demonstrated binding free energies of -94, -142, and -103 kJ/mol, respectively.

Conclusion: Irinotecan displayed a binding activity similar to other known inhibitors but has the advantage of being approved by the FDA. We recommend further in vitro and in vivo investigations of Irinotecan for its potential in targeting IRE1α in cancer therapy.

Keywords: Dutasteride, irinotecan, molecular dynamics simulation, zafirlukast.

Acknowledgement: The numerical calculations performed in this study were provided with the support of TUBITAK-TRUBA and the National Center for High-Performance Computing of Türkiye. (UHeM) under grant number 1016382023.

OP-27

Evaluation of the semen microbiome for fertility in obese men with next-generation sequencing

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Objective: In this study, it was aimed to examine the microbial content and diversity in semen samples of obese men, to determine the differences between infertile and fertile groups, and to investigate the effects of seminal microbiota on semen parameters, sperm DNA fragmentation (SDF), sperm chromatin condensation, and total antioxidant capacity (TAC).

Methods: Thirteen obese infertile men aged 18-55 years with BMI over 30 kg/m^2 and five obese fertile men as the control group included in the study. For microbiome analysis, the V3 and V4 regions of the 16S rRNA gene were sequenced using the amplicon sequencing method, which is one of the next-generation sequencing techniques. SDF was analyzed by TUNEL test, TAC was analyzed by ELISA test and histone-rich sperm percentage was analyzed by the aniline blue staining method.

Results: It was seen that the most abundant bacteria in both groups belonged to the phylum of *Bacillota*, *Pseudomonadota*, *Actinomycetota* and *Bacteroidota*. The most common bacteria at the genus level were *Pseudescherichia*, *Staphylococcus*, *Paenibacillus*, *Streptococcus*, *Klebsiella*, and *Moraxella*, which had similar distributions in both groups. No statistically significant difference was observed between the groups in terms of alpha diversity (p=0.161). It was determined that *Brevibacterium, Paenibacillus, Alistipes, Lactiplantibacillus, Rhizobacter, Sphingomonas* and *Venlonella* genera were correlated with SDF; *Pantoea, Devosia, Bacteroides, Acidovorax* genera were correlated with TAC; *Fusobacterium* genera was correlated with the percentage of aniline-positive sperm, and *Corynebacterium, Hydrogenophaga,* and *Paenalcagenes* genera were correlated with BMI.

Conclusion: Bacterial species in semen may play a role in male infertility by affecting semen quality, sperm chromatin condensation, SDF or TAC. Considering the relatively small size of the study sample, more meaningful results can be expected with a larger sample.

Keywords: Male infertility, obesity, seminal microbiome, sperm DNA fragmentation, total antioxidant capacity.

OP-28

The analysis of the contribution of pleomorphic adenoma gene-like 2 (PLAGL2) on apoptosis in keratinocytes

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Objective: The outermost layer of the skin, known as the epidermis, has a renewing capacity. New skin cells are constantly generated in the basal layer, and as they mature, they undergo apoptosis to make space for newer cells. Impaired regulation of apoptosis in this process is often associated with skin developmental abnormalities and skin diseases. Pleomorphic Adenoma Gene-like 2 (PLAGL2) is a leucine-zipper transcription factor that has been shown to regulate apoptosis in different kinds of cells. Nevertheless, there has been no prior research into the impact of PLAGL2 on apoptosis in keratinocytes. Therefore, the aim of this study is to investigate the role of PLAGL2 in keratinocyte-specific apoptosis in terms of skin homeostasis.

Methods: The CRISPR/Cas9n system was used to silence the expression of *PLAGL2* in human keratinocyte HaCaT cells. Cloning and Sanger sequencing were used to identify CRISPR/Cas9n-mediated insertions and deletions (INDELs) on both alleles. Western blot was used to analyze PLAGL2 expression in PLAGL2 knock-out clones and control cells with anti-PLAGL2 antibody. Apoptosis was analyzed using flow cytometry to quantify Annexin-V/Propidium iodide (PI) stained cells and Western blot to determine apoptotic markers including Caspase3 and PARP.

Results: Following CRISPR/Cas9n-mediated PLAGL2 targeting, clones, in which PLAGL2 expression was shown to be silenced by Western blot, were analyzed for INDELs and two clones with INDELs causing frame-shift mutations in both alleles were chosen for the following analysis of apoptosis. Annexin-V/PI staining and apoptotic marker analysis demonstrated that silencing of *PLAGL2* expression in HaCaT cells significantly increased apoptosis compared to control.

Conclusion: Silencing the transcription factor PLAGL2 induces apoptosis in keratinocytes, implying that PLAGL2 may be involved in the regulation of epidermal homeostasis via controlling keratinocyte survival.

Keywords: CRISPR-Cas systems, gene knock out, HaCaT cells, PLAGL2 protein, programmed cell death.

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OP-29

The effect of ketogenic diet on mitochondrial cardiomyopathy

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Objective: As the major energy plant of the cell, genetic defects of mitochondrial bioenergetics cause an astonishingly wide spectrum of diseases. Currently, there are no treatments for mitochondrial diseases; their management is usually limited to supportive measures against symptoms and complications only. Ketogenic diet (KD) is a low-carbohydrate, high-fat diet that shifts metabolism from glycolysis to mitochondrial beta-oxidation. This study aimed to investigate the effect of KD on mitochondrial cardiomyopathy in a mouse model with a heart and skeletal muscle-specific deletion of the *Dars2* gene (hmKO), which covalently attaches aspartate to its tRNA during mitochondrial translation.

Methods: Five control and five hmKO mice were fed ad libitum with either KD or normal chow diet post-weaning. The study assessed whether KD improved phenotypic and molecular aspects of mitochondrial cardiomyopathy. Phenotypic assessments were based on lifespan, body weights, and behavioral experiments. Molecular assessments included mitochondrial DNA quantification; cardiomyopathy, mitochondrial biogenesis, antioxidant response, and beta-oxidation markers (protein/mRNA levels,) and ATP content.

Results: KD had no impact on the lifespan or body weights of hmKO mice compared to their chow-fed littermates. Similarly, behavioral tests showed no improvement in parameters such as movement, exercise capacity, and muscle strength. Molecular experiments revealed an increase in mitochondrial biogenesis, a shift towards fatty acid metabolism, and reduced antioxidant response as expected with KD. However, some of these molecular improvements (e.g., ATP levels) did not manifest at the phenotypic level. Furthermore, an increased heart-to-body weight ratio and elevated mitokine levels post-KD suggested cardiomyopathy progression.

Conclusion: The lack of phenotypic improvement is likely due to KD's inability to extend the lifespan of hmKO animals, necessitating the termination of KD intervention at week six. A three-week KD treatment proved insufficient to observe phenotypic amelioration, despite some molecular improvements, in a severe form of early-onset mitochondrial cardiomyopathy.

Keywords: Cardiomyopathies, ketogenic diet, mice, mitochondrial diseases.

Acknowledgement: This project was supported by the Scientific and Technological Research Council of Türkiye (TÜBİTAK) research grant 321S547 to Sukru Anil Dogan.

OP-30

Investigation of the effects of cisplatin and juglone on breast cancer cell lines

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Objective: In our study, it is aimed to examine the effects of the combination of juglone and cisplatin on breast cancer cells, to determine the expression changes in BLACAT1, miR-155-5P and CCR2 RNAs for this purpose, to investigate their roles in breast cancer and to reveal their possible biomarker properties and then to determine their possible roles in metastasis.

Methods: In this study, breast cancer cell lines MDA-MB-231 and MCF-7 were used. The cytotoxic effects of juglone and cisplatin were investigated by CCK-8 analysis. The effects of the substances applied in the cell lines on the expression levels of BLACAT1, miR155 and CCR2 ncRNAs were examined by qPCR, and their invasiveness was analyzed by the Transwell invasion assay.

Results: IC50 values were calculated as 6.24 µM/ml at 72nd hour for MCF-7 and 7.64 µM/ml at 72nd hour for MDA-MB-231 in cisplatin administration, 7.43 µM/ml at 48th hour for MCF-7 and 8.61 µM/ml at 48th hour for MDA-MB-231 in juglone administration. In the expression changes, a 5.98 -fold decrease in BLACAT1 and 2.09 -fold decrease in CCR2 was observed in the application of juglone to MCF-7 cells. miR-155 increased 2.13-fold. No significant change was observed in the expression of BLACAT1 in cisplatin administration, CCR2 increased 4.55 times and miR155 decreased 3.19 times. In the combined application, while the expressions of miR155 and CCR2 did not change, the expression of BLACAT1 decreased by 2.11 times. In the application of juglone to MDA-MB-231 cells, the expression of BLACAT1 increased 3.8-fold and miR155 increased 2.59-fold, and no significant change was observed in CCR2. While the expression of BLACAT1 did not change in cisplatin administration, the expression of CCR2 increased by 2.45 times and that of miR-155 decreased by 9.6 times. In the combined administration, the expression of BLACAT1 did not change, CCR2 increased 3.03 times and miR-155 increased 10.34 times. In invasion analysis, separate administration of substances in MCF-7 cells decreased invasion compared to the control group, and combined administration affected invasion less than single administration and control group. In MDA-MB-231 cells, juglone administration reduced invasion more than cisplatin. In the combined application, it affected the invasion less compared to the single application and the control group.

Conclusion: In addition to chemotherapy, the use of phytochemicals in the treatment of breast cancer, which is one of the highest incidence types of cancer in the world, is increasing day by day. Juglone, one of the important phytochemicals, is likely to be a potential therapeutic agent effective on metastasis and invasion in invasive breast cancer. In this context, we think that our study will contribute to the world literature.

Keywords: BLACAT1, breast cancer, CCR2, Cisplatin, Juglone, miR-155-5p.

Acknowledgement: This study was financially supported by Pamukkale University Scientific Research Projects Coordination Unit through project numbers 2022SABE015.

OP-31

Effect of chemotherapy-induced autophagic secretome on natural killer cell activity

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Objective: Autophagy induced by hypoxia or metabolic stress also plays an important role in the regulation of inflammatory pathways. In cancer cells, this may lead to secretion of pro- and/ or anti-inflammatory cytokines and chemokines which may help the immune escape of the tumor. Our aim is to investigate whether chemotherapy-induced autophagy-associated secretome has the potential to modulate the NK cell-mediated anti-tumor immune responses.

Methods: Initially, western blot and immunostaining analyses were performed for autophagy markers (LC3I/II and p62) to demonstrate that Etoposide (Eto) induces autophagy in MCF-7 cells. LC/MS-MS and cytokine array analysis were performed to determine the content of chemotherapy-induced autophagic secretome in supernatants of autophagy-induced MCF-7 cells. Finally, how the chemotherapy-induced autophagic secretome effects the capacity of DNAM1-NK-92 cells to target MCF-7 cells was determined by degranulation assays.

Results: We demonstrated Etoposide (Eto) induces autophagy in MCF-7 cells as confirmed by detection of autophagy markers including LC3I/II and p62 by WB and by immunostaining analysis. LC/MS-MS results revealed that, metabolic enzymes, tumor antigens, chaperones and metastasis-related proteins were secreted during etoposide induced autophagy which could be reduced by use of Chloroquin. In the cytokine array results, it was seen that the amounts of cytokine/chemokine and growth factors detected in the content of each autophagic secretome differed between the groups.When wildtype or DNAM1 overexpressing NK-92 cells were treated with autophagic secretomes, it was observed that there were differences in the capacity of targeting MCF-7 cells.

Conclusion: This study provides new insights in the field of chemo-immunotherapy by characterizing the chemotherapy-induced autophagic secretome and determining its possible effect on NK cells.

Keywords: Autophagy, etoposide,MCF-7, NK cells.

OP-32

Comparative RNA-Seq analysis between smooth and ulcerated plaque materials of asymptomatic carotid artery stenosis patients

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Objective: Carotid artery stenosis (CAS) is a cellular disease characterized by plaque accumulation in the arteries supplying the brain. CAS patients are divided into two groups: symptomatic and asymptomatic. Even if the stenosis degree is ≥70% in asymptomatic patients, stroke-related neurologic symptoms are not observed, complicating early diagnosis and treatment. Regardless of the stenosis degree, plaques are smooth or ulcerated according to their surface morphology, while they are grouped as stable or sensitive according to their biology. Unlike smooth or stable plaques, ulceration can increase the risk of stroke by creating vulnerable plaques prone to rupture. Biological characterization of plaques according to their morphology is important for establishing reliable treatment protocols in asymptomatic patients. In the current study, the biological characteristics of smooth and ulcerated plaques in asymptomatic CAS patients were determined at the transcriptome level, and effective biomarkers in the progression of ulceration were investigated.

Methods: Four asymptomatic CAS patients with compatible clinical features, 50% (n=2) of whom had ulcerated plaques and whose plaque morphology was determined by Doppler USG, were included in the study. RNA samples obtained from plaques resected by carotid endarterectomy were characterized by Agilent Bioanalyzer. Appropriate RNAs were taken for RNA-Seq analysis using the AmpliSeq transcriptome panel in the IonGeneStudio™S5 NGS system. Data was processed with CLC Workbench and IonReporter™. After identifying 1765 differentially expressed genes (DEG) between the two groups based on $p<0.05$&log2FC±1.5-fold, matching genes between the dataset and the literature and associated pathway analyses were performed using Ingenuity Pathway Analysis (IPA).

Results: According to IPA, the most significantly upregulated DEGs in the ulcerated group were *AVPI1* (10.13), *ID3* (3.071), *ECM2* (2.264), and *ADRA2C* (2.13); downregulated ones were found as *CCL19* (5.050), *CHI3L2* (4.118), *CXCL13* (3.76), *CCL21* (2.9) and *C15orf48* (2.16). It was detected that the main cellular functions cumulatively affected by the DEGs between the groups were cell death and survival, immune system-related cellular movement, and cell-cell interaction. The DEGs and changing cellular functions were determined to primarily affect actin cytoskeleton signaling, integrin, and p21-activated kinase (PAK) signaling pathways.

Conclusion: Our results show that altered regulation of PAK signaling, decreased cell-cell interaction, and chemokine signaling, which affects the immune system, play a role in distinguishing ulcerated plaques from smooth ones regarding biological properties in asymptomatic CAS patients.

Keywords: Asymptomatic, carotid artery stenosis, plaque biology, RNA sequence analysis, transcriptomic.

Acknowledgement: This research was supported by the Scientific Research Projects Unit of Bursa Uludag University (Project No: TAY-2022-592).

OP-33

Oncogenic protein phosphatase 1D/wild-type p53-induced phosphatase 1 phosphatase enhances basal and chemotherapy-induced autophagy in cancer cells

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Objective: PPM1D/Wip1 is a member of the PP2C family phosphatases Upon activation by genotoxic stress, Wip1 dephosphorylates key elements of DDR such as ATM, YH2AX, Chk1, Chk2, and p53 and contributes to the termination of DDR. Wip1 is overexpressed in common human cancers and thus recognized as an oncogene. Oncogenic regulates genotoxic stress-induced cellular responses such as cell cycle arrest, apoptosis, and senescence. Recent data suggest that Wip1 plays a role in the induction of autophagy. Autophagy is a catabolic process in which cellular contents are degraded and recycled. Autophagy plays a tumor-suppressive role in the early stages of cancer but supports tumor survival and contributes to resistance to cancer treatment in advanced stages of cancer. Currently, the molecular mechanisms underlying its role in treatment resistance remain largely unknown. In this study, we examined the role of oncogenic Wip1 in the induction of basal and chemotherapy-induced autophagy in cancer cells.

Methods: MCF-7, D283-med, and IMR32 cells were used. Induction of autophagy was achieved by etoposide. Chloroquine and GSK2830371 were used to inhibit autophagy and Wip1 respectively. WB analysis was used to assess LC3 I-II formation, p62 degradation, Wip1 Ulk1, p53, and p21 expressions. Confocal microscopy was used to test the co-localization of Wip1 and Ulk1. Co-IP analysis was performed to test the Wip1-Ulk1 interaction.

Results: Here we showed that oncogenic Wip1 enhances basal and chemotherapy-induced autophagy which further promotes cancer cell survival. Wip1 interacts with Ulk1 and dephosphorylates at Ser757 and Ser638 in breast, neuroblastoma, and medulloblastoma cells cancer. Chemotherapy-induced autophagy is accompanied by increased p53 levels and cell cycle arrest. Chemical inhibition of Wip1 by the allosteric inhibitor GSK2830371 and/or autophagy by hydroxychloroquine caused induction of apoptosis by preventing the degradation of pro-apoptotic proteins.

Conclusion: Our data provide evidence that oncogenic Wip1's play important role in promoting basal and chemotherapy-induced autophagy which contributes survival of cancer cells. Utilizing Wip1 and autophagy inhibitors may be a promising therapeutic strategy and warrants further investigations.

Keywords: Cancer, chemotherapy-induced autophagy, MCF-7.

OP-34

Investigation of LSM14A as a potential biomarker for Alzheimer's disease

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Objective: mRNAs are essential for neurons to continue cellular processes such as synaptic plasticity and memory formation. Processing bodies (P-bodies) bind the mRNAs, which are critical to the progression of synaptic plasticity at dendritic translation sites. These mRNAs may be silenced prior to translation and stored in the P-bodies, which consist of three main proteins: DDX6, 4E-T, and LSM14A. LSM14A plays essential roles in mRNA translation, storage, and degradation. Dendritic damage is observed in the early stages of neurodegenerative diseases. While it is not clear how much dendritic substance is released into the extracellular space due to damage, some studies have shown the presence of exosomes and mRNA in the cerebrospinal fluid (CSF). The presence of mRNA in dendritic regions with P-bodies suggests that mRNA could potentially exit into CSF via P-bodies. In this study, we investigated the potential of LSM14A as a biomarker for the early phase of Alzheimer's disease (AD) by measuring LSM14A levels in CSF of AD patients.

Methods: In this study, the level of LSM14A protein was detected by ELISA in CSF of 20 mild cognitive impairment (MCI), 42 AD, and 19 subjective cognitive impairment patients. Raw data was analyzed by GraphPad InStat DTCG 3.06 with a one-way ANOVA method.

Results: The level of LSM14A in the MCI group is significantly higher than in the control group, and the level of LSM14A increases proportionally with age of patients. In AD patients, LSM14A levels were high in the first three years of the disease.

Conclusion: According to our results, the level of LSM14A in the CSF of AD is high in the early phase of the disease. As a result, our results show that CSF LSM14A protein might be a biomarker for the early stage of AD. Our studies continue with the increasing number of patients.

Keywords: Alzheimer's disease, biomarker, LSM14A, neurodegeneration.

OP-35

Targeting ELF3 using anti-sense oligonucleotides in chondrocytes: a novel in vitro approach to osteoarthritis therapy

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Objective: Chondrocytes are the main cells that produce the extracellular matrix of articular cartilage and have a highly differentiated structure. Cartilage has a limited ability to regenerate itself, which is an important health problem worldwide. This limitation leads to cartilage degeneration in arthritic conditions such as inflammation-prone genetic diseases, rheumatoid arthritis and osteoarthritis (OA). ELF3 is a transcription factor, which is responsible from the regulation of genes related with inflammation, also remarkably increases its own expression through a super enhancer. Our research group, is currently focused on antisense oligonucleotide (ASO) based gene therapy options, has established a chondrocyte-inflammation model (CIM) in vitro to test the effect of ELF3 suppression to find a potential therapeutic target for inflammation-caused OA.

Methods: The CIM was generated by IL-1B (interleukin 1 beta) treatment in chondrocytes differentiated from mesenchymal stem cells (MSCs). In order to enlighten the inflammatory signature of the model at the transcriptomic level, RNA-sequencing was performed. Then ASOs were designed to suppress ELF3 expression on this model. Different concentrations of ELF3-ASO transfection was performed, and the gene expression was evaluated with qRT-PCR.

Results: At a concentration of 1000 nM ELF3-ASO was transfected into chondrocytes, ELF3 expression was found to be decreased by 60%. Moreover, the gene expressions of IL-1B and IL-6, genes associated with inflammation, were also decreased. The expression of MMP13, the main player of chondrocyte matrix destruction, was also decreased after ELF3 suppression.

Conclusion: After ELF3-ASO treatment, it was shown that the genes related with inflammation and chondrocyte matrix destruction were decreased. According to our results, ELF3-targeted therapies may be an effective strategy to reduce inflammation in the OA process which could lead to a potential target for treatment.

Keywords: Anti-sense oligonucleotides (ASO), chondrocyte, inflammation, osteoarthritis (OA), transcriptomics.

OP-36

Discovery and efficacy of new IL-17A inhibitors in the suppression of inflammation in multiple sclerosis with in silico, in vitro and in vivo approaches

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Objective: Multiple sclerosis (MS) is a complex autoinflammatory nervous system disease in which IL-17 plays a central role. Drug repositioning studies with computational biology methods are a current approach that enables rapid and efficient drug discovery in complex diseases. Therefore, our aim in this study is to explore new potential IL-17A inhibitors and their molecular mechanisms of action in MS using in silico, in vitro and in vivo approaches.

Methods: The receptor binding sites of the IL-17A ligand and the homodimer crystal structure of the ligand were prepared by in silico methods. Using molecular docking, MM/GBSA calculations and molecular dynamics (MD) simulations, the FDA-approved drug library was screened, and candidate molecules that would potentially inhibit receptor-ligand binding were listed. In vitro inhibition potentials of candidate molecules were determined using the ELISA method and HEK-Blue reporter cells expressing IL-17R. The cuprizone MS model was used to evaluate the in vivo activity of active molecules.

Results: Of approximately 2300 FDA-approved molecules, 12 candidate molecules that could inhibit IL-17A/IL-17RA binding were identified. The therapeutic activities of these molecules and their binding energies were calculated by MD simulation calculations. In vitro studies showed that 9 molecules inhibited the IL-17A/IL-17RA interaction almost 100%. The IC50 values of Berotralstat, Epirubicin and Mitaxantrone molecules were determined as the molecules showing the highest inhibition at 5mM and below. In the cuprizone MS model performed with selected candidate molecules, it was observed that the level of proinflammatory cytokines and T cell-subtypes responsible for inflammation, decreased as a result of IL-17A inhibition.

Conclusion: Nine of the 12 candidate molecules determined by the in-silico approach inhibit the IL-17A/IL-17RA interaction. The potential of using molecules known to have no toxic effect by drug repositioning studies as IL-17A inhibitors in MS has been determined by pre-clinical studies.

Keywords: Cuprizone, drug repurposing, ELISA, HEK-Blue IL-17 cell, IL-17A, multiple sclerosis.

OP-37

Common finding in rare neuromuscular diseases: mitochondrial protein import defect in skeletal muscle

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Objective: Mitochondrial proteins must be transported into the organelle correctly for proper functioning. Impaired mitochondrial protein import has a primary/secondary effect on the pathogenesis of various diseases. Besides, abnormal mitochondrial morphology, decreased membrane potential and ATP production, as well as increased reactive oxygen species affect mitochondrial protein import. However, no study has investigated the role of this mechanism on secondary mitochondrial damage detected in neuromuscular diseases. In our previous studies, common microRNAs (miRNAs) that play a role in the regulation of mitochondrial damage in different rare neuromuscular diseases (DMD, Megaconial Congenital Muscular Dystrophy (CMD), Ullrich CMD, and alpha-dystroglycanopathy) were identified. The possible target genes of these miRNAs were found to be associated with mitochondrial protein import. In this study, we studied the possible relationship between mitochondrial import mechanism and secondary mitochondrial damage detected in ethiopathogenesis of DMD, Megaconial CMD and Ullrich CMD.

Methods: MitoGFP plasmid was created by cloning the mitochondrial signal sequence of the Aconitase 2 (ACO2) protein into pcDNA3-GFP vector, and transfected to the primary myoblasts of DMD (n=2), Megaconial CMD (n=1), and Ullrich CMD (n=2) patients and 2 control individuals. In order to analyze mitochondrial protein import, transfected cells were immunofluorescently stained with the mitochondrial marker TOM20, and co-localization ratio of TOM20 and MitoGFP was analyzed by using the "Colocalization Threshold" plugin in the Fiji (v2.13.1) program.

Results: A statistically significant decrease in MitoGFP-TOM20 co-localization was observed in primary myoblasts of all patients compared to control cells.

Conclusion: We detected a defect in mitochondrial protein import mechanism (pre-sequence/TIM23 import pathway) in DMD, Megaconial CMD, and Ullrich CMD skeletal muscle cells. Therefore, this mechanism could be used as a common innovative therapeutic target for rare neuromuscular diseases with secondary mitochondrial damage.

Keywords: Mitochondrial damage, mitochondrial protein import, neuromuscular disorders, primary myoblast cell culture.

Acknowledgement: This study is supported by the Hacettepe University Scientific Research Projects Coordination Unit (Project No: TSA-2021-19199).

OP-38

Combinatorial effect of temozolomide, 5-fluorouracil and epirubicin treatment on glioblastoma

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Objective: Glioblastoma (GBM) are highly malignant primary brain tumors, characterized by rapid progression and high resistance to treatment. The resistance of the drug is highly correlated with chemotherapy failure. So far, temozolomide is the common standard chemotherapy prescribed for glioma tumors. Nonetheless, there are considerations about its therapeutic efficacy that exhibits some limitations in the apoptotic process. This study aims to overcome the resistance of glioma to TMZ by defining the ability of combined therapies' in enhancing temozolomide sensitivity and increasing its anticancer effect, by examining the impact of Epirubicin and 5FU combined with TMZ- induced apoptosis.

Methods: The U87MG cell line was treated TMZ resistant over a period of 16 days. Cells resistant to 200µM TMZ concentration were then tested under different combinations of Epirubicin and 5FU. Cell viability was evaluated by the MTT method. The IC50 values of the cells were determined for drugs in different combinations. At effective combined concentrations, ROS activity in U87MG cells was investigated by using DCFH-DA dye, and apoptosis evaluation by flow cytometry using Annexin V dye. Colony formation assay was performed to evaluate the effect of drugs on cancer stem cell potential.

Results: It has been shown that when U87MG cells resistant to 200µM TMZ concentration were given TMZ together with 200 nM Epirubicin and 5FU, nearly 100% of the cells lost their viability. While the determined 3-drug combination was determined as the most effective combination, the limited efficacy of different combinations was also shown. When an effective triple combination is applied, it has been shown that ROS activity in cells increases and cells are triggered to apoptosis. When the colony forming potential of cancer cells was evaluated, it was shown that triple therapy maximally limited the colony forming ability.

Conclusion: Our results demonstrate that the combination of Epirubicin and 5FU with TMZ can overcome TMZ resistance in glioblastoma cells. In addition, our study is the first study in which three chemotherapeutics with DNA binding properties were used together and epirubicin was used against drug resistance in glioblastoma.

Keywords: 5FU, combined therapy, epirubicin, glioblastoma, TMZ.

OP-39

Investigation of the effects of thiostrepton and cisplatin combination on anticancer and DNA repair genes in lung adenocarcinoma cells

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Objective: One of the factors contributing to drug resistance in chemotherapy is the activation of DNA repair genes. It has been reported that the FoxM1 transcription factor activates DNA repair genes in cancer cells, leading to chemotherapy resistance. In this study, the anticancer effect of simultaneous treatment of cisplatin as a chemotherapeutic drug and the FoxM1 inhibitor thiostrepton in lung adenocarcinoma cells (A549) and its changes on DNA damage response genes were also examined.

Methods: The effects of thiostrepton and cisplatin, administered separately and concurrently, on A549 cell viability were analyzed using the MTT assay. Their effects on cell migration were assessed using a wound healing assay. Changes in DNA repair genes were examined using real-time PCR.

Results: Simultaneous treatments of cisplatin and thiostrepton induced a significant cytotoxic effect in lung cancer cells. Combined treatments were observed to significantly reduce A549 cell migration according to the wound healing assay. Gene expression analyses showed that thiostrepton reduced FoxM1 expression and significantly decreased the expression of XRCC1, CSK1, Exo1, and Skp2 genes compared to the control in combined treatments.

Conclusion: These findings suggest that the combination of thiostrepton with cisplatin may enhance anticancer effects in lung adenocarcinoma cells and inhibit DNA damage repair induced by chemotherapy. Further analyses in resistant cell lines are recommended to elucidate the role of FoxM1 inhibition in overcoming chemotherapy resistance.

Keywords: Chemotherapy resistance, cisplatin, DNA repair, FoxM1, thiostrepton.

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OP-40

CXCR4 expression does not affect regenerative capacity of bone marrow multipotent mesenchymal stem cells in a model of intestinal damage

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Objective: We have previously shown that use of Trypsin results in a significantly decreased surface expression of CXCR4 by bone marrow mesenchymal stem cells (BM-MSCs). In this study, we aimed to evaluate the effects of CXCR4 expression on the regeneration of damaged tissues by BM-MSCs in a Mouse model of inflammatory bowel disease (IBD).

Methods: RAG2$^{-/-}$ mice were treated with 3% DSS for 5 days to induce colitis and animals were clinically assessed for weight loss, changes in stool and other signs of disease using the disease activity index (DAI) scoring system (DAI=0, healthy; DAI=6 colitis and DAI=12 severe colitis). Balb/c derived BM-MSCs collected with Trypsin or non-enzymatic methods were associated CXCR4 expression with flow cytometry. Animals with a DAI score of at least 6 where transplanted with CXCR4^{high} or CXCR4^{low} BM-MSCs intraperitoneally. Animals were fed FITC-Dextran to assess intestinal permeability through oral gavage 4 hours before dissection. Serum and colons were collected and FITC-Dextran levels were determined using fluorescent absorbance spectometry. Colons were fixed and paraffin embedded, followed by staining with Hematoxylin/Eosin to assess colon anatomy and epithelial layer integrity and with Alcian Blue to assess the mucus lining.

Results: Medium severity (DAI 7) colitis-like disease developed in all DSS-treated Rag2$^{-/-}$ mice. Colon lengths decreased significantly after DSS treatment ($p<0.05$) and intestinal permeability increased. Transplantation of both CXCR4^{high} and CXCR4^{low} BM-MSCs resulted in normalization of colon lengths ($p<0.005$) and intestinal permeability. Similarly, significant improvement ($p<0.0001$) was observed in both groups compared to the DSS group after histological evaluations. DAI scores decreased from 7 in DSS-treated non-transplanted mice to 0.3 in the CXCR4^{high} group and 2.3 in the CXCR4^{low} group.

Conclusion: CXCR4 is known to be one of the most important chemotactic receptors for MSCs. In this study, in an in vivo mouse model of IBD we found a similar improvement after transplantaton of CXCR4^{high} and CXCR4^{low} BM-MSCs, indicating that the regenerative effects of BM-MSCs may be largely independent of their migration and settlement in damaged tissue. We believe therefore that paracrine effects (through secretion of exosomes or cytokines) of BM-MSCs may play an important role in their regenerative capacities. Further studies in the DSS-induced IBD mouse model should determine the migration rate of BM-MSCs to the injured intestinal tissue and assess possible paracrine factors.

Keywords: Bone marrow, CXCR4 expression, intestinal damage, multipotent mesenchymal stem cells.

OP-41

Patient-originated high-grade glial tumor organoids maintained the tumor subtype heterogeneity

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Objective: Because high-grade gliomas are heterogeneous, therapeutic strategies are challenging to develop with 2-dimensional *in-vitro* models, which are limited in preserving tumors' cellular and mutational diversity. For this reason, it has become essential to develop new organoid models that preserve the heterogeneity and genome stability of tumors and simulate the structure and function of human organs. The present study aims to show that the patient's tumor characteristics are preserved by forming primary organoids from tumor samples of high-grade glioma patients.

Methods: Tissues diagnosed as high-grade glial tumors on the frozen evaluation were divided into two for pathological analysis and organoid culture. In order to determine the histopathological diagnosis of tumor tissues and subtypes of tumors, the expression status of KI67, GFAP, Olig-2, p53, and ATRX proteins was performed by immunohistochemical staining (IHC) analysis, and IDH-1 mutation status was performed by sequence analysis. For organoid culture, the tissues were dissected into 0.5-1 mm dissection and grown in GBO medium for 15 days in a CO_2 incubator with an orbital shaker. IHC and genomic analyses were performed again to show that the tumor characteristic of the patient was preserved after organoid formation.

Results: The histopathological diagnosis of 2 organoids grown in cell culture media was determined as high-grade glial tumors. The rate of KI67 in the first organoid developed was 10%, while this rate was 30% in patient tumor tissue. The rate of KI67 of the second organoid was 5%, while this rate was 35% in patient tumor tissue. The decrease in KI67 ratios of organoids by 30% in the first organoid and 20% in the second organoid compared to the tumor tissue of the patient was interpreted as apoptosis and necrosis developing due to hypoxia in the middle part of the organoids. GFAP positive, Olig-2 positive, ATRX negative, and IDH-1 negative were determined in both organoid and patient's tumor tissues. The p53 mutation was negative in the first organoid and positive in the second. In the histopathological and molecular evaluations of both organoids, it was observed that there was no significant difference in GFAP, Olig-2, ATRX, p53, and IDH-1 results when compared with the patient's tumor tissue ($p<0.05$).

Conclusion: Due to the preservation of tumor-specific features on organoid models, creating a biobank with patient materials with different characteristics and determining individual-specific treatments will be possible.

Keywords: High grade brain tumor, intra-tumor heterogeneity, organoid culture.

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OP-42

In vitro modeling of acute graft versus host disease using Rag2-/- mouse colonoids

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Objective: This study aimed to model Dacute graft versus host disease *in vitro* using colon organoids (colonoids) obtained from immunodeficient Rag2$^{-/-}$ mice.

Methods: Rag2$^{-/-}$ mouse colons were collected, cleared of stool, and cut into 2 mm pieces. Colon pieces were incubated with Gentle Cell Dissociation Reagent at room temperature and colon pieces were precipitated by filtering. Colon crypts were suspended in Matrigel, seeded in a dome shape in 24-well plates and cultured in organoid growth medium. CD3 T cells were isolated from C57BL/6 spleens to induce an aGvHD-like inflammatory state. Spleen T cells stimulated with CD3ϵ and CD28 were cultured in suspension with passage 3 colonoids and followed using live-cell imaging for 4 days. Colonoids were embedded in paraffin for immunohistochemistry staining and Hematoxylin/Eosin (H&E) staining. In order to determine the cellular organization; colonoids were stained for active caspase 3 to assess apoptotic cells, anti-cytokeratin 20 to stain colonocytes and anti-MUC2 to visualize goblet cells. To measure intestinal permeability, FITC-Dextran was added to the culture medium and photographs were taken at 15-minute intervals under a fluorescent microscope.

Results: Colonoids were successfully developed from RAG2$^{-/-}$ mice, and intestinal crypts surrounding a lumen were found in H&E stained sections. After immunofluorescence staining, Cytokeratin 20 expression was detected revealing the presence of colonocytes that covered luminalized crypt surfaces. Muc2-labeled goblet cells were detected among the epithelial cells on the crypt surfaces. Some of the crypt cells were marked with active-caspase 3. An increase in apoptotic cell rates and intestinal permeability was seen after RAG2$^{-/-}$ colonoid spleen T cell co-cultures. Live colonoid imaging showed that T cells attacked and destroyed the organoids.

Conclusion: In this study, we succesfully obtained colonoids from RAG2$^{-/-}$ immunodeficient mice. An aGvHD-like inflammatory environment was induced using organoids/T cell co-cultures. Future studies aimed at the development of drug or cellular therapies for aGvHD can used this model.

Keywords: Acute graft versus, Rag2-/- mouse colonoids, MUC2.

OP-43

Effect of thiacloprid on androgen receptor gene expression

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Objective: Androgens are hormones that are essential for sex differentiation and development. They act by binding to receptors such as the androgen receptor, which can function as a ligand-dependent transcription factor and is a member of the nuclear hormone receptor superfamily. Androgen receptor is expressed in various tissues, and has important effects on bone, muscle, prostate, adipose tissues, and reproductive, cardiovascular, immune, nervous, and hematopoietic systems. Thiacloprid is an insecticide that functions through nicotinic acetylcholine receptors. The effect of this frequently used pesticide on the expression of the gonadal androgen receptor gene was investigated.

Methods: Negative control group and three different treatment groups of thiacloprid (2.5, 5 and 10 mg/kg) were formed. 10 Swiss albino mice in the 10 mg/kg thiacloprid group and 8 Swiss albino mice in the other groups were used. Thiacloprid was administered subcutaneously to mice with a total of 11 injections for 21 days. At the end of the experiment, the gonads of the mice were removed under anesthesia and the expression levels of the androgen receptor gene were investigated by quantitative real-time PCR. Total RNA was isolated, purity was determined, and cDNA synthesis was performed accordingly. The expression levels of the androgen receptor gene were normalized according to GUSB and TFRC housekeeping genes and determined compared to the control group using the ΔΔCt method.

Results: Androgen receptor gene expression was found to be 0.25-, 0.11-, 0.16-fold in the 2.5, 5 and 10 mg/kg thiacloprid treatment groups, respectively ($p<0.05$). Thiacloprid inhibited the expression of the androgen receptor gene.

Conclusion: A dose-independent inhibition of the androgen receptor was detected after treatment of different doses of thiacloprid. Suppression of androgen receptor gene expression can lead to the development of different diseases, including reproductive disorders. The pathway by which this effect occurs should be investigated in detail.

Keywords: Gene expression, gonad, pesticide.

OP-44

Fluopyram suppresses gonadal androgen receptor expression

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Objective: Fluopyram is a pesticide that functions as a mitochondrial complex II inhibitor, has a very long half-life in soil and has low water solubility. Because of its permanence in nature, it may reach humans even in low doses. It is known that pesticides can have effects on the androgen receptor. Androgens, function through the androgen receptor, have an important role in many systems, especially reproduction. The effect of fluopyram on the androgen receptor was investigated.

Methods: Fluopyram groups at 3 different doses (0.5, 1, 2 mg/kg) and negative control group were formed, with 8 Swiss albino mice in each group. Fluopyram was administered to mice by subcutaneous injection every other day for 21 days, after which the testes and ovaries of the mice were removed. After total RNA isolation from tissues, cDNA synthesis was performed, and the expression levels of the androgen receptor gene were determined compared to the negative control group with the StepOnePlus qRT-PCR device. qPCR data were normalized according to GUSB and TFRC reference genes and evaluated through the REST2009 program.

Results: Similar results were seen in all groups, in a dose-independent manner. Accordingly, after 0.5, 1 and 2 mg/kg fluopyram treatment, the fold change of the androgen receptor gene was determined as 0.11, 0.13, 0.09, respectively ($p<0.05$).

Conclusion: Fluopyram suppressed androgen receptor gene expression at all doses in similar manner. The androgen receptor is essential in the development of genital organs and secondary sex characters and in the normal function of the reproductive system in both males and females. Androgen receptor deficiency may pave the way for the development of different diseases, including infertility, and different cancers. The inhibitory effect of fluopyram on androgen receptor gene expression reveals that the effect of this pesticide on the androgen receptor signaling pathway needs to be determined.

Keywords: Androgen receptor, gene expression, pesticides.

OP-45

Investigation of the effect of the TMED9 gene on multiple myeloma cell lines

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Objective: Multiple myeloma (MM) is a hematological malignancy characterized by abnormal immunoglobulin accumulation in plasma cells. The product of the TMED9 gene is carrier protein expressed in endoplasmic reticulum membrane. Our previous transcriptome study, we found that TMED9 gene has a high RPKM value in MM patients. This study, we aimed to investigate the function of TMED9 in cell lines due to its role protein transport in MM.

Methods: TMED9 gene expression was suppressed by siRNA-mediated electroporation in MM cell lines U266 and RPMI8226. In both cell lines, TMED9 was overexpressed using expression vector. The change in gene expression levels was determined by qPCR and calculated by ΔΔCT method. Changes the protein level of TMED9 were analyzed by Western blot. All experimental groups, cells were stained with Apopxin-Green, 7-AAD and CytoCalcein Violet dyes for apoptosis determination and visualized with confocal microscope. ImageJ program was used to determine the number of apoptotic and viable cells. The effect of TMED9 on cell proliferation was analyzed by MTT assay.

Results: TMED9 expression was found to be suppressed by 74%, 78% at 24h and 50%, 65% at 48h in U266 and RPMI8226, respectively. In the vector group, TMED9 was highly expressed especially at 24 hours and was found to be increased 100-fold in U266 and 80-fold in RPMI8226 cells. The changes of TMED9 was demonstrated at the protein level by chemiluminescence imaging. MTT results revealed that cell proliferation of siRNA-treated groups decreased compared to the control group. Especially at 24 hours, cell viability was found to decrease by 60% in U266 cells and 45% in RPMI8226 cells. Apoptosis assay results, it was calculated that the apoptotic cell rates were above 50% in the siRNA treated groups at 24 and 48 hours in both cells. However, TMED9 overexpression was shown to increase cell viability and proliferation compared to control groups.

Conclusion: In this study, we found that suppression of TMED9 gene expression decreased the viability of MM cells and triggered apoptosis. Our results are promising findings in MM as TMED9 has been associated with poor prognosis in different cancer types. In future studies, we plan to investigate the pathways through which this association occurs. This is the first functional study targeting the TMED9 gene in U266 and RPMI8266 cells.

Keywords: Apoptosis, cancer, proliferation, siRNA, TMED9.

OP-46

Effect of rosuvastatin on apoptosis and autophagy in the rat model of CLP-induced sepsis

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Objective: The sepsis is a systemic inflammatory response syndrome and one of the important life-threatening causes of death in intensive care units. Although it affects many organs, the most common fatal damage occurs in the lungs. Statins have been shown to be effective in regulating the response to inflammation and repairing damage associated with sepsis. Rosuvastatin exhibits higher pleiotrophic effects as well as greater enzyme suppression properties. Our aim was to evaluate the dose-dependent effect of rosuvastatin on apoptosis and autophagy in lung tissue in the rat model of sepsis induced by cecal ligation and puncture (CLP).

Methods: The sprague Dawley rats were randomly divided into four groups: Sham, CLP, CLP+Rosuvastatin (10 mg/kg), CLP+Rosuvastatin (20 mg/kg). Rosuvastatin was given orally 4 hours before the CLP protocol. The rats were sacrificed 16 hours after the CLP protocol by monitoring their mortality.The expressions of Bcl-2 and Bax genes for apoptosis, and beclin-1 and LC3 genes for autophagy were analyzed using the real-time RT-PCR method. The ratio of the expression level of the anti-apoptotic Bcl-2 gene to the pro-apoptotic Bax gene was compared between the groups.

Results: It was observed that apoptosis was significantly suppressed in the group treated with 10 mg/kg rosuvastatin and induced in the 20 mg/kg group. LC-3 and beclin-1 are responsible for the formation of the autophagosome and the phosphatidylinositol-3-kinase complex, respectively. LC3 gene expression level decreased significantly in the group treated with 20 mg/kg rosuvastatin. Beclin-1 gene expression level decreased significantly in both dose groups. Although rosuvastatin had a protective effect on both cellular steps leading to tissue damage, 10 mg/kg was effective in apoptosis and 20 mg/kg was effective in autophagy.

Conclusion: These findings may contribute to the inclusion of the widely used rosuvastatin in new treatment strategies.

Keywords: Cecal ligation and puncture, lung, rosuvastatin, sepsis.

OP-47

Effects of vitamin D on the liver in rats fed a high-fat and high-fructose diet

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Objective: In this study, we aimed to investigate the effects of vitamin D treatment on liver degeneration in the metabolic syndrome (MetS) model caused by a high-fat and high-fructose diet.

Methods: The study involved four experimental groups: a healthy control group (HC), a control group treated with vitamin D (K+VD), a MetS group (MetS), and a MetS group treated with vitamin D (MetS+VD). Vitamin D supplementation (oral 170 IU/week) was initiated in the treatment groups starting from the third week of the experiment and continued until the end of the 15th week. During the study, the weights, fasting blood sugar, waist circumference, and calorie intake of all groups were measured. At the end of the experiment, the liver tissues were fixed and embedded in paraffin blocks. Hematoxylin-Eosin, PAS, and Van Gieson staining were performed on the tissue sections taken for morphological evaluation and detection of glycogen accumulation and fibrosis. In addition, the immunohistochemistry method was used to analyze the expression of various proteins (TGF-β1, α-SMA, NLRP3, GSDMD, GPX-4, PCNA, VDR) in liver tissue sections. Apoptotic cell death was measured using the TUNEL method, and serum insulin levels were assessed using the ELISA method. Statistical analysis was performed on all the obtained results.

Results: Fasting blood sugar and serum insulin levels ($p<0.001$), weight, and waist circumference measurements were found to increase in the MetS group compared to all other groups, while all these values decreased in the MetS+VD group. It was shown that TGF-β1, α-SMA, NLRP3, GSDMD, GPX-4 protein expressions, and apoptotic index were significantly higher in the MetS group compared to other groups ($p<0.001$). Especially with vitamin D treatment, a significant decrease was detected in the expression of these proteins and the apoptotic index ($p<0.001$).

Conclusion: Vitamin D application was found to reduce fasting blood sugar levels in MetS, regulate the liver's response to insulin, and have positive effects on oxidative stress, inflammation, fibrosis, cell death, and proliferation.

Keywords: Cell death, liver degeneration, metabolic syndrome, vitamin D.

OP-48

Investigations of microtubule plus end tracking proteins in an in vitro SMA model

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Objective: Spinal muscular atrophy (SMA) is a neurodegenerative disease caused by SMN protein deficiency. SMN loss causes dysregulations of proteins regulating the neuronal cytoskeleton, including microtubules. Alterations of proteins regulating microtubule dynamics have been previously described in SMA model systems.We previously showed downregulation in microtubule plus end-binding 3 (EB3) protein and increased comet numbers in SMN-depleted motor neuron-like cells. EB3 is a hub for binding of other plus-end tracking proteins (+TIP) on growing microtubule ends. Here, we hypothesized that dysregulations in EB3 could have an impact on +TIPs . Therefore, in this study, we created a protein interaction network of SMN with microtubule-associated proteins (MAPs), including +TIPs using bioinformatic tools, and analyzed microtubule plus end tracking $p150^{Glued}$ and CLIP170 proteins in an in vitro SMA model.

Methods: To construct a protein interaction network, we retrieved the list of all MAPs, and both direct and indirect interactions with SMN were collected. Among all, levels of EB3-interacting proteins, namely, $p150^{Glued}$ and CLIP170 were analyzed by Western blot using SMN knockdown motor neuron-like NSC34 cells. Immunofluorescence stainings and confocal imaging were performed for quantitative image analysis of $p150^{Glued}$ and CLIP170 comet structures by Image J.

Results: Several candidate MAPs, having potential to functionally interact with SMN were identified via bioinformatic analysis. In SMN-depleted cells, significant upregulation was found in $p150^{Glued}$ but not CLIP170 protein level compared to controls. Quantitative microscopic analysis showed no alterations in CLIP170 comets, however, $p150^{Glued}$ comet numbers were significantly increased at the proximal and distal part of neurites in SMN-depleted cells.

Conclusion: We concluded that dysregulations in $p150^{Glued}$ could have an impact on microtubule dynamics as well as retrograde transport since it is the major subunit of dynactin. The findings indicate that motor proteins may have a role in the pathomechanisms of SMA and have been investigating in our ongoing studies.

Keywords: Microtubule, microtubule plus end tracking proteins, spinal muscular atrophy.

Acknowledgement: This study is supported by The Scientific and Technological Research Council of Türkiye (TÜBİTAK, Project number; 121S884).

OP-49

Determination of endoplasmic reticulum sensor protein IRE1-RNAase activity dependent miRNAs and target genes in triple negative breast cancer

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Objective: Stress factors affect the protein folding function of the Endoplasmic Reticulum (ER) and activates Unfolded Protein Response (UPR). IRE1 is a bifunctional UPR stress sensor protein with kinase and RNase activity. Under ER stress conditions, IRE1 cuts mRNAs and intracellular specific miRNAs located in ER by activating its RNase function. miRNAs have been shown to play important roles in Triple negative breast cancers (TNBC) and miRNAs have been also shown as key regulators of UPR signaling pathway. However, there are no studies in the literature regarding miRNAs regulated by IRE1-RNase activity in TNBC. In this study, we aimed to find the relationship between critical proteins playing a role in TNBC development and miRNA expressions regulated by IRE1 RNAse activity.

Methods: We isolated miRNAs from MDA-MB-231 and MCF10A cells treated with the IRE1-RNAase inhibitor/DMSO and we performed miRNome analysis by using microRNA PCR-array. miRNAs with altered expression in MCF10A cells were excluded from the study. Then, differentially expressed 10 miRNAs were selected for target gene prediction by using "mirWalk2.0" and ‘’Target scan’’ bioinformatic tools. Finally, to test the molecular connections of miRNAs regulated by IRE1 with important proteins involved in BC, we verified the miRNA-target gene relationship for each miRNA by using miRNA modulator molecules.

Results: Our results show that more than 100 miRNAs were differantially regulated by IRE1-RNAase activity in TNBC. hsa-miR-32-5p, has-miR-200c and let-7f-5p miRNA expressions were downregulated, hsa-miR-362-5p and hsa-miR-646 expressions were upregulated when IRE1-RNAse activity inhibited. We transfected cells with these miRNA’s modulators and observed that let-7a regulates PIK3CA, hsa-miR-32-5p regulates FRS2 and hsa-miR-362-5p regulates CDK6 under control of IRE1-RNAse activity.

Conclusion: Our findings revealed functional links related to breast cancer- miRNAs and UPR. In addition, miRNAs regulated by the IRE1 RNase activity, and the genes targeted by these miRNAs were identified for the first time in TNBCs by this study.

Keywords: Endoplasmic reticulum stress, inositol-requiring enzyme-1 (IRE1), microRNA, triple negative breast cancer.

Acknowledgement: This study was supported financially by the Scientific and Technological Research Council of Türkiye. (TÜBİTAK, project number: 120Z719)

OP-50

Investigating the role of SPINK2 in cell death and proliferation in acute myeloid leukemia

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Objective: It has been determined Serine Protease Inhibitor Kazal Type 2 (SPINK2) is overexpressed in Acute Myeloid Leukemia (AML) patients. However, the exact function of high SPINK2 expression in AML hasn't been fully elucidated. This study aimed to investigate the effect of SPINK2 expression on cell proliferation and cell death in AML cell line THP-1 by suppressing through lentivirus-mediated.

Methods: The impact of suppressing SPINK2 expression on cell proliferation was examined in THP-1 cells using the MTT test, and its effect on cell death was investigated by a flow cytometer, Western blot, and qRT-PCR methods.

Results: When SPINK2 was suppressed, a 28% decrease in cell viability was observed at 24 hours, and reductions of 38% and 40% were found at 48 and 72 hours, respectively. Flow cytometer analyses showed a 27% decrease in cell viability at 24 hours and a 39% decrease at 48 hours. Western blot analysis using pro/active-caspase-3 antibody revealed presence of pro-caspase-3 protein in both SPINK2-suppressed cells and control cells, but active caspase-3 was not observed. During the study, a publication suggested potential association between SPINK2 and ferroptosis. Thus, the study also investigated the impact of SPINK2 suppression on ferroptosis. It was observed that SPINK2 suppression led to a 90% decrease in STEAP3 expression, but there was no change in SLC7A11 gene expression.

Conclusion: This study demonstrated suppressing SPINK2 in THP-1 cells is associated with cell death. The results suggest cell death caused by SPINK2 suppression occurs independently of caspases and is not related to ferroptosis. The discrepancy with previous studies may be attributed to TP53 mutations in THP1 cells and the p53-mediated role of STEAP3 in ferroptosis induction. TP53 mutations are commonly associated with a poor prognosis and treatment resistance in AML. Our study suggests inducing cell death in TP53-deficient cell lines suppressing SPINK2 expression proposes SPINK2 as a potential therapeutic target in AML patients with TP53 mutations.

Keywords: 3rd generation lentivirus, acute myeloid leukemia, ferroptosis, SPINK2 expression.

OP-51

The impact of endocrine disrupting chemicals-induced oxidative stress on the detoxification organs and disease pathogenesis

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Objective: Endocrine-disrupting chemicals (EDCs) are widely used in industrial products, including cosmetics, food and drink packaging, drugs, toys, households, medical devices, pesticides, personal care products, and paints. Phthalates exert endocrine-disrupting effects in humans and wildlife associated with the pathogenesis of various diseases, including diabetes, obesity, infertility, cardiovascular diseases, metabolic syndrome, and cancer. We investigated the impact of *in-utero* exposure to DHP and DCHP in rats associated with liver metabolism.

Methods: Pregnant albino Sprague Dawley rats were divided into control groups administered only with corn oil as a vehicle and treatment groups treated with di-*n*-hexyl phthalate (DHP) and dicyclohexyl phthalate (DCHP) prepared in the corn oil, respectively (n=10). DHP and DCHP administration was performed at dosages of 0 (corn oil=vehicle), 20, 100, and 500 mg/kg bw/day prepared in corn oil by gavage from GD 6 to GD 19. After birth, male and female rats were grown and sacrificed at day 90. Animal weight, hematological, and serum biochemistry biomarkers were measured. Histopathological and biochemical parameters regarding oxidative stress-induced liver damage were evaluated.

Results: Relative and absolute liver weights, triglyceride, alanine transaminase (ALT), lactate dehydrogenase (LDH), and alkaline phosphatase (ALP) levels were altered upon DCHP and DHP administration. Histopathological changes, including congestion, sinusoidal dilatation, inflammatory cell infiltration, cells with a pyknotic nucleus, lysis of hepatocytes, and degeneration of hepatic parenchyma have been observed in the liver samples of DHP and DCHP dose groups. Moreover, increased glutathione s-transferase (GST), glucose 6-phosphate dehydrogenase (G6PD), and glutathione reductase (GR) activities have been found in the liver samples of DHP and DCHP-treated rats addressing impaired oxidative stress metabolism.

Conclusion: For the first time in the literature, we showed that *in-utero* exposure to DHP and DCHP leads to liver damage caused by impaired oxidative stress metabolism in male and female rats.

Keywords: Animal model, liver damage, oxidative stress.

OP-52

The effect of amyloid beta 1-42 on the expression of mitochondrial dna-encoded genes

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Objective: Mitochondrial dysfunction is a common feature in the pathology of various neurodegenerative diseases. Mitochondrial damage is an early characteristic of Alzheimer's disease (AD). The major peptide in AD pathology, amyloid beta 1-42 (Aβ1-42), has been shown to localize within mitochondria. However, our knowledge regarding the specific roles of Aβ1-42 within mitochondria is limited. Mitochondrial dysfunction in AD may also involve changes in the expression of genes encoded by mitochondrial DNA (mtDNA). Altered mitochondrial gene expression has been reported in AD patients compared to control groups. Moreover, our previous studies and existing literature have demonstrated that the nuclear localization of Aβ1-42 can bind to promoter regions of nuclear DNA, affecting gene expression. This study aims to investigate the effect of different doses of Aβ1-42 peptide on the expression of mtDNA-encoded genes.

Methods: HEK 293T cells were treated with 0.1μM and 1μM Aβ1-42. RNA isolations were performed at 24 and 48 hours after treatment. The expression of 13 respiratory complex genes, 2 mitochondrial rRNA genes, and 3 mitochondrial tRNA genes encoded by mtDNA was investigated through qRT-PCR following cDNA synthesis. Statistical analysis of the results was carried out using one-way ANOVA test in GraphPad Prism 8.

Results: It was determined that the gene expression levels of MTND3, MTND4, MTND4L, MTND5, MTATP6 in cells treated with 0.1μM Aβ1-42 for 24 hours were significantly decreased compared to the control group. MTCO2 gene expression level in the group treated with 0.1μM Aβ1-42 for 48 hours, and MTND4L and MTATP8 gene expression levels in the group treated with 1μM Aβ1-42 were significantly decreased compared to the control group.

Conclusion: The decrease in the expression levels of certain mitochondrial genes following Aβ1-42 treatment suggests that this peptide may be involved in the regulation of mitochondrial gene expression. These findings, indicating that Aβ1-42, a pathological component of AD, may directly or indirectly influence the expression of mtDNA-encoded genes, will contribute to our understanding of mtDNA gene expression changes in AD patients and shed light on the limited-known functions of this peptide within mitochondria.

Keywords: Alzheimer's disease, amyloid beta 1-42, mitochondria, mitochondial DNA.

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OP-53

Interaction of LZTR1 and NUAK2 proteins and their effects on the mitochondrial apoptosis pathway

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Objective: Leucine Zipper Like Post Translational Regulator 1 (*LZTR1*) has tumor suppressor functions in glioblastoma multiforme (GBM) and hepatocellular carcinoma (HCC), while NUAK Family Kinase 2 (*NUAK2*) has an oncogenic function. The aim of this study is to investigate the relationships between LZTR1 and NUAK2 and their effects on mitochondrial apoptosis pathway-induced cells.

Methods: For this purpose firstly, human embryonic kidney 293 (HEK293) cells stably expressing Flag-tagged LZTR1 were prepared. The interactions of LZTR1 and NUAK2 proteins and their intracellular localizations were examined by immunoprecipitation (IP) and immunofluorescence (IF) staining methods, respectively. Apoptosis was triggered by treating cells with 0.4 mM hydrogen peroxide (H_2O_2) for 10 h. BCL-2, BAX, p53, phosphorylated (p)-p53-Ser15, p-p53-Ser46, Survivin, Caspase 3, active Caspase 3, PARP1, and active PARP1 levels in parental HEK293 cells and stably LZTR1-expressing clones that were transiently transfected/untransfected with NUAK2 expression plasmid or treated/non-treated with H_2O_2 were examined by western blotting (WB) method, using specific primary antibodies.

Results: IP experiments indicate that LZTR1 and NUAK2 interact. IF analyzes showed that LZTR1 and NUAK2 protein are found mostly in cytoplasm and nucles, respectively, alone; while in the presence of LZTR1, the localization of NUAK2 alters to be in the cytoplasm. Our WB results showed that active PARP1 protein levels increased with H_2O_2 treatment, LZTR1 levels decreased in apoptotic cells, LZTR1 caused decrease of NUAK2 levels, LZTR1 and NUAK2 co-expression in apoptotic cells caused decrease of Survivin but increase of p-p53-Ser46 levels.

Conclusion: With this study, interactions of LZTR1 and NUAK2 proteins, LZTR1-induced intracellular localization change of NUAK2, and the effects of LZTR1 and NUAK2 on H_2O_2-induced apoptosis were shown for the first time. These findings have potential to contribute to elucidation of the functions of LZTR1 and NUAK2 proteins and their roles in diseases, which they were associated with.

Keywords: LZTR1 and NUAK2 proteins, mitochondrial apoptosis pathway.

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OP-54

Interaction of alpha synuclein protein with mitochondrial DNA

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Objective: Mitochondrial dysfunction, which is one of the common mechanisms of neurodegenerative diseases, is among the early indicators that occur before clinical symptoms in Parkinson's disease (PD).The localization of alpha-synuclein (α-syn), which contributes to PD pathology, in the mitochondria has been demonstrated, and its role in mitochondrial biogenesis has been reported. In substantia nigra tissues from PD patients, changes in the expression of genes encoded by mitochondrial DNA (mtDNA) have been shown compared to control groups. Furthermore, our previous studies have revealed alterations in mtDNA gene expression in the leukocytes of PD patients. It is known that α-syn, an amyloidogenic protein, acts as a transcription factor, binds to nuclear DNA, and affects the expression of many genes. Additionally, α-syn can work in conjunction with nuclear transcription factors. Therefore, our study investigates the interaction of α-syn protein with mtDNA and its impact on the mtDNA binding capacity of mitochondrial transcription factor A (TFAM) as a result of the overexpression of the SNCA gene.

Methods: In our study, differentiated LUHMES cells were used. Mitochondrial chromatin immunoprecipitation experiments with α-syn and TFAM antibodies were conducted under physiological conditions and in cells with overexpression of the SNCA gene.Purified DNA fragments were analyzed using qPCR with primer pairs covering the mitochondrial genome. The results were expressed as fold enrichment compared to the negative control.

Results: Our results indicate that in the group with overexpression of the SNCA gene, α-syn interacts with specific regions of mtDNA. In the group with overexpression of the SNCA gene, we observed specific changes in TFAM binding signals along the mtDNA genome, which were dependent on particular regions.

Conclusion: According to our findings, α-syn, known to influence histone proteins and some transcription factors in the nuclear genome, may impact the transcription of mtDNA-encoded genes. Therefore, the observed changes in mtDNA gene expression in Parkinson's disease could occur due to α-syn's transcriptional influence. These insights also provide a fresh perspective on the α-syn-mitochondrial relationship in PD.

Keywords: Alpha synuclein, mitochondrial DNA, Parkinson’s disease.

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OP-55

Somatic variations in hepatocyte nuclear factor-1 alpha gene in glial tumors and their impact on survival

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Objective: The hepatocyte nuclear factor-1 alpha (HNF1A) gene is a crucial transcription factor for the expression of genes specific to the liver. Despite the analysis of HNF1A gene expression and variations in different cancer types, its association with glial tumors has not been clearly understood. Our study aims to investigate somatic variations of the HNF1A gene in patients with low and high-grade glial tumors and examine their impact on survival.

Methods: In our study, the exon-3 and exon-4 of the HNF1A gene, known to frequent variations in solid tumors, were analyzed using next-generation sequencing. After DNA isolation from four low-grade and twelve high-grade glial tumors, library preparation was performed for next-generation sequencing. Single nucleotide polymorphisms (SNPs) and deletions-duplications (Indels) identified through Archer analysis were analyzed for clinical significance in cancer research platforms such as My Cancer Genome, cBioportal, Clinvar, and COSMIC. Survival analyses of patients were conducted using the Kaplan-Meier analysis.

Results: A frameshift variant NM_000545.8 (HNF1A):c.864del (p.Pro291fsTer51) (rs762703502), previously reported as pathogenic for monogenic diabetes, was detected in two low-grade and three high-grade gliomas. This variant, with an allele frequency of 0.00112 reported in the ExAC database, was reported for the first time in glial tumors. Survival analysis between patients with and without this variation showed a significant difference (log-rank p=0.022).

Conclusion: While somatic variations in the HNF1A gene have been previously reported in various cancers, this study reports the c.872del (p.Pro291fsTer51) variant, previously known to be pathogenic for monogenic diabetes, for the first time in glial tumor patients. This somatic variation was found in five out of sixteen patients (31%). Despite the limited number of cases, patients with variation had a higher survival rate. Our study highlights the significance of somatic variation in the HNF1A gene in glial tumors and their association with survival, but further studies with a larger number of cases are needed.

Keywords: Glial tumor, hepatocyte nuclear factor-1 alpha, next-generation sequencing, survival analysis.

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OP-56

CCDC84, associated with microcephalic primordial dwarfism, directly interacts with pre-mRNA splicing factor PRPF3

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Objective: In our previous study on a MPD family, by homozygosity mapping, candidate gene approach and systematic mutation analysis we identified an autosomal recessively inherited splice site deletion (g.17185_17188delAAGT) causing a frameshift and premature termination codon (p. Lys269Serfs10Stop) in the *CCDC84 (CENATAC)*, whose function is unknown. The aim of this study was to identify the interaction partners of CCDC84 protein to contribute to understanding the molecular mechanism of the MPD.

Methods: Immunoprecipitation, SDS-PAGE separation of immunoprecipitated proteins, and subsequent mass spectrometry analysis of separated protein bands were used to determine the potential CCDC84 interaction partners. Both immunofluorescence and immunoblot analysis were used to confirm the identified candidate CCDC84-interacting protein. *In vitro* pull down assays were utilized to determine if the identified interaction is direct or indirect as well as which domain of the CCDC84-interacting protein mediates this interaction. The impact of the identified CCDC84 mutation on the interaction was also assessed at the same time.

Results: Mass spectrometry analysis revealed a promising interaction between CCDC84 and PRPF3. While immunofluorescence staining showed that both proteins were localized in the nucleus, immunoblot analysis verified the interaction. In vitro pull-down analysis showed a direct interaction between CCDC84 and PRPF3, and PRP3 domain of the PRPF3 mediates this interaction. The CCDC84-PRPF3 interaction was considerably disrupted both in vivo and in vitro in the presence of the CCDC84 gene mutation that we identified.

Conclusion: This study shows a direct interaction between CCDC84 and PRPF3, which is essential for pre-mRNA splicing. The findings not only demonstrate the importance of CCDC84 for splicing but also highlight the critical role of splicing in the molecular basis of MPD.

Keywords: Coiled coil domain containing-84, microcephaly, PRPF3, splicing.

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OP-57

The effect of human astrocyte-derived α-synuclein increase on trophic factor release in neuron-astrocyte synaptic components and neurodegeneration

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Objective: Astrocytes are necessary to form a proper synapse structure, and dysfunctions in astrocytes cause loss of synaptic connections and neurodegeneration. Astrocytes are the most essential source of trophic factors. Trophic factors have critical roles in neuron survival and the formation of synaptic connections. Our previous study reported that α-synuclein overexpression in human astrocytes alters the production and release of trophic factors. In the present study, the extent to which the increase in astrocyte-derived α-synuclein affects neurons that do not have any pathology by affecting the release of growth factors required for neuron survival and synapse formation, the potential to trigger the degeneration of synaptic connections and promote neurodegeneration has been investigated.

Methods: A co-culture system consisting of human precursor neural cells LUHMES, and human primary astrocyte cells was established. In this system, only astrocytes were transfected with the plasmid carrying the human SNCA gene. Then, these SNCA overexpressing astrocytes were brought together with neurons, and it was determined whether the increased α-synuclein in astrocytes moved toward neurons or not. The movement of α-synuclein was followed by live cell imaging. The levels of synaptic proteins such as thrombospondin, synaptophysin, PSD-95, and NGF were determined by the western blot method. Raw data for each group were analyzed via one-way ANOVA followed by a Tukey-Kramer multiple comparisons test (MCT) when data were normally distributed or a Kruskal-Wallis test followed by Dunn's multiple comparisons test when data were not normally distributed in GraphPadInStat DTCG 3.06. $p<0.05$ was considered statistically significant. All data are presented as means (SD) in figures.

Results: Our data showed that astrocyte-derived α-synuclein migrated to LUHMES cells, α-synuclein pathology increased neuronal cytotoxicity and decreased NGF expression level. On the other hand, it was determined that it increased the levels of thrombospondin, synaptophysin, and PSD-95 protein, which are involved in synapse formation.

Conclusion: These findings lead us to consider that α-synuclein pathology increases synaptic vesicle formation in astrocytes, possibly to facilitate the release of neurotrophic factors. Synaptic vesicle formation associated with α-synuclein accompanies the release of these factors and enhances the release or production of the factors that play a role in synapse formation.

Keywords: Astrocyte, α-synuclein, neuron, neurotrophic factors, synaptic communication.

OP-58

Investigation of anticancer effects of curcumin in human head and neck cancer cells in hypoxic environment

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Objective: Curcumin (*Curcuma longa L.*) is an active ingredient of the herbal medicine and dietary spice turmeric, which has phenolic pigment derived from the rhizom, belonging to the *Zingiberaceae* plant family, native to Asia. Due to its molecular properties, it quickly penetrates cells and enters the cytosol by passing through the plasma membrane. It is used for chemopreventive, antimetastatic and anti-angiogenic purposes. The role of natural compounds such as curcumin in the modulation of the anticancer potential of chemotherapeutic drugs is increased during the last years. Hypoxia is one of the major characteristics of solid tumors leading to resistance to radiotherapy and chemotherapy. In this study we investigated whether curcumin has potential to modulate the anti-cancer responses including, cell cycle arrest apoptosis and senescence in response to etoposide treatment in 2A3-head and neck cancer cells in hypoxic conditions. Additionally we analysed the expression levels of p53, p21, HIF-1α and glycolitic enzymes expression under hypoxic conditions.

Methods: Cell culture: 2A3 HPV(+) was purchased from ATCC. It was maintained throughout study with 88% DMEM + 10% fetal bovine serum (FBS) +1% Penicillin-Streptomycin and 1% Sodium Pyruvate. Hypoxic incubation was carried out in a hypoxic chamber at 37 °C in 1% O_2 plus 5% CO_2 and HIF-1α knock-down was achieved 4 different HIF-1α shRNA plasmid transfection. Apoptosis, cell cycle and senescence were measured by Annexin V/7 AAD, BrdU/PI analysis and SAß-gal staining, respectively. p53, p21, HIF-1α and glycolitic enzymes analyse with Western Blot.

Results: Here we show that It has been shown that curcumin and etoposide treatment 2A3 cells affect cell viability of 2A3 cells. The treatment of curcumin and curcumin+etoposide combination againsts proliferation by halting the cell cycle in the G0/G1 phase. Curcumin and etoposide combination mainly induces apoptosis but not senescence in 2A3 cells. Induction of apoptosis mediated by p53 and p21 expressions. In addition Hif-1α knock-down sensitizes 2A3 cells to apoptosis in response to etoposide and curcumin treatment.

Conclusion: 2A3 cells are resistant to etoposide in both normoxic and hypoxic conditions. Hif-1α knock-down enhances the effect of both curcumin and etoposide in hypoxia via increasing the cell cycle arrest and apoptosis in 2A3 cells. Results from this study may contribute to the determination of new therapeutic targets and strategies in head and neck tumors and to the development of more effective treatment responses.

Keywords: Curcumin, human head and neck cancer cells, hypoxic environment

OP-59

Investigation of efficacy time of conditioned medium derived from the human amniotic membrane in cancer cells: determination of anti-cancer property of the amniotic membrane

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Objective: In recent years, the amniotic membrane has become an encouraging tool for developing an anti-cancer drug. Thus, we aimed to evaluate the anti-cancer ability of conditioned medium generated from human amniotic membrane (hAM-CM) on cancer cells.

Methods: Human placentas (40 weeks) were obtained by cesarean section. The amniotic membrane was manually stripped from placenta and cut into pieces which were placed in a sterile physiological solution. Later, they were incubated with DMEM with 10% FBS and 1% PSA. To find the efficacy value of conditioning medium derived from human amniotic membrane (hAM-CM), the medium was collected after 24, 48, and 72h incubation and XTT proliferation assay was used to determine the impact of hAM-CM on healthy (HUVEC, HEK293, BV2, L929) and cancer (HEp2, PANC, MDA, A549) cell lines. We also performed a morphological analysis of the cells by light microscope after being treated with hAM-CM.

Results: Cell viability was significantly changed by hAM-CM in a dose- and time-dependent manner. The 72-hour incubation of hAM-CM is more effective than 24 and 48-hour incubations on cancer cells. While HEK293 and L929 cells exposed to hAM-CM showed significant but moderately increased proliferation (respectively, 110%; $p<0.05$ and 103%; $p<0.05$); HUVEC and BV2 cells showed reduced proliferation (respectively, 70%; $p<0.0001$ and 82%; $p<.0001$) compared to control cells (100%). However, PANC and MDA cells revealed the lowest viability by 60% and 40%, respectively ($p<0.001$), while HEp2 and A549 cells showed similar proliferation activity as healthy cells (77% $p<0.01$ and 87% $p<0.05$, respectively).

Conclusion: This study may support the importance of AM for cancer treatment, especially for some cancers like breast and pancreatic cancers. Our next plan is to identify active compounds responsible for anti-proliferative activities of hAM-CM.

Keywords: Amniotic membrane, cancer, conditioned medium, cytotoxicity, proliferation.

OP-60

Demonstration of the presence of nuclear transcription factors in mitochondria and investigation of their interactions with alpha synuclein

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Objective: Transcription factors (TFs) are proteins that play a role in the regulation of gene expression and participate in the complex formation binding to DNA before the initiation of gene expression. The expressions of genes encoded in mitochondrial DNA (mtDNA) are also regulated by specific transcription factors. One of the common mechanisms in neurodegenerative diseases involves mitochondrial dysfunction and disruptions in energy supply. Alpha-synuclein (aSyn), a dominant component of Parkinson's disease, can alter the expression of many genes encoded in both nuclear and mtDNA. Changes in gene expression can occur through direct binding to DNA as well as indirectly by interacting with TFs. The presence of some general TFs in mitochondria has been demonstrated. This study aimed to answer the question of whether other nuclear TFs are present in mitochondria and whether aSyn interacts with these TFs.

Methods: In the study, differentiated LUHMES cells were used. While no treatment was applied to one group of these cells, in the other group, overexpression of aSyn was performed, and mitochondria were isolated from the cells. Lysates were obtained from isolated mitochondria and processed using a commercial kit designed to investigate TF-TF interactions, specifically with nuclear TFs. In brief, immunoprecipitation was carried out with an antibody targeting aSyn from the lysate, and the resulting sample was applied to wells of a 96-well plate, each containing specific oligonucleotides for 48 different TFs. Signals based on interactions were detected through fluorometric measurements, determining which TFs were present in the mitochondria and interacted with aSyn.

Results: According to our analyses, it has been determined that many nuclear TFs are present in mitochondrial lysate, and some of them interact with aSyn. Among these, it has been identified that HIF, SMAD, and GATA have the highest interaction with aSyn in the aSyn overexpression group.

Conclusion: According to these findings, it has been observed for the first time in the literature that many nuclear TFs can also be present in mitochondria. The interaction of nuclear TFs with mtDNA under physiological conditions is a separate research topic. On the other hand, TFs with increased interaction with aSyn in mitochondria due to aSyn overexpression may be a contributing factor to altered mtDNA gene expressions under pathological conditions.

Keywords: Alpha synuclein, mitochondrial DNA, transcription factors.

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OP-61

Investigation of the effects of cisplatin and juglone on neuroblastoma cancer cell and bronchial epithelial cell lines

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Objective: The aim of our study is to investigate the cytotoxic effects of cisplatin and juglone combination on neuroblastoma and bronchial epithelial cell lines and to determine the expression levels of ADH1B, BTNL9, SDC1 genes.

Methods: In our study, neuroblastoma cell line SH YS5Y and BEAS-2B human bronchial epithelial cell lines were used. Juglone and cisplatin were applied to the cell lines in combination. The effects of juglone and cisplatin applied to BEAS 2B and SH YS5Y cell lines were investigated by CCK-8 analysis. Then, quantitative Real-Time PCR was performed under appropriate conditions with primers specific to the ADH1B, BTNL9, SDC1 genes and SYBR Green master mix. Expression changes were evaluated by the $2^{-\Delta\Delta CT}$ method.

Results: IC50 values for juglone were calculated as 7.43 µM/ml at the 72nd hour for BEAS 2B and 4.13 µM/ml at the 72nd hour for SH YS5Y. In cisplatin application, BEAS-2B was calculated as 9.6 µM/ml at the 72nd hour, and for SHSY5Y, it was calculated as 8.74 µM/ml at the 72nd hour. According to the results of expression change analyses, juglone application to BEAS 2B cells resulted in a 19.97-fold increase in the ADHL1B gene, a -32.22-fold decrease in BTNL9, and a 36.76-fold increase in SDC1. In cisplatin application, a 38.59-fold increase was observed in ADHL1B, while -165.42-fold decreases were observed in BTNL9 and -13.00-fold decreases were observed in SDC1. In the combined application of juglone and cisplatin, a -213.78-fold decrease was observed in the ADHL1B gene, -410.15-fold decrease in BTLN9, and a 60.13-fold increase in SDC1. In SHSY 5Y cells, with juglone application, there was a 445.75-fold increase in ADHL1B and a -144.01-fold decrease in SDC1. While no significant change was observed in the BTNL9 gene with juglone and cisplatin application, it increased 4.59 times with juglone and cisplatin combined application. While ADHL1B decreased by -13.64 times in cisplatin application, SDC1 decreased by 6.92 times. In juglone and cisplatin combined application, there was a 40.50-fold decrease in ADHL1B and a 28.44-fold increase in SDC1. In ADHL1B in SHSY 5Y cells, cisplatin and combination treatments enhanced the chance of survival. There was no discernible difference between BTLN9 and the control group. Compared to the control groups, the expression rate of SDC1 was reported to decrease significantly.

Conclusion: In our study, we provide evidence that juglone, cisplatin, and their combination can inhibit Neuroblastoma cell line survival, proliferation, migration, and invasion. We believe it will contribute to the literature on Neuroblostoma, one of the most prevalent childhood cancers that develops in infancy and has a very poor prognosis.

Keywords: BEAS 2B, cisplatin, juglone, SHYS5Y.

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OP-62

Oleuropein suppressed drug resistance in 5-fluorouracil-resistant gastric cancer cells under normoxic and hypoxic conditions

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Objective: Gastric cancer (GC) is one of the world's most common gastrointestinal system cancers and the leading cause of cancer-related deaths. Although standard chemotherapy treatment has a positive effect on the course of the disease, drug resistance developing in GC patients negatively affects the treatment of this disease. For this reason, new therapeutic approaches that are effective in breaking the developing chemo-resistance are needed. The current study aimed to break chemo-resistance by suppressing tumor aggressiveness with Oleuropein (OL) by developing 5-fluorouracil (5-FU) resistance in GC cells.

Methods: A resistant cell line (AGS-FUR) was developed by treating AGS cells with increasing 5-FU doses (5-100µM) concentrations. To confirm acquired 5-FU resistance, simultaneous cell proliferation and colony formation analyses for tumor aggressiveness were performed with the xCELLigence device. For chemo-resistance interactions, the effect of OL-only and in combination with 5-FU on cell death was examined by Annexin V analysis, effect on reactive oxygen species Oxidative Stress kit and its effect on suppressing the aggressiveness of tumor cells Colony formation and wound healing analysis under normoxic and hypoxic conditions.

Results: In AGS-FUR cells, it was determined that 25µM (IC50) and 100µM (maximum dose) 5-FU, which are effective on AGS cells, did not inhibit proliferation for 24-48h ($p>0.05$). It was proven that drug resistance occurred successfully, as the same doses reduced colony formation in AGS cells ($p<0.05$) but did not suppress colony formation in AGS-FUR cells ($p>0.05$). While the IC50 dose of OL in AGS cells was 73.86 µM, this dose was found to be 94.58 µM in AGS-FUR cells ($p<0.05$). OL caused apoptotic death in 66% of AGS cells and 42.5% of AGS-FUR cells ($p<0.05$). OL+5-FU treatment caused apoptotic death in 63.7% of AGS cells and 23.9% of AGS-FUR cells ($p<0.05$). OL+5-FU suppressed ROS in AGS and AGS-FUR cells. It was determined that the invasion of AGS and AGS-FUR cells was suppressed by at least 70% by OL and 5-FU under normoxic and hypoxic conditions ($p<0.05$). Additionally, OL+5-FU treatment reduced the number of colonies compared to untreated AGS and AGS-FUR controls ($p<0.05$).

Conclusion: Current findings show that OL may be a candidate that can be used in further studies to develop new therapeutic approaches that can reverse the acquired 5-FU resistance in GK cells and suppress the aggressiveness of these cells in different physiological conditions.

Keywords: Acquired 5-FU resistance, gastric cancer, oleuropein.

Acknowledgement: This research was supported by Bursa Uludağ University Scientific Research Projects Coordination Unit (HDP(IMYO)-2020/18).

OP-63

Investigating the etiology of polymicrogyria using in silico methods

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Objective: Polymicrogyria (PMG) is a neurologic condition in which the cortical gyri on the surface of the brain are smaller and more numerous than normal. Clinical manifestations of PMG may include mild or moderate mental retardation, spastic hemiparesis, muscle weakness and epilepsy. PMG is diagnosed by magnetic resonance imaging (MRI) and currently only symptomatic treatment is provided. In the literature, PMG is thought to be caused by neuronal disorders and associated with structural defects in the cytoskeleton. The use of current systems biology approaches to elucidate the etiology of diseases is becoming increasingly common and successful results are being obtained. In this study, we aimed to elucidate the etiology by using systems biology approaches on the PMG-associated gene list.

Methods: Genes involved in the etiology of PMG were obtained through reviews, databases and panels in the current literature. FZA was performed by accessing the Gene Ontology (GO) database through the GeneSCF program. The first 20 statistically significant ($p<0.05$) terms were listed from the obtained data. Through the Cytoscape program, the STRING database was accessed to create a PPE network with a high confidence score (>0.7) and the proteins that play a central role in the pathway were identified with the Centiscape tool. Finally, the cell signaling pathway was drawn using popular pathway databases such as KEGG, Reactome, Wiki pathways in PathVisio program.

Results: Through reviews, panels and databases, 68 genes involved in the etiology of PMG were identified. Within the first 20 terms obtained as a result of FZA, 4 main groups were identified. These are: "Neurodevelopment and Nervous System", "Cell Division and Cycle", "Cytoskeleton" and "Others". In the PPE network through Cytoscape, 46 of the 68 genes formed a meaningful network. Of these, 7 were identified as hub proteins playing a central role. In the PPE network, proteins associated with "Cytoskeleton" and "Membrane Structure" were found to form groups.

Conclusion: Results consistent with the literature data were obtained and important processes involved in the etiology of PMG were identified. For the first time, PMG-associated cell signaling pathway was drawn by identifying proteins critical for the disease.

Keywords: Cell signaling pathway analysis, functional enrichment analysis, interaction network, polymicrogyria.

OP-64

Screening of therapeutical targets and molecular pathways of melatonin against medullary thyroid cancer: a bioinformatics study

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Objective: Melatonin is a neuroendocrine, synthesized and secreted by the pineal gland. Melatonin regulates various biological functions. There has been considerable evidence from experimental and clinical studies suggesting that melatonin can be used to prevent and treat cancer. Melatonin exhibits a range of beneficial effects against cancer via apoptotic, antiangiogenic, antiproliferative, and metastasis-inhibitory pathways. Limited number of studies reported beneficial effects of melatonin on thyroid cancer. Also, no studies were reported the effects of melatonin on medullary thyroid cancer (MTC). In this study, comprehensive screening of molecular targets of melatonin in the treatment of MTC was aimed via bioinformatics tools.

Methods: Drugbank, Superpred, Genecards and Swiss Target Prediction databases were used to determine the target proteins of melatonin. Simultaneously, MTC-related target genes were collected from Genecards, and DisGeNET databases. The intersected protein targets of melatonin and MTC were determined. Function related target to target protein network was developed using STRING database. Hub genes were determined via Cytoscape software. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyzes were performed using ShinyGO database.

Results: 959 melatonin and 2114 MTC targets were obtained from the databases, while 359 of them shared interaction. TP53, STAT3, AKT1, and JUN were determined as hub genes. GO and KEGG enrichment analyzes exhibited multiple signalling pathways that involve melatonin against MTC. The hub genes were found to be highly related to cancer pathways, and response to endogenous stimulus.

Conclusion: Melatonin possibly regulates apoptosis as well as HIF-1, cAMP, MAPK, and PI3K-AKT signaling pathways through hub genes. Considering all the bioinformatics and network pharmacology findings, melatonin could be a potent agent for treating MTC.

Keywords: Bioinformatics, medullary thyroid cancer, melatonin, network pharmacology, target prediction.

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Mapping sensory innervations of the whole murine hearts at healthy state and post-myocardial infarction

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Objective: The cardiac function is tightly regulated through autonomic neural circuits innervating the heart. Previous reports indicate structural and functional abnormalities in cardiac innervations leading to cardiac pathologies. Although sympathetic and parasympathetic neural-cardiac interactions have been well studied, the sensory innervations and their function remains largely unknown. Thus, understanding the sensory neural network at the whole healthy heart and post-ischemic states are critical for both molecular and functional aspects.

Methods: To reveal the afferent (sensory) innervations at the 3D whole heart level, double transgenic strain of Vglut2-Cre::tdTomato were generated to trace Vglut2-expressing sensory fibers. Ischemic damage was mediated by a myocardial infarction (MI) model in mice and investigated in comparison to SHAM controls. Murine hearts were collected from healthy, acute and chronic post-MI states. Following immunostaining with anti-RFP antibody to enhance tdTomato signal, sensory neural network and nerve ending morphologies were imaged by LSM880 confocal imaging. Nerve diameter analysis were performed by Neutube1.0 program.

Results: Cardiac afferent innervations by Vglut2-expression was imaged at the intracardiac ganglia, atria and ventricles at both dorsal and ventral sides of the whole hearts. Significantly denser neural innervations and larger axonal fibers were detected on the dorsal side compared to ventral side, revealed by nerve diameter analysis. High resolution mapping of cardiac afferents demonstrated various nerve-end morphologies including flower-spray and end-net terminals on atria and ventricles, with exclusive intramuscular terminations at the ventricles. Evaluation of post-MI hearts demonstrated denervation at the infarct zone, hyperinnervation and emerging nerve sprouts at the border zone in comparison to the remote area and SHAM controls.

Conclusion: This study described a complete 3D map of cardiac afferent innervations at the whole heart level providing a crucial information regarding neurocardiac interactions. Our findings indicated that the abnormal sensory remodelling post-MI could potentially contribute to cardiac malfunction that awaits further functional studies.

Keywords: Cardiac afferent, heart innervations, myocardial infarction, sensory neurons, whole-organ imaging.

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OP-66

Investigation of target genes of miR-409-3p associated with secondary mitochondrial dysfunction in skeletal muscle

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Objective: Mitochondrial dysfunction and morphological abnormalities are observed as a common factor in many neuromuscular diseases with different clinical, genetic, and pathological backgrounds. Studies showed that secondary organelle damage in skeletal muscle plays a role in the early stages of muscle degeneration, and it is known that microRNAs (miRNAs) have potential functions in this pathway. Our previous studies identified microRNA profiles associated with secondary mitochondrial dysfunction in skeletal muscle in different rare neuromuscular diseases (DMD, Megaconial Congenital Muscular Dystrophy (CMD), Ullrich CMD, and alpha-dystroglycanopathy). 10 of 17 miRNA targets were found to be associated with mitochondrial pathways, and 6 of them were associated with more than one pathway and were identified as priority candidates by bioinformatic analysis. Cell-based functional analyses showed that the overexpression of miR-409-3p amongst these miRNAs reduced ATP synthesis and disrupted mitochondrial morphology in skeletal muscle cells. Therefore, we aimed to determine the target gene/s of this miRNA associated with mitochondrial damage pathway.

Methods: Target gene prediction tools (*miRWalk, mirDB, TargetScan, etc.*) were used to predict targets of miR-409-3p. Potential mitochondrial target gene list was created by overlapping all potential target genes of miR-409-3p with the mitochondrial genes in Human MitoCarta3.0 list. Pathway analysis was performed on potential targets of miR-409-3p with *GENEONTHOLOGY-PANTHER* database and candidates for miR-409-3p were selected. The effects of miR-409-3p overexpression on the expression of the selected candidate were investigated at RNA and protein levels through cell-based functional analyses.

Results: We identified all potential mitochondrial target genes for miR-409-3p and concluded that overexpression of miR-409-3p in C2C12 myoblast cells reduced the expression of our primary candidate Mitofusin-1 *(MFN1)*.

Conclusion: miR-409-3p and/or its targets have the potential to be therapeutic targets to prevent/revert mitochondrial damage observed in skeletal muscle, which is a common and secondary finding in the pathogenesis of rare neuromuscular diseases.

Keywords: MicroRNA, mitochondria damage, neuromuscular diseases, target gene.

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OP-67

Exosome characterization by transmission electron microscopy in prostate cancer

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Objective: Prostate cancer is the second most common cancer in men, particularly affecting older men. The incidence of prostate cancer is constantly increasing in almost all countries, but despite its high morbidity, its etiology is not fully known. Exosomes are the smallest members of extracellular vesicles, varying in size between 20-100 nm. What makes exosomes interesting is that they vary in size, shape and structure depending on the cargo content they carry and the cells from which they originate. Our study aimed to determine the differences in size and shape of exosomes between patient and control individuals.

Methods: In our study; exosomes were isolated from plasma samples taken from 75 patients (25 BPH, 25 Localized prostate cancer patients and 25 Metastatic prostate cancer patients) and 21 control individuals. Exosomes were characterized by transmission electron microscopy to evaluate them in terms of size and shape and exosome sizes were measured using the Image J program.

Results: A difference was observed in the shapes of exosomes between the control group and the disease groups. While the exosome shapes were more circular and smooth in the control group, it was determined that the exosome shape was distorted in the cancer groups, including the BPH group, and gained a more oval and even pointed appearance. According to the image sizing results in our study, exosome sizes in all groups ranged between 21.36-137.28 nm. While these values are in the range of 21.36-98.94 nm in the control group, 22.35-104.07 nm in the BPH group, 42.94-137.28 nm in the LPCa group and 38.11-124.33 nm in the MPCa group.

Conclusion: These results show that exosome sizes increased significantly, especially in the cancer group compared to the control and BPH groups. While a statistically significant difference in exosome size was determined between the control group and cancer groups (LPCa, MPCa) ($p<0.005$), no significant difference could be determined between the control group and the BPH group.

Keywords: Exosome, LPCa, prostate cancer.

OP-68

Intratumor heterogeneity-related aneuploidy determines therapy resistance in cancer

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Objective: The effect and progression of aneuploidy and chromosomal instability (CIN), two characteristics of cancer, are not well understood as of today. Intratumoral heterogeneity (ITH) is a phenomenon wherein tumor cells compete for survival in their microenvironment under constantly shifting circumstances, giving rise to more aggressive subpopulations with various mutations and/or epigenetic changes. In cancer cells, whole genome duplication (WGD) increases ITH and is associated with accelerated cell migration.

Methods: In our study, we labeled two near-diploid and three near-tetraploid clones using five different fluorescent colors to examine whether 4N clones that have undergone WGD acquire a phenotype that enables them to outcompete their 2N counterparts and to evoke a resistance to various cancer drugs in order to study the role of WGD in cancer progression. In vivo examination of the ploidy-related invasive abilities of these clones was performed in xenograft mice.

Results: Our results showed that 4N clones had a broad range of chromosome number counts, had various amplifications, deletions and translocations, were affected by the chemotherapeutics 5-fluorouracil, oxaliplatin, irinotecan, and gemcitabin at a higher, by paclitaxel at a lower level, and exhibited a more migratory but not a more invasive profile, when compared to 2N clones.

Conclusion: Here, we were able to pinpoint particular genetic alterations in the 4N colorectal cancer cell clones that were associated with the acquisition of resistance to chemotherapy and radiotherapy.

Keywords: Aneuploidy, anticancer therapy resistance, chromosomal instability, intratumor heterogeneity.

OP-69

Evaluation of immunological and hematological parameters in patients with pulmonary embolism due to Covid-19 infection

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Objective: Coagulation mechanisms are reported to be impaired in patients with pulmonary embolism (PE) due to Covid-19, however, the relationship between cellular interactions and immunological and hematological mechanisms have not been fully elucidated. Our study aimed to investigate serum glycoprotein 2b3a (GP2b3a) and alpha 2-antiplasmin (α2AP) levels in patients with PE due to Covid-19.

Methods: Among 80 people diagnosed with Covid-19, patients who had not had PE (n=26), patients with PE who received treatment for 3 months (n=26), and patients with PE who received treatment for 6 months (n=28) were included in the study. Serum GP2b3a and α2AP levels were determined by ELISA. Windows PASW Statistics-18 program was used. Normality was done with the Shapiro-Wilk test, three group comparisons were made with the Kruskal-Wallis test. Mann-Whitney test was applied for subgroup comparisons.

Results: There was no significant difference in GP2b3a levels and α2AP levels between patient groups with and without PE due to Covid-19 ($p>0.05$).

Conclusion: Changes in hematological and immunological parameters have been reported in patients with PE due to Covid-19. Platelet GP2b3a receptors cause thrombus formation through fibrinogen bridges. Inflammation and clotting tendencies increase in Covid-19. In our findings, no significant difference was observed in GP2b3a levels. α2AP, one of the fibrinolytic system elements, is an inhibitor of plasmin. In PE studies in mice, α2AP has been shown to reduce thrombus dissolution and increase mortality during PE. In our findings, no significant difference was observed in α2AP levels. Covid-19-associated coagulopathy and high D-dimer levels have been reported and are associated with high mortality. In our findings, it was observed that D-dimer increased in relation to embolism and its duration. There are studies showing that Pct increases in Covid-19 and inflammation. In our findings, it was observed that Pct increased in relation to embolism and its duration. Detailed studies are needed to elucidate the role of platelets and fibrinolysis mechanisms in order to guide treatment in Covid-19 patients.

Keywords: Alpha 2-antiplasmin, Covid-19, glycoprotein 2b3a, pulmonary embolism.

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OP-70

The role of the WWOX gene and its association with the hippo signaling pathway in tamoxifen resistance

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Objective: Tamoxifen (Tam) is an estrogen receptor modulator that is a crucial component of the treatment protocol for a majority of estrogen receptor-positive (ER+) breast cancer patients. There is a need to minimize the side effects of TAM and develop treatment protocols to combat drug resistance. Understanding the relationship between the Hippo pathway and TamR mechanism is believed to contribute to the development of new strategies to overcome resistance. While the involvement of *WWOX* in the development of various cancers is known, limited information is available regarding the impact of *WWOX* knockout (KO) on the TamR mechanism in breast cancer. Therefore, this current study aims to investigate the effect of *WWOX* gene knockout (KO) and overexpression (OE) on TamR associated with the Hippo signaling pathway using the CRISPR/Cas9 method.

Methods: The clinical and in-vitro study included 50 patients who received 20 mg/day of tamoxifen treatment. RNA was isolated from paraffinized tumor and normal tissues for cDNA synthesis. Subsequently, *WWOX*-KO-MCF-7 and *WWOX*-OE-TamR-MCF-7 cell lines were established using CRISPR/Cas9, and RNA was isolated from these cells for cDNA synthesis. The expression levels of *WWOX, WBP2, YAP,* and *TAZ* genes were evaluated using RT-qPCR. Quantitative data obtained were evaluated using normality-tests, t-tests, X2-tests and correlation analysis.

Results: An increase in the expression of *WWOX, WBP2, YAP,* and *TAZ* was observed compared to normal tissue, and a correlation was found between the expressions of *WWOX* and *WBP2, YAP, TAZ* (*p<0.001). A correlation was observed between TamR development in patients and *YAP, WWOX, WBP2* (p<0.05). Additionally, a correlation was noted between *YAP* expression levels and necrosis (p=0.041), perineural invasion (p=0.048), and tumor size (p=0.035). Significant differences were found in *TAZ* expression with necrosis (p=0.031), tumor grade (p=0.017), and venous vascular invasion (p=0.014). According to expression results, fold-change values of *WWOX, WBP2, YAP, TAZ* genes were -1.785, -1.022, -1.454, -1.873 in MCF-7-KO cell line, respectively (*p<0.05), and in MCF-7-OE cell line, they were 0.035, 0.027, 0.649, 0.178, respectively (*p<0.05).

Conclusion: The present study demonstrates the relationship between *WWOX* and the Hippo pathway, and suggests that loss of *WWOX* may lead to tamoxifen resistance. Moreover, overexpression of *WWOX* may re-sensitize TamR cells to Tam. The findings provide significant insights into the prognostic role of *WWOX* in TamR and contribute to the development of targeted therapies.

Keywords: MCF-7-OE cell line, tamoxifen resistance, WWOX gene.

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OP-71

Determination of alterations in the characteristics of triple negative breast cancer cells when Piwi interacting RNAs are inhibited

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Objective: In this study, we aimed to determine what kind of changes could be detected in the metastatic properties of MDA-MB-231 triple negative breast cancer cells by the inhibition of piR-651 and piR-823 regions.

Methods: Lipofectamine was used to transfect non-target, anti-piR-651 and anti-piR-823 sequences into HUVEC and MDA-MB-231. Proliferation and motility were determined in both cell lines at 24th, 48th, and 72nd hours. MDA-MB-231 cells were exposed to an invasion assay with crystal violet staining based on the motility data. Ki-67, HIF-1α, MMP-2, and MMP-9 gene expressions were determined during the 48th hour, when the optimum impact of inhibition was detected in both cell lines.

Results: The 48th hour proliferation was selected as the optimal hour for anti-piR-651 and anti-piR-823 transfected MDA-MB-231 cells compared to control group ($p<0.001$) with the help of decreased motility and invasion assay results at the 48th hour ($p<0.001$). However, no significant difference was observed on proliferation and motility of anti-piR-651 and anti-piR-823 transfected HUVEC cells compared to control group ($p>0.05$). Except for MMP-2 in anti-piR-823 transfected HUVEC cells and HIF-1α in anti-piR-823 transfected MDA-MB-231 cells; gene expressions of Ki-67, HIF-1α, MMP-2 and MMP-9 decreased in both cell lines compared to control groups ($p<0.001$).

Conclusion: Although proliferation and motility of anti-piR-651 and anti-piR-823 treated healthy cells were unaffected, reductions in Ki-67, HIF-1α, and MMP-9 expression suggest the cells' genetic potential. Both inhibitions produce a reduction in motility, invasion, and proliferation in MDA-MB-231 cells, and gene expression data support cell movement data. piR-651 and piR-823 inhibitions do not have any effect on the survival of healthy cells, but they significantly reduce survival and metastasis in metastatic cancer cells. piR-651 and piR-823 as the potential inhibitors for breast cancer metastasis and invasion may be a good idea for future cancer gene therapy or genetic manipulation studies.

Keywords: Invasion, metastasis, Piwi interacting RNA, triple negative breast cancer.

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Evaluation of gankyrin and cysteine dioxygenase type 1 genes promoter methylation changes as a new target for early diagnosis and follow-up of gastric cancer

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Objective: Abnormal DNA methylation plays an important role in carcinogenesis by altering the expression of tumor suppressor genes and oncogenes. Reversibility of DNA methylation allows reactivation of tumor suppressor genes silenced by promoter hypermethylation. *Cysteine dioxygenase type 1 (CDO1)* and *PSMD10 (Gankyrin*) are genes that affect intracellular carcinogenic pathways and their expression is mainly regulated by DNA methylation. It is known that precancerous lesions caused by chronic gastritis are risk factors for gastric cancer and no study has been found to investigate the DNA methylation and expression status in the promoter region of *Gankyrin* and *CDO1* in comparison with gastric tumors. In our study, we aimed to investigate the potential biomarker feature that can be used in the early stage and follow-up of gastric cancer by determining the methylation and expression levels of *Gankrin* and *CDO1* genes and comparing them with clinicopathological data.

Methods: The expression status of Gankyrin and CDO1 determined by immunohistochemically in 40 individuals diagnosed with chronic gastritis and 60 patients diagnosed with gastric cancer and the promoter methylation status was detected by DNA sequencing after bisulfite conversion.The results were compared with clinicopathological data.

Results: CDO1 promoter methylation levels in the patient group were found to be significantly higher than those in the control group. It was determined that CDO1 promoter methylation levels increased significantly depending on the clinical stage, tumor size and lymph node involvement. No significant change was observed in the methylation and expression status of the Gankrin gene and clinicopathological comparisons between the two groups. **Conclusion:** CDO1 promoter methylation status has predictive and prognostic potential for gastric cancer and may be a target for epigenetic therapy of gastric tumors.

Keywords: DNA methylation, epigenetics, expression, gastric cancer.

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OP-73

Development of a chemical agent induced retinal degeneration animal model for exploring anti-inflammatory gene transfer strategies in genetic blindness

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Objective: The development of effective animal models is crucial for advancing research in retinal diseases, including age-related macular degeneration (AMD), diabetic retinopathy, and retinitis pigmentosa. In this study, we present a novel $CoCl_2$-induced animal model of retinal degeneration, offering a valuable platform for investigating anti-inflammatory gene transfer strategies to combat these blinding diseases.

Methods: Our approach involved the induction of retinal degeneration in rodents through controlled exposure to cobalt chloride ($CoCl_2$). We characterized the progression of retinal damage, including microglial activation in this model. The $CoCl_2$-induced animal model was carefully validated using histological and molecular assessments.

Results: Our chemical agent-induced animal model successfully replicated key aspects of retinal degeneration observed in AMD, diabetic retinopathy, and retinitis pigmentosa, including microglial activation. The model demonstrated a progressive loss of retinal structure, closely resembling the pathophysiology of these retinal diseases.

Conclusion: We have established a robust $CoCl_2$-induced animal model of retinal degeneration, offering a valuable experimental system for the evaluation of anti-inflammatory gene transfer strategies. This model holds significant promise for advancing our understanding of retinal diseases and developing innovative therapeutic interventions, emphasizing the importance of anti-inflammatory approaches in the treatment of these blinding conditions.

Keywords: Genetic blindness, gene transfer strategies, retinal degeneration.

OP-74

The role of SERPINB1 expressions in glioblastoma drug resistance to temozolomide

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Objective: Glioblastoma (GBM) is a highly malignant grade 4 glial tumor with an average survival duration of approximately 12-18 months. Resistance to conventional tumor treatments remains a pivotal factor hampering the success rates of eradication. Temozolomide (TMZ), a primary chemotherapeutic agent for GBM, faces a challenge as nearly half of GBM cells exhibit resistance, making complete eradication elusive. Consequently, adjuvant therapies are required, and the quest for novel prognostic markers becomes imperative. SERPINB1, a serine protease inhibitor, is found to be associated with cancer cell migration and invasion in various tumor cells. Moreover, SERPIN clad-B proteins have been implicated in promoting the survival of GBM cancer stem cells, contributing to tumor recurrence. The aim of this study was to investigate the role of SERPINB1 expression levels in GBM drug-resistance development and its prognostic implications.

Methods: To achieve this, we modulated SERPINB1 expression profiles in various GBM cell lines. SERPINB1 overexpression was attained through gene transfection, while downregulation was achieved via siRNA-mediated gene silencing. Subsequently, the in vitro therapeutic impact of SERPINB1 on drug resistance in these cell lines was meticulously assessed.

Results: The results showed that SERPINB1 expression levels in GBM cells increased cell death and sensitized some cells to TMZ. Therefore, for the first time this study reports the involvement of SERPINB1 in TMZ resistance in GBM and underlines the prognostic importance of SERPINB1 expressions for GBM therapy.

Conclusion: This study unveils the previously unrecognized role of SERPINB1 in mitigating TMZ resistance in GBM. Importantly, it underscores the prognostic importance of SERPINB1 expression levels in GBM therapy. The comprehensive exploration of the intricate association between SERPINB1 and GBM, coupled with the elucidation of the molecular mechanisms underpinning TMZ resistance, holds the potential to enhance our understanding of disease progression and chemoresistance. Ultimately, these findings may lead to the refinement of standard therapies and the establishment of innovative therapeutic approaches for GBM. Further investigations on SERPINB1 expressions and the molecular mechanisms related to TMZ resistance may contribute to improved prognostic outcomes and novel therapeutic strategies for this devastating disease.

Keywords: Chemotherapy, drug resistance, glioblastoma, SERPINB1, temozolomide.

OP-75

Investigation of prognostic gene markers in cholangiocarcinoma cancer with bioinformatics tools

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Objective: Malignant cancer called cholangiocarcinoma (CCA) arises from epithelial cells in the biliary tree. It is a type of cancer that accounts for 15% of all primary liver malignancies and is rapidly growing throughout the world. Biomarker research is a potential strategy for the early detection and treatment of CCA. Recent research has shown COL1A1 (Collagen Type I Alpha 1 Chain), COL1A2 (Collagen Type I Alpha 2 Chain), and MMP7 (Matrix Metallopeptidase 7) as possible cholangiocarcinoma (CCA) biomarker candidates and shown that COL1A1 and COL1A2 may be able to distinguish between intrahepatic CCA (iCCA) and extrahepatic CCA (eCCA). COL1A1 and COL1A2 may have a role in the development and prognosis of cancer. MMP7 has been connected to tumor metastasis and inflammatory processes.The purpose of this study is to ascertain the efficacy of COL1A1, COL1A2, and MMP7 as CCA biomarkers.

Methods: Utilizing bioinformatics analytical techniques and tools, a thorough investigation of the relationship between the COL1A1, COL1A2, MMP7, and CCA genes was conducted. In the study, the novel study pipeline was constructed by including DisGeNET and SNP tools together with the utilization of Cbioportal, GeneDisteller and R-language programming. Basic information about gene mutations was obtained using the DisGeNET database. SNP databases have investigated the relationship between genes and diseases, and additional CCA characteristics have been examined for potential biomarker identification. The commercial statistics for these genes were examined again in Cbioportal. Using Gene Disteller, text mining research was carried out to look at the genetic connections to other tumors.

Results: Analysis revealed that substantial quantities of these genes were present in CCA samples in particular.

Conclusion: Indeed, by indicating their roles in extracellular matrix regulation and tissue remodeling, the results have highlighted the significance of using the COL1A1, COL1A2, and MMP7 genes to recognize the emergency of various cancers of CCA.

Keywords: Bioinformatic, biomarker research, cholangiocarcinoma, COL1A1, COL1A2, MMP7.

OP-76

The effect of xCT inhibitor imidazole ketone erastin on 5-fluorouracil therapeutic potential in colorectal cancer cells

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Objective: Colorectal cancer is a concerning malignancy ranking third in incidence and second in mortality worldwide. The inadequacy of traditional chemotherapy approaches due to potential side effects and drug resistance requires the development of new treatment strategies, such as the combination of conventional chemotherapeutic drugs with antioxidant mechanism inhibitors. In our study, we focused on the depletion of glutathione (GSH), which plays an important role in the antioxidative mechanism by inhibition of SLC7A11 carrier protein. The effects of GSH inhibition on the efficacy of the traditional chemotherapeutic drug 5-fluorouracil (5-FU) in cancer cells were investigated.

Methods: Imidazole ketone erastin (IKE) was used to reduce GSH activity by inhibition of SLC7A11 in HCT-116 colorectal cancer cells. The effects of IKE, 5-FU and their combination on cell viability were determined by Sulforodamine-B test, effects on GSH&GSSG amounts by GSH&GSSG assay, intracellular cystine levels by cystine uptake assay, effects on ROS level by flow cytometry, and effects on cell death by Hoechst-33342&PI staining.

Results: The effects of 5-FU on cell viability in HCT-116 cells and IC_{50} value for the combination were determined by Sulforodamine-B (SRB) test. The effect of IKE molecule on cell viability was investigated and concentrations of 0.1 µM, 1 µM and 4 µM were chosen for use in further experiments. By determining the amount of GSH&GSSG and the levels of cystine influx to the cell, it was shown that IKE provides SLC7A11 inhibition. It has been observed that the combination of 5-FU with IKE increases the level of ROS in HCT-116 colorectal cancer cells, but does not trigger cell death.

Conclusion: It has been shown in our project that inhibition of the antioxidant system by IKE contributes to the efficacy of 5-FU. Therefore, we anticipate that future translational studies may evaluate the addition of small molecule inhibitors such as IKE to cancer therapy.

Keywords: Anticarcinogenic agents, antineoplastic combined chemotherapy protocols, colorectal neoplasms, fluorouracil, glutathione, imidazole ketone erastin.

Acknowledgement: This study was supported by TUSEB Group A R&D Project with the code 16470.

OP-77

Investigation of RSL3-induced ferroptotic cell death in GSTM3 silenced prostate cancer cells

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Objective: Prostate cancer ranks as the most prevalent malignancy among men, with approximately 1.6 million new cases diagnosed annually worldwide. Notably, a significant proportion, roughly 20%, of prostate cancer cases present at advanced stages, and the intricate landscape of tumor heterogeneity complicates therapeutic approaches. Consequently, a comprehensive understanding of the molecular mechanisms and genetic background of prostate cancer assumes critical importance in the investigation of novel and more effective treatment modalities. The GSTM3 gene, also known as Glutathione S-Transferase Mu 3, is a member of the glutathione S-transferase (GST) family of genes. GSTs play a crucial role in detoxifying various harmful compounds by catalyzing the conjugation of glutathione with these substances. The association between GSTM3 genetic polymorphisms and prostate cancer and its response to treatment was demonstrated. Understanding the genetic aspects of GSTM3 and its role in detoxification processes is essential in the study of cancer susceptibility and personalized medicine approaches. RSL3 is a small molecule compound that has gained significant attention in the field of cancer research, specifically in the context of a type of programmed cell death called ferroptosis. In this study, it is aimed to investigate the possible effect of GSTM3 expression and RSL3 induction in prostate cancer.

Methods: The GSTM3 gene was knocked down using siRNA in prostate cancer cells. The effects of silencing were examined after 48 hours. GPX4 gene expression level was evaluated with RT-PCR. The viability of RSL3-induced cells was measured by MTS. ROS detection was performed using H2DCFDA in flow cytometry.

Results: Overexpression of the GSTM3 gene was observed in transcriptome analysis using GSEA analysis. The GPX4 gene an important target of ferroptosis, increased by silencing the GSTM3 gene. An elevation in reactive oxygen species (ROS) production was observed in prostate cancer cells where GSTM3 had been silenced and subsequently induced with RSL3, in comparison to the control cells.

Conclusion: The development of targeted therapies for prostate cancer is vital to cure the disease and eliminate side effects.Our findings indicate that the strategic targeting of GSTM3 enhances the susceptibility of prostate cancer cells to ferroptosis-induced cell death.

Keywords: GSTM3, prostate cancer cells, RSL3-induced ferroptotic cell death.

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OP-78

Tricetin enhances the therapeutic potential of 5-fluorouracil on colorectal cancer cells

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Objective: The third most prevalent cancer type in the world, colorectal cancer (CRC), currently lacks an efficient treatment. The fight against CRC requires the development of innovative treatments. Recent research reveals a new approach to halting the spread of cancer by combining organic compounds with conventional treatments. One of these organic compounds is tricetin, a type of flavonoid with an anticancer potential. Here, the anticancer effects of tricetin on the CRC cell line HCT-116 cells were examined and the first experimental evidence of tricetin and 5-FU's positive effects in an *in vitro* model was established. The purpose of this study was to determine whether this combination might be employed in translational oncology.

Methods: The effects of tricetin alone or in combination with a conventional chemotherapeutic 5-fluorouracil (5-FU) on cell viability were determined by Sulphorodamine-B test, the effects on cell death by Hoechst 33342/PI technique, and the effects on the apoptotic pathway-associated protein expression levels by Western blotting.

Results: Combining 5-FU with tricetin further decreased the viability of HCT-116 CRC cells (39%) when compared to their use alone (45%). In the subsequent experiments, tricetin IC_{30} (40 µM) and the 5-FU IC_{50} (20 µM) were used. Protein expressions of PARP and Caspase-3 were determined by Western blotting. Caspase-3 cleaves PARP, a nuclear enzyme activated in response to environmental stress and involved in DNA repair, during apoptotic signaling and deactivating its capacity for DNA repair. A recognized indicator of apoptotic signaling is the presence of cleaved PARP. Based on our findings, PARP cleavage by caspase-3, 5-FU and tricetin has been determined to have an apoptotic effect on HCT-116 cells through this pathway.

Conclusion: We have discovered that tricetin enhanced the therapeutic ability of 5-FU in CRC and it could be used as a therapeutic adjunct in oncological treatment. Translational clinic studies are required to further investigate the use of tricetin in patients.

Keywords: Apoptosis, colorectal cancer, combination therapy, flavonoid, tricetin

OP-79

Role of La-related protein 7 in osteogenic matrix maturation

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Objective: La-related proteins are a family of RNA-binding proteins that play important roles in RNA metabolism. Alazami syndrome is a genetic disease that occurs as a result of homozygous mutations in the La-related protein 7 (LARP7) gene and is characterized by growth and mental retardation, facial dysmorphic findings and skeletal system disorders. Since RNA-binding proteins show tissue/cell-specific expression, disease models are needed to elucidate their functions. Our goal is to clarify LARP7's role in the osteogenic differentiation.

Methods: Osteogenic differentiation was induced in human primary mesenchymal stem cells and LARP7 expression was monitored throughout this process. The day with the highest expression level was identified, LARP7 was silenced by RNA interference. Then, the global gene expression profile was analyzed by high-scale RNA sequencing (RNA-seq) method and the validation of the identified target genes was completed with qRT-PCR.

Results: The mRNA expressions of RNA-binding proteins were analyzed during cellular differentiation processes, an interesting pattern revealed in the osteogenic differentiation, in line with the disease phenotype. An increase in expression was observed in LARP7 and the majority of other superfamily members in matrix-maturation. At the stage when LARP7 expression is at its highest, it was suppressed with gene-specific siRNAs and osteogenic differentiation was shown to decline. In addition, RNA-seq was performed and differentially expressed genes were identified.

Conclusion: It was found that LARP7 plays a role in matrix-maturation during osteogenesis, and in transcriptomic analyzes examining the effect of LARP7 deficiency on the gene expression pattern, it was observed that LARP7 suppression caused a decrease in the expression of mRNAs with high copy numbers. It is predicted that LARP7 may indirectly affect the processing and stability of various RNA molecules, including transcription factors and signaling molecules involved in osteogenesis, through RNA metabolism.

Keywords: Alazami Syndrome, COL1A1, La-related protein 7 (LARP7), matrix-maturation, osteogenesis.

OP-80

Exploring novel vicinal diaryl-substituted isoxazole and pyrazole derivatives as cytotoxic compounds for targeting breast cancer: Mechanisms and therapeutic potential

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Objective: Breast cancer (BC) is a malignant tumor that forms in the cells of the breast, making it the most frequently diagnosed cancer among women and the most prevalent cancer overall. The global burden of BC is expected to increase in the coming decade, driven by factors such as late menopause, hormonal stimulation of breast cells, age, and obesity. Due to gene mutations and the deregulation of numerous critical signaling pathways, BC exhibits resistance to conventional chemotherapy. It is crucial to design and develop novel therapeutics against BC. In this study, we investigated the molecular mechanisms of newly discovered cytotoxic vicinal diaryl-substituted isoxazole and pyrazole compounds on BC.

Methods: After assessing compound cytotoxicity via real-time cell electronic sensing (RT-CES), we analyzed cell cycle, cell death, and DNA damage through flow cytometry. Transcriptome changes were examined using a PanCancer panel of 770 cancer-related genes. Senescence-associated β-galactosidase activity and oxidative stress were evaluated via immunostaining. Cell cycle, senescence, and oxidative stress-related pathways were analyzed by western blot. *In vivo,* the anti-cancer effects of compounds were assessed by a tumor xenograft assay.

Results: From over 60 compounds, 11 and 85 proved highly cytotoxic against breast cancer cells, with low doses. Time-and dose-dependent growth inhibition upon treatment with 11 and 85 was observed due to oxidative stress-induced DNA damage resulting in senescence, cell cycle arrest in the G0/G1 phase, and apoptosis. Cell cycle pathway and related genes were the most differentiated pathways and genes by analysis of the cancer panel. The expressions of G0/G1 cyclins were also changed in protein level with treatment. Furthermore, the administration of both 11 and 85 led to a significant reduction in tumor size in nude mice.

Conclusion: The demonstrated anti-tumor effects of compounds 11 and 85 affirm their potential as prospective anti-cancer agents for breast cancer.

Keywords: Anti-cancer activity, breast cancer, cytotoxicity, therapeutics.

Acknowledgement: This study was supported by a Research Grant from TUBİTAK (#215S015).

OP-81

Determination of the frequency of BCL-2 gene (rs2279115 and rs4987856) and LIF gene (rs929271) polymorphisms in patients with congenital anomalies of kidney and urinary tract anomaly (CAKUT)

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Objective: Congenital anomalies of the kidney and urinary tract (CAKUT) are characterized by several malformations. Its prevalence is 0.3-0.6% in live births. BCL-2 (B-cell Lymphoma) gene regulates apoptosis and LIF (Leukemia Inhibitory Factor) gene takes a role in many biological processes such as blastocyst growth and uterine preparation for implantation. In this study, two single nucleotide polymorphisms (SNPs) of the BCL-2 gene (rs2279115 and rs4987856) and one SNP of the LIF gene (rs929271) were investigated in CAKUT patients for the first time in the literature.

Methods: We used the PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism) for rs2279115 and rs929271, and SNaPshot for rs4987856. A Total of 129 CAKUT patients and 105 controls were enrolled in the study.

Results: The results of the analysis showed that the difference in the distribution of allele frequencies for rs2279115 and rs4987856 polymorphisms of the BCL-2 gene and rs929271 polymorphism of the LIF gene was found to be statistically significant (p=0.000, p=0.000, p=0.000, respectively) between the patient and control groups.

Conclusion: This study, which is the first time in the literature, showed that changes in BCL-2 and LIF genes are associated with CAKUT disease.

Keywords: BCL-2, CAKUT, LIF, rs2279115, rs929271, rs4987856.

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OP-82

Investigating the role of sonic hedgehog pathway and the of temozolamide resistance in glioma

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Objective: This study aims to examine the effect of the Sonic Hedgehog (SHH) cellular pathway on drug resistance in IDH1 wild-type and mutant gliomas and to increase sensitivity to temozolomide by inhibiting the SHH pathway. Additionally, we investigate the relationship between IDH1 expression and SHH pathway activity.

Methods: We employed the naturally Temozolomide-resistant LN-18 cell line. Cyclopamine was used to examine the relationship between the SHH pathway and Temozolomide activity. Cyclopamine inhibits the SHH pathway by binding to the SMO protein. Plasmids with IDH wild-type and IDH R132H mutation were transfected into the LN-18 cell line using Lipofectamine 3000. Cells were treated with 5-10-20 µM cyclopamine and 50-100-250 µM Temozolomide, and the effect of concentration changes on cell viability over 72 hours was examined by MTT assay. The IDH-wild type and IDH-mutant LN-18 subgroups were treated with 10 µM cyclopamine and 100 µM Temozolomide, and the effect of cyclopamine on Temozolomide resistance was examined by MTT assay to assess cell viability. SMO, GLI1, PTCH1, IDH1, TGF-beta, and GAPDH molecules were quantitatively examined at the mRNA and protein levels by qRT-PCR and western blotting. Apoptosis, ROS, and cell cycle analyses were performed after combined drug use.

Results: Cells exhibited resistance to 50 and 100 µM Temozolomide, and the drug did not produce significant success. However, 250 µM Temozolomide caused toxicity compared to the control at the 24th hour. When Temozolomide was applied simultaneously with Cyclopamine at varying concentrations, it was observed that 50 and 100 µM Temozolomide were effective, and the effectiveness of 250 µM Temozolomide increased. In cell viability experiments conducted with 100µM Temozolomide and 10µM Cyclopamine in LN-18 cells, it was observed that cyclopamine increased the effectiveness of Temozolomide in all three groups. Compared to the ninety percent viability rate of cells applied with temozolomide alone, the combined application reduced the viability rate of the cells to less than fifty percent. It has been proven that it can prevent Temozolomide resistance, especially after 48 hours. In the mRNA analysis, it was observed that the SMO mRNA level increased 3-fold in cells with IDH overexpression, suggesting that IDH overexpression may cause an increase in SHH activity. According to the results of mRNA and protein analysis in cells treated with cyclopamine and Temozolomide, it was observed that SMO, PTCH1, and GLI1 levels decreased significantly at the 24th hour. At the 48th hour, there was a slight increase in GLI1 levels, independent of the still low SMO level. Additionally, although TGF-beta activity was very low at 0 hours, the mRNA level started to increase at 24 hours after combined drug administration, and the increase continued at 48 hours. This result showed us that SHH pathway members have meaningful interactions with the TGF-Beta pathway, independent of SMO levels. After cyclopamine application, it was observed that the activity of the SHH pathway decreased, leading to increased apoptosis and ROS activity.

Conclusion: Our observations indicate that the success rate of the chemotherapeutic effect on IDH1 wild-type and mutant glioma cells increases when Temozolomide is applied in combination with Cyclopamine in LN-18 cells that are resistant to Temozolomide. When gene expression levels were examined, it was observed that PTCH1 and SMO gene expressions decreased after cyclopamine application, but GLI1 expression fluctuated and TGF-B expression increased, supporting this rise. Our studies suggest that the combination of cyclopamine will increase the success and effectiveness of treatment in Temozolomide-resistant glioma patients.

Keywords: Cyclopamine, glioma, LN-18 cell line, sonic hedgehog pathway, temozolomide.

OP-83

The combined use of olive oil, olive leaf extracts, and lycopene increases the efficacy of sorafenib on Hep40 cells

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Objective: Liver cancer (HCC) is one of the most common cancers in the world and the primary chemotherapy for HCC is Sorafenip (Srp). The fact that HCC patients do not respond well to Srp and long-term use of Srp results in primary or secondary drug resistance highlights the need for new therapeutic strategies to optimize the efficacy of Srp. In recent years, interest in bioactive compounds in the development of drugs has increased and it has been shown in many studies that some plant-based bioactive compounds show synergistic effects with drugs used in clinical applications. In this context, the present study evaluated the synergy of combination therapy with olive oil phenolic extract, natural phenolic compounds including olive leaf extract (OLE) and lycopene (Lyc) to increase the efficacy of Srp and to reduce the dose of Srp with all possible combinations.

Methods: The liver cancer cell line Hep40 was treated with Srp alone or simultaneously in combination with these extracts. Cell proliferation was investigated by MTT assay and apoptosis responses by annexin V assay. The CompuSyn algorithm was used to determine the combination index (CI). In addition, liquid chromatography (HPLC) and gas chromatography (GC) analyses were used to determine the chemical composition of olive oils from four different regions of Türkiye to determine fatty acid, sterol, and polyphenol compositions.

Results: Olive oil extract was found to markedly potentiate the therapeutic efficacy of Srp. In contrast, OLE and Lyc did not enhance the efficacy of Srp against Hep40 cells as much as olive oil extract, while concurrent treatment with olive oil extract and Lyc and OLE potentiated Hep40 cell cytotoxicity. In addition, the combinations including olive oil extract and OLE showed a synergistic effect with Srp. It was observed that the chemical composition of olive oil varied greatly depending on the variety and regional differences.

Conclusion: Olive oil was shown to inhibit Hep40 cell growth specifically. Detailed molecular analyses are required to understand the properties of phenols in olive oil that potentiate the Srp response. Additional studies are required to examine the in-vivo potential of combined treatment of Srp and phenolic extracts as a therapeutic strategy in human liver cancer.

Keywords: Drug combinations, liver cancer, lycopene, sorafenib, olive oil, olive leaf.

OP-84

Effects of oleuropein on inflammation in the U937 monocytic cell model

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Objective: Olives contain many potentially bioactive compounds that may have antioxidant, antimicrobial, antihypertensive, antiviral, anti-inflammatory, hypoglycemic, neuroprotective and anticancer properties. Oleuropein is a substance that is found more in the early stages of the olive fruit, and its amount decreases over time as it metabolizes as ripening progresses, giving the fruit bitterness. Oleuropein is a glycosylated secoiridoid, a type of phenolic bitter compound found in green olives, skin, flesh, leaves, argan oil and seeds. Recent basic research studies and observational epidemiological studies strongly support that the disease-preventing effects of natural products are partially attributed to antioxidants. This study aimed to determine the effects of oleuropine on inflammation in the U937 monocytic cell model.

Methods: The effect of oleuropein on cell viability and the doses to be applied were determined by the MTT test. Expression levels of IL-1β and IL-6 genes were determined by qRT-PCR method. Statistical evaluation was performed using Minitab 14 program for MTT test, mRNA and wound healing assay. The averages and standard deviations of the numerical data obtained with UV Spectro for the MTT test were calculated and compared with control cells using ANOVA.

Results: U937 cells were treated with different concentrations of oleuropein (3 mg/ml, 2 mg/ml, 1 mg/ml, 500 µg/ml, 250 µg/ml, 100 µg/ml, 50 µg/ml, 30 µg/ml, 10 µg/ml) at different time intervals (24 hours, 48 hours, 72 hours) were incubated. MTT was then performed to determine the viability of the cells. Experiments were designed to include 6 and at least two independent experiments at a time. In U937 cells, a statistically significant decrease in cell viability was observed at 250 µg/ml concentration compared to the control group with oleuropein at 24, 48 and 72 hours ($p<0.05$). The increase in cytotoxic effect correlates with the increase in dose. The 250 µg/ml dose of oleuropein increased the expression of the IL-1β gene by 3.21 times compared to the control ($p=0.026$) and increased the expression of IL-6 by 2.71 ($p=0.043$).

Conclusion: Oleuropein has been shown to increase chronic and acute inflammation response by increasing IL-1β and IL-6 gene expression. The study will be expanded by showing the changes that oleuropein causes at the protein level of these genes.

Keywords: Inflammation, oleuropei, U937 monocytic cell model.

OP-85

Methylation analysis of histone-related gene HIST1H4F and its effect on gene expression in bladder cancer

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Objective: Recently, aberrant DNA methylation of HIST1H4F gene (encodes Histone 4 protein) has been shown in many types of cancer which may act as a promising biomarker for early cancer diagnosis. However, the correlation between DNA methylation of HIST1H4F gene and its role in gene expression is unclear in bladder cancer. Consequently, the first aim of this study is to explore the DNA methylation pattern of the HIST1H4F gene and after that, further elucidate its effect on HIST1H4F mRNA expression and histone 4 protein expressions in bladder cancer.

Methods: In bladder tissue samples and bladder tumor cell line T24 cells, the methylation pattern of HIST1H4F gene was analyzed by sequencing and the effects of this gene's methylation profiles on HIST1H4F mRNA expression were examined through qPCR in bladder cancer.

Results: Sequencing analysis revealed significantly higher methylation frequencies of HIST1H4F gene in bladder tumor samples relative to normal samples ($p<0.0001$). Also, when we evaluated the correlations between hypermethylation of HIST1H4F and the clinicopathological parameters (tumor stage, tumor grade, lymph node metastasis, muscle-invasive), a significant difference was not found between the groups ($p>0.05$). Subsequently, we evaluated to the role of hypermethylation of HIST1H4F gene on HIST1H4F gene expression and histone 4 protein expressions. Hypermethylation of HIST1H4F gene did not affect only HIST1H4F mRNA expression levels in bladder cancer. Also, we did not determine any change in HIST1H4F methylation profile although HIST1H4F expression decreased or increased in T24 cells (HIST1H4F gene is hypermethylated) in a time-dependent manner.

Conclusion: Hypermethylation of HIST1H4F gene can be used as a promising early diagnostic biomarker and prognostic marker for cancer patients. Hypermethylation of HIST1H4F may play a role in bladder cancer tumorigenesis by affecting chromatin structure.

Keywords: Bladder cancer, DNA methylation, *HIST1H4F* gene, histone 4,

OP-86

Synergistic effects of SN38-OA/Cur combination on triple negative breast cancer cell line

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Objective: Triple-negative breast cancer (TNBC) is a heterogeneous group of tumors with the lowest survival rate among breast cancer subtypes. Due to the resistance to chemotherapy in TNBC patients, treatment options are limited in the clinic. Therefore, new drug discoveries and alternative therapies are needed for the treatment of TNBC. Irinotecan (Ir) is a chemotherapeutic agent commonly used for the treatment of cancer. The active metabolite of Ir, SN-38, binds to the topoisomerase I-DNA complex and induces double-strand breaks, thus exerting anti-tumor effects. At pH≤4.5, SN-38 is converted to lactone, the active form with antitumor activity, while at pH≥7.5 it exists in the inactive carboxylate form, a less stable form with limited therapeutic effect. Because of this limiting effect, structural modification of SN38 is needed for more effective treatment. In the present study, it was planned to synthesize a new drug by binding oleic acid (OA) to preserve the lactone form of SN38 and to develop a more effective combination therapy model that can be used in the treatment of TNBC by preparing a combination of SN38-OA with curcumin (Cur), which has known antioxidant activity to increase the potential efficacy of SN38-OA.

Methods: SN38-OA prodrug was modified by ester synthesis and characterized by NMR, FTIR, and UV-VIS methods. The effects of SN38, SN38-OA, and Cur and their combinations on cell viability, cell death, cell cycle, and migration in MDA-MB-231 and MCF10-A cell lines were analyzed by MTT, AO/EtBr, Annexin V, cell cycle, and scratch assay.

Results: It was determined that SN38-OA showed a low dose IC50 value (75nM-*$p<0.05$) compared to SN38 at 48 hours, which is the effective time. Compared to their use alone, the SN38-OA/Cur combination (25nM/25mM) showed a synergistic effect and inhibited cell viability by 85%, while no statistically significant toxic effect was observed in healthy cells. SN38-OA and SN38-OA/Cur combination increased the apoptotic cell rate from respectively, compared to control. SN38-OA and SN38-OA/Cur combination induced cell arrest in the G2/M phase and inhibited cell migration by approximately 80% (*$p<0.001$).

Conclusion: The findings suggest that SN38-OA/Cur combination enhances the potential efficacy of SN38 on the MDA-MB-231 cell line and the combination may be a promising therapeutic approach in the treatment of TNBC.

Keywords: Oleic acid, SN38, synergistic effect, triple negative breast cancer.

OP-87

Investigation of the synergistic effects of remdesivir and quercetin combination in triple negative breast cancer cells

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Objective: Triple negative breast cancer (TNBC) is the most aggressive subtype among breast cancers. Due to the limited treatment options and the development of resistance to current therapies in TNBC, new treatment strategies are needed. Previous studies have shown that the metabolisms of cancer cells and parasites are similar and that antiparasitic agents may have anti-cancer effects. Remdesivir (Rem) is an RNA-dependent RNA polymerase (RdRp) inhibitor used as an antiparasitic agent. Although Rem has demonstrated anti-cancer effects in cancer types including prostate cancer and melanoma, its use is limited due to its high toxicity. Flavonoids are known for their anti-inflammatory, antioxidant, and anti-cancer properties. Quercetin (Que) is a flavonoid that has been shown to have a synergistic effect with various chemotherapeutic agents in breast cancer. This study aims to investigate the synergistic effects of Rem-Que in TNBC cells.

Methods: The effects of Rem, Que, and Rem-Que on MDA-MB-231 cancer cells were evaluated by WST-1, Annexin V, cell cycle analysis, and acridine orange (AO)/ethhidium bromide (EB) staining.

Results: Rem, Que, and Rem-Que significantly inhibited cell viability in MDA-MB-231 cells at 72 hours at a statistical rate ($p<0.01$). Cell viability after exposure to Rem was determined to be 52.04% and 42.5% at 25 µM and 50 µM, respectively, while exposure to Que, it was 51.3% and 44.41% at 40 µM and 50 µM, respectively. Subsequent combination experiments show that cell viability decreases to 40.23% at 25-50 µM, where the IC50 value of the synergistic effect with the Compusyn program was determined. Morphological analysis demonstrate that Rem-Que combination increased cytoplasmic damage and reduction in cell size compared to the use of Rem alone. Moreover, after treatment with 25 µM Rem, 50 µM Que, and 25+50 µM Rem-Que, the percentage of cells in the G2/M phase increased from 44.8% to 52% and 59.3%, respectively, compared to the control, whereas this rate was increased to 48.3% in the Rem-Que combination.

Conclusion: The study demonstrates that Rem has cytotoxic effects and its combination with Que has a synergistic effect in TNBC cells, for the first time. These results highlight Que is promising to enhance the effectiveness of Rem when used at low doses in TNBC treatment.

Keywords: Quercetin, remdesivir, triple negative breast cancer.

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OP-88

Investigation of the expressions of NEAT1 and miR-410-3p in breast cancer cell lines and determination of their effects on metastasis

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Objective: In this study, we aim to investigate the expressions of NEAT1 and its target miR-410-3p in breast cancer cell lines MCF-7 and MCF10A and determine their roles in metastasis.

Methods: In our research, MCF-7 was used as the breast cancer cell line, and MCF-10A cell line were used as the healthy breast cell line as a control. Our cultured cells were removed with lysis reagent, and total RNA isolation was performed. In order to detect the change in expression at the RNA level, cDNA synthesis was performed from the isolated RNAs, and then expression levels were determined by qRT-PCR. Transwell invasion experiment was performed to determine the invasion capacity of the cells, and the cells were stained and visualized at the end of 24 hours of incubation.

Results: NEAT1 and miR410 expressions were determined by qRT-PCR in cell lines. NEAT1 expression increased 2.30-fold in the MCF-7 cell line compared to the MCF-10A cell line (p=0.000145). miR-410 expression decreased 0.35-fold in the MCF-7 cell line compared to the MCF-10A cell line (p=0.000001). Matrigel-invasion test was performed to determine the different invasion capacities between cell lines. After staining the invading cells using crystal violet dye, it was seen that the MCF-7 cell line had invasive properties, in line with the literature, but the MCF10A cell line did not acquire invasive properties even in the presence of chemoattractants.

Conclusion: In this study, it was observed that, in accordance with the literature, NEAT1 expressions increased in the breast cancer cell line MCF-7 compared to the normal breast cell line MCF-10A and suppressed miR-410-3p expressions, resulting in increased invasion and metastasis.

Keywords: Breast cancer, metastasis, miR-410-3p, NEAT1.

Acknowledgment: This study was supported by PAU Scientific Research Projects Coordination (Project No: 2020SABE028).

OP-89

To investigate the effect of chemical chaperones on the production of trastuzumab antibody in HEK293T cell line

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Objective: The aim of this study was to investigate the effect of chemical chaperones on the production of trastuzumab antibody in HEK293T cell line.

Methods: After the bacterial stock reconstitution was performed in liquid medium, DNA was isolated from the plasmid by alkaline lysis method. Simultaneously, HEK293T cell line was grown in appropriate medium. After transfection, the cell line was selected to match the antibiotic resistance gene present on the plasmid. Passaging was carried out for more than one month using hygromycin medium to ensure that the cell forms a stable cell line after transfection. RNA isolation from the stable cell line pellet and Cdna library formation were completed. Actin PCR was performed to control the formation of the Cdna library. In order to demonstrate the trastuzumab production of the stable cell line at the gene expression level, PCR steps related to the antibiotic resistance gene in the plasmid structure and the heavy and light chains of the constant region of the trastuzumab antibody were completed. Trastuzumab antibody production was demonstrated at protein level by western blot method. 4-PBA, a chemical chaperone, was applied to the stable cell line.

Results: Gene expression and protein level production of trastuzumab antibody in the 4-PBA treated cell line was compared with the production in the cell line without 4-PBA.

Conclusion: As a result of the experiments, it was shown that the cell line could be transfected with the relevant plasmid and trastuzumab production was realised at gene expression level and protein level. It was determined that antibody production increased after the application of 4-PBA, a chemical chaperone, to the stable cell line. In parallel with the studies in the literature, it was shown in this study that chemical chaperones can increase protein/antibody production.

Keywords: Chemical chaperones, HEK293T cell line, trastuzumab.

OP-90

Effect of vitamin D on endoplasmic reticulum stress in testicular tissue of rats with metabolic syndrome

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Objective: Metabolic syndrome (MetS) might develop in the presence of diabetes, hypertension, and dyslipidemia and, is an important risk factor for cardiovascular diseases. The relationship of MetS with male infertility has been discussed in recent studies. It was reported that endoplasmic reticulum stress (ERS) induced as a result of environmental conditions such as hypoxia and nutritional effects or factors that disrupt calcium homeostasis is observed in the testicular tissues of infertile cases with MetS. The regulatory effect of Vitamin D (Vit D) on MetS and ERS has been shown in studies. This study aimed to investigate the effect of Vit D on the ERS pathway triggered by MetS in testicular tissue.

Methods: The study group consists of MetS (n=10), Vit D-supplemented MetS (MSD, n=10), and healthy control groups (n=10). MetS model was induced by feeding Sprague-Dawley male rats with 10% fructose water and a diet containing 17% fat and 17% fructose for 15 weeks. VitD was supplemented to MSD group in addition to MetS diet starting from 3rd week. Experimental group was sacrificed at 15th week and testicular tissues were dissected. Tissues were snap-frozen and stored at -80°C until RNA isolation. After homogenization of tissues, total RNA isolation and cDNA synthesis were performed. Gene expression analysis was performed by quantitative real-time PCR (qRT-PCR) method and relative quantitation (RQ) values were calculated by comparative Ct method. All samples were studied in triplicates. The expressions of ERS and Vit D-related genes, glucose-regulated protein 78 (*Hspa5*), activating transcription factor 6 (*Atf6*), and endoplasmic reticulum localized 57 (*Pdia3*) genes were analyzed. Values of the actin beta (*Actb*) gene were used as a control. Statistical significance was compared using the student T-test.

Results: While no significant change in *Atf6* gene expression was observed in the testicular tissue between the MS and MSD groups, an increase in the expression of *Hspa5* (p=0.005) and *Pdia3* (p=0.004) genes was observed in the MS group.

Conclusion: These results suggest that regulation of ERS and Vit D-related genes in testicular tissue may differentiate in presence of MetS and that male infertility may be associated with the affected spermatogenesis process.

Keywords: Endoplasmic reticulum stress, metabolic syndrome, testicular tissue, vitamin D.

OP-91

Investigation of the effect of neural system on cancer in human and mouse breast cancer cell lines

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Objective: The nervous system is closely related to various physiological processes, from development, growth, tissue homeostasis, and repair in the body. Studies have shown that the nervous system plays an active role in some types of cancer, such as pancreatic, colon, breast, head and neck cancer. In addition, neural infiltration has been associated with tumor progression and poor prognosis. Although breast cancer is the most diagnosed cancer type and causes the most death among women worldwide, it is a type of cancer in which neural infiltration is observed very intensely. However, studies have not been able to explain how neurons affect their behavior by creating an effect on cancer cells

Methods: In this direction, bioimageable Beta-III Tubulin GFP neurons and cancer lines with mCherry expression were used. Human (MDA-MB-231) mouse (EMT6) breast cancer cell lines were used in the study and the cells were co-cultured with DRGs. Some time after the culture step, the cells were visualized under a confocal microscope. Afterwards, STAT3 expression levels in both neurons and cancer lines were examined by Western Blot and Quantitative PCR methods.

Results: In the Western blot results obtained from the control and co-culture groups, DRGs grown with cancer cells showed a significant reduction in STAT3 protein expression compared to DRGs from the control group. A significant reduction in STAT3 expression was observed in the EMT6+AKG co-culture group compared to the EMT6 control group. Based on the Western Blot results obtained from the control and co-culture groups of MDA-MB-231, a significant rise in STAT3 protein expression was observed within the DRGs coexisting with MDA-MB-231 cancer cells when compared to the control group's DRG. Between the MDA-MB-231+DRG co-culture group and the MDA-MB-231 control group, no significant difference in STAT3 expression was observed.

Conclusion: The results revealed that neurons and cancer cells affect each other bidirectionally and showed that targeting the neural system in treatment approaches may be a potential strategy for cancer treatment.

Keywords: Cancer cell line, dorsal root ganglia, EMT6 Cell line, MDA-MB-231 Cell line, STAT3.

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OP-92

Evaluation of CTLA-4 gene methylation profiles of CD8+ T cells after renal transplantation

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Objective: CTLA-4 is one of the immune checkpoint molecules and inhibits autoreactive T cells, especially at the initial stage of naive T cell activation. While the CTLA-4 response attracts a lot of attention, especially in cancer studies, data to understand its role in organ transplantation is limited. Our aim is to determine the methylation levels in the CTLA-4 promoter region of kidney transplant patients before and after transplantation and to determine its relationship with gene expression levels.

Methods: Bisulfite modification was perfomed to CD8+ T cell DNA samples isolated from 30 kidney transplant patients whose CTLA-4 gene expression levels were determined before and after transplantation in our previous project, which we completed in 2022. Then, a PCR reaction was performed with CTLA-4-specific primers and the methylation levels of 4 CpG islands in the CTLA-4 gene promoter were determined by Sanger sequencing. In addition, CTLA-4 protein levels were determined by Western blotting method and serum-soluble sCTLA-4 levels were determined by ELISA method.

Results: In 2 patients, CpG island 1 was hemi-methylated before and after transplantation, while islands 2-3-4 were methylated. It was determined that CpG islands 1 and 2 of 2 patients were methylated before the transplantation but became hemi-methylated after the transplantation. While a relationship was found between the gene expression levels and methylation levels of 2 patients, no relationship was found between the gene expression levels and methylation levels of the other 2 patients. Although gene expression changes and protein levels were found to be compatible with each other, no significant relationship was found between them and sCTLA-4 levels. Considering the patients' post-transplant clinical conditions (rejection attack) and the medications they used, no significant relationship was found between CTLA-4 methylation levels and protein levels and the clinical status.

Conclusion: Based on the idea that gene expression levels are not only controlled by gene methylation, we are planning studies on the effects of other epigenetic control mechanisms, such as determining the miRNA profile related to the CTLA-4 gene and determining the changes in the expressions of histone acetylase and deacetylases.

Keywords: CTLA-4, DNA methylation, renal transplantation.

Acknowledgment: This study was supported by Tubitak 1002 program with project number of 321S274.

OP-93

Evaluation of eye and bone pathologies in lrp5 mutant zebrafish

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Objective: Osteoporosis-Pseudoglioma (OPPG) syndrome, a disease associated with loss-of-function mutations in the *LRP5* gene, is characterized by autosomal recessive inherited congenital or early-onset blindness, severe and early-onset osteoporosis, skeletal fragility, and sometimes learning difficulties. Considering that the family members examined in the previous study, which is the starting point of the study, did not have bone fractures, childhood osteoporosis complaints, and there were no other eye findings other than microphthalmia, the patients were evaluated as isolated microphthalmia to date. Based on this, it is thought that c.2827+1G>A splice site mutation, which was found as homozygous in our study for the first time, may cause an anomaly in the eye, especially microphthalmia, rather than bone. To date, OPPG syndrome has not been specifically studied in zebrafish. From this point of view, our objective was to create and evaluate the relevant mutation.

Methods: We used to *lrp5* sa11097 zebrafish line for this study. H&E staining was used to examine the histologic structure in the eye. Alcian blue and alizarin red were used to examine cartilage and bone structure, respectively.

Results: In this study, it was determined that the head size was significantly smaller in 8-day-old mutant larvae compared to 8-day-old wild AB type larvae. When the eye size was examined, no significant difference was observed between the groups. When 8-day-old wild-type and mutant larvae were examined for bone and cartilage structure, it was determined that the development of the notochord structure of mutant larvae was slower than that of wild-type larvae.

Conclusion: The study was the first in the literature to evaluate eye and bone-cartilage findings together. In this study, there was no finding in favor of microphthalmia, but a significant difference in notochord development was observed. However, more detailed studies are needed to understand whether this difference is mutation specific.

Keywords: Eye and bone pathologies, lrp5, mutant zebrafish.

OP-94

Investigation of combined treatment possibilities in resistant glioblastomas with sanguinarine stimulation

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Objective: Glioblastoma accounts for approximately 70% of primary brain tumors in adults. Both hereditary and environmental factors have an impact on the occurrence and development of glioblastoma. Despite simultaneous Temozolomide (TMZ) chemotherapy and radiotherapy treatment following the surgical procedure, the tumor may recur within 15 months and turn into high-grade glioma and cause high mortality. Genomic instability occurs in the cell and ROS levels increase. Increased ROS disrupts cell homeostasis and causes cell death by increasing cell cytotoxicity. However, TMZ may not be equally effective in every glioblastoma patient, and patients may develop resistance to TMZ. In order to eliminate this chemotherapeutic resistance developing in glioblastoma patients, combined treatment applications with new therapeutic agents that strengthen TMZ activity and increase the TMZ sensitivity of the cell are important. In this study, it was aimed to increase the sensitivity of the cell to TMZ by using molecules that increase the intracellular ROS level, which plays an important role in cell homeostasis.

Methods: U87-MG and TMZ-resistant U87-MG were used as cancer cells, and HUVEC cell line was used as healthy cell. Sanguinarin (SNG) and Mitoxantrone (MIT), Ketoconazole (KET) and Brexipiprazole (BREX) molecules were used to increase ROS formation. Cell viability, intracellular ROS level were measured and colony formation was monitored.

Results: In cell viability analysis, IC50 values were found 57.9µM for SNG, 1.9µM for MİT in U87-MG cell at the end of the 48th hour. IC50 values in the HUVEC cell were 64.61µM for SNG and 2.21µM for MIT. In TMZ resistant U87-MG cells, the IC50 value was found 47.99µM for SNG and 1.93µM for MIT. The IC50 value of KET and BREX molecules in all cell lines was in the range of 150-200µM. While KET + BREX combined treatment did not affect cell viability in any cell line, it was observed that combined applications of other molecules reduced cell viability approximately 10-fold. Compared to single applications of the molecules, combined applications increased the intracellular ROS level approximately 5-fold and reduced colony formation.

Conclusion: SNG, MIT, KET and BREX molecules increase cell death in high-stage and TMZ-resistant glioblastomas. Combined application of molecules can be considered as an alternative approach instead of TMZ, which is widely used in the treatment of glioblastoma.

Keywords: Glioblastoma, mitoxantrone, ROS, sangunarine.

OP-95

Circulating markers of netotic and pyroptotic cell death and their prognostic significance in Covid-19 disease

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Objective: It is suggested that various types of cell death in leukocytes may play a role in the pathophysiology of Covid-19 caused by SARS-CoV-2 infection. In this study, we investigated the relationship between the severity of Covid-19 disease and netotic and pyroptotic cell death.

Methods: Serum samples were collected from 150 Covid-19 patients with mild (M), moderate (Mo), and severe (ICU) courses, as well as 89 healthy individuals. Serum levels of neutrophil elastase (NE), myeloperoxidase (MPO), cell-free DNA (cfDNA), citrullinated histone H3 (CitH-H3), gasdermin-D (GSDMD), IL-18, and IL-8 were analyzed by ELISA method. The relationships between circulating netosis (NET) and pyroptosis markers and disease severity and clinical parameters were investigated.

Results: Levels of NE, CitH-H3, and GSDMD were significantly higher in the overall patient group compared to the healthy group. In ICU patients, age, D-dimer, ferritin, CRP, and neutrophil levels were significantly higher, while lymphocyte and hemoglobin levels were lower. CitH-H3 levels were significantly higher in ICU patients, while NE levels were significantly higher in M+Mo patients. In deceased patients, IL-8 levels were low, CRP was high, and age, CRP, and CitH-H3 levels were significantly high in intubated patients, with low IL-8 levels.

Conclusion: Our results indicate that circulating netosis and pyroptosis markers in Covid-19 patients are associated with inflammation, dysregulated hemostasis, and fibrinolysis, and they may play a role as prognostic indicators of disease severity. These findings suggest that they could be considered as potential targets in the treatment of Covid-19.

Keywords: Comorbidity, COVID-19, netosis, pyroptosis, SARS-CoV-2.

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OP-96

Optimized number of in silico tools and random forest algorithm predicts variation of unknown significance variants: a new approach

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Objective: FMF is complex hereditary autoinflammatory diseases which is commonly observed in Mediterranean region. However, according toThe International Study Group for Systemic Autoinflammatory Diseases (INSAID) consensus criteria half of the *MEFV* gene variants are uncertain. In this study, we combine multiple tools by implementing Random Forest (RF) algorithm. Therefore, we aim to assign variation of unknown variants (VOUS) to disease-causing or benign categories.

Methods: We extracted variants of the *MEFV* gene from the Infevers database, and single nucleotide alterations in coding regions were included; others were excluded from the study. We then determined the optimal number of in-silico tools for our model. On the training dataset, we conducted a RF classifier on known *MEFV* gene variants. The prediction dataset included 168 VOUS variants.

Results: We included 266 of the 381 *MEFV* gene variants and four computational tools (Revel, MetaLR, SIFT, and Polyphen-2) in a study. Overall, 98 variants were classified as benign or disease-causing variants. However, the remaining 168 variants were detected as having uncertain significance. According to power analysis, our sample size was sufficient to conduct a RF classifier model. Therefore, we selected 98 known variants as a training dataset, of which 49 were evaluated as disease-causing; however, only 49 were evaluated as benign. After that, the RF algorithm was conducted, and 100% accuracy was obtained. The remaining 168 variants were predicted to be benign or disease-causing.

Conclusion: As many *MEFV* gene variants categorized into uncertain categories, it is essential to accurately predict *MEFV* gene variants. Functional investigations play a crucial role in the identification and characterization of VOUS variants. Nevertheless, it is both time-consuming and costly. Employing a RF method to train an optimized number of *in silico* tools could offer a viable strategy for predicting VOUS variants in routine clinical practice, hence aiding in clinical decision-making.

Keywords: FMF, In silico, *MEFV* gene variants.

OP-97

NOTCH3 gene sequence variants and prostate cancer risk

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Objective: Prostate cancer has been defined as the second most common type of cancer in men, especially in older ages, and its incidence is constantly increasing in almost all countries, but its etiology is not fully known despite its high morbidity. age, race and family history are known risk factors. Notch signaling is one of the cell interaction mechanisms that has been preserved throughout evolution and plays a role in determining cell fate during development. It is closely related to carcinogenesis because it affects tumor suppressor and oncogenic mechanisms. There are four Notch genes (Notch 1-4) in mammals. Notch members that are expressed in the prostate gland and whose expression differences were determined in prostate cancer are Notch1 and Notch3. Accordingly, it was aimed to sequence the promoter regions of the Notch3 gene expressed in prostate tissue and to determine serum Notch3 protein levels in our study.

Methods: The Notch3 gene promoter in 25 benign prostatic hyperplasia (BPH), 25 localized prostate cancer (PCA), 25 metastatic prostate cancer (MPCA) patients and 15 control individuals was sequenced by the Sanger sequencing method and serum Notch3 levels were determined using Elisa kits. The results were evaluated with appropriate statistical methods and a p value of less than 0.05 was considered significant.

Results: In the analysis in which the entire data set for the Notch3 gene region was blasted, the most common variation, the C>T variation at position 6758, was determined in 67 samples (rs1044009). Notch3 serum levels were significantly lower in the BPH group (p= 0.002), MPCA group (p <0.001) and PCA group (p <0.001) compared to the control group.

Conclusion: In line with the study data, it is thought that the Notch3 gene is a candidate gene for prostate cancer risk and serum protein levels can be confirmed in larger populations and used in diagnosis.

Keywords: Benign prostatic hyperplasia, Notch3 gene, prostate cancer, sanger sequencing.

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OP-98

Development of nerve guidance conduit modified with gold nanoparticles and neural factors for rat spinal cord injury regeneration

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Objective: Spinal cord injuries are very common worldwide and dramatically affect life quality of the patients. Although there is a partial regeneration capacity for peripheral nerves, the functional recovery is very difficult due to the inhibitory microenvironment of the central nervous system. The developments in the fields of biomaterials and nanomedicine are very promising considering the difficulties and unsatisfactory results in traditional treatments. The aim of this study was to develop a nerve guidance conduit with micro-channeled topography, gold nanoparticle (AuNPs) conductive and modified with BDNF/NGF/IKVAV-pentapeptide molecules for regeneration of the rat spinal cord injury.

Methods: The smooth surfaced and micro-channeled (1 micron width) polycaprolactone (PCL)/polylactic-co-glycolic acid (PLGA) hybrid film scaffolds were fabricated using electron beam lithography and spin coating techniques. The surfaces of the scaffolds were made conductive by using two different materials (AuNPs and polypyrrole: PPy). In addition, the scaffold surfaces were modified with various biomolecules (BDNF/NGF/IKVAV-pentapeptide). The prepared tubular nerve guidance conduits were implanted into the right lateral hemisection injury site performed in the *Sprague Dawley* rats. Behavioral studies, histological staining and Western blot analyses were performed at the 5th and 10th weeks following implantation.

Results: The designed PCL/PLGA implant material generally showed good integration with the tissue and was degraded in the process. The most optimal results for nerve regeneration were obtained from the micro-channeled and AuNPs conductive group modified with neural factors. Micro-topography, neural factors, and suitable surface conductivity (AuNPs) alone did not provide effective regeneration, but all together promoted optimal neural recovery with synergistic effect. On the other hand, the PPy modified group and the smooth surfaced scaffold group did not provide a desired level of nerve regeneration.

Conclusion: In this study a functional channeled and AuNPs conductive PCL/PLGA tubular nerve guidance conduit with modified BDNF/NGF/IKVAV-pentapeptide molecules has been developed for regeneration of the rat spinal cord injury in long-term period. The developed micro/nano-designed PCL/PLGA scaffolds can be used as nerve conduits to provide optimal axonal guidance and neural regeneration required for recovery of function after various nerve injuries.

Keywords: Gold nanoparticles, neural factors, rat spinal cord injury, regeneration.

Acknowledgment: This study was supported by The Scientific and Technological Research Council of Türkiye (Project Number: 119S141).

OP-99

Investigation of cytogenetic damage by micronucleus method in head and neck cancer patients treated curative chemoradiotherapy and post-operative radiotherapy

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Objective: Radiotherapy (RT) is one of the main treatments with surgical resection and chemotherapy for head and neck cancers. Radiation induces double-stranded DNA breaks, resulting in mutations and chromosomal abnormalities, including dicentric and acentric fragments, leading to genomic instability. The frequency of micronuclei (MNi), considered an indicator of genomic instability, is an important biomarker of genotoxicity. RT also causes toxicity in normal cells. Our study aims to examine the chromosomal damage at peripheral lymphocytes and the individual radiotoxicity in patients undergoing RT and chemo-radiotherapy (CRT).

Methods: Twelve patients underwent RT, 10 underwent CRT, and 10 healthy controls enrolled. Heparinized peripheral blood samples were collected from patients three times: before, during, and after treatment. Two separate tubes were used to culture blood samples, one after *ex vivo* irradiation with 2 Gy X-ray and the other without irradiation. Cytochalasin B was added at the 44th hour to block cytokinesis and then harvested at the 72nd hour. The procedures were also performed on the samples obtained from the control group. The slides were stained with Giemsa and 1000 cells were aimed to evaluate according to FENECH's criteria.

Results: Before treatment, the frequency of MN was higher in the RT and CRT groups than in the control group. However, there was no significant difference between RT and CRT groups. In the RT group, there was no significant difference in MN frequencies between pre-treatment, mid-treatment, and post-treatment. However, in the CRT group, there was a significant difference between pre-treatment and post-treatment, and between mid-treatment and post-treatment. A relationship was also observed between MN frequencies and therapy-related severe side effects. In the RT case group, a significant relationship was found between the difference in MN frequency before treatment and xerostomia, and in the CRT case group, a significant relationship was found between the difference in MN frequency at the end of treatment and dysphagia.

Conclusion: Our study showed that examining the frequency of MN is significant in determining radiosensitivity.

Keywords: Chemoradiotherapy, genomic instability, head and neck cancers, micronucleus, radiotherapy.

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OP-100

Expression of novel gene clusters in ACTH-secreting PitNET

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Objective: Cushing's disease (CD) is a rare disease caused by exposure to excessive amount of endogenous glucocorticoids due to ACTH-secreting pituitary neuroendocrine tumor (PitNET). The aim of this study was to investigate the expression of cell cycle regulator, deubiquitinating enzyme, transcription factor, and cell signaling genes, as well as the methylation of *CDKN2A* and *USP8* genes, which are likely to play a role in the development of ACTH-secreting PitNET.

Methods: Pituitary tumor tissue samples from thirty-two patients with corticotroph adenomas and fifteen anterior pituitary tissue obtained from fresh autopsies were investigated. Gene expression analysis of *USP8*, *CABLES1*, *USP2*, *STAM2*, *VPS28*, *HDAC2*, *IL-6*, *SMARCA4*, *EGFR*, *WEE1*, *CDKN2A*, *CCND1*, *NR4A1*, *NEUROD1* and *RIPK1* was performed by qRT-PCR, and methylation analysis was performed by MS-PCR.

Results: Expression of *CABLES1*, *NR4A1*, *CCND1*, *NEUROD1*, *USP2* and *WEE1* genes changed significantly. There was a significant correlation between *RIPK1*, *SMARCA4* and *USP2* expression and pre-op cortisol levels; *WEE1* expression and pre-op ACTH levels; *CDKN2A* expression and urinary cortisol levels; *CABLES1*, *NEUROD1*, *SMARCA4* and *STAM2* expression and post-op 48 h cortisol levels; *CCND1* expression and adenoma size, and finally *WEE1* expression and remission status. We found that the *CDKN2A* gene was partially methylated, and the *USP8* gene was fully unmethylated.

Conclusion: The results of PCA demonstrated that the altered expression of *USP2*, *CABLES1*, *CDKN2A* and *WEE1* genes related to development of the ACTH-secreting PitNET. There were four co-acting genes for ACTH-secreting PitNET, which were correlated with disease development and parameters: *USP2, CABLES1, CDKN2A* and *WEE1*. *WEE1* is likely to be used as a target gene for predicting remission.

Keywords: ACTH-secreting pituitary neuroendocrine tumor (PitNET), Cushing's disease, methylation.

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OP-101

Endothelin-converting enzyme-1b genetic variants could increase the hyperlipidemia risk and be involved in neoatherosclerosis formation in the stented coronary arteries

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Objective: The possibility that increased LDL-cholesterol that leads to atherosclerosis may be a risk factor for in-stent restenosis (ISR) that was characterized by neoatherosclerosis formation in stented coronary arteries has been bolstered by recent publications. Membrane-bound endothelin converting enzyme-1 (ECE-1) is involved in the maturation process of endothelin-1 (ET-1) of which is known as the most powerful vasoconstrictor agents of human body. Recent studies suggest that polymorphisms in the ET gene family are associated with the development of atherosclerosis. Thus, in the current study, we studied the effects of rs213045 and rs2038089 polymorphisms in ECE-1 gene to determine whether they could be associated with a genetic predisposition to ISR.

Methods: All patients were diagnosed with coronary artery disease (CAD) confirmed by coronary angiography (CAG). According to the results of CAG at least six-months after stent-implantation, the patients were categorized two groups as ISR (n=102) and Non-ISR (n=74). Real-time PCR method was used for the detection of genotypes.

Results: Genotype distributions of rs213045 and rs2038089 variations in ECE-1 gene were found similar between study groups. However, type II diabetes (T2DM) and hyperlipidemia were determined as risk factors for the development of ISR ($p<0.010$, $p<0.001$, respectively). When further analysis was performed to compare the frequencies of age, gender, hypertension, T2DM and rs213045 and rs2038089 SNPs of ECE-1b gene between hyperlipidemic and non-hyperlipidemic ISR patients, rs213045 T allele ($p=0.017$), hypertension ($p<0.001$), T2DM ($p<0.001$) and female gender ($p=0.007$) were found higher in hyperlipidemic ISR patients. The regression analysis, showed that rs213045-T allele ($p=0.036$) and T2DM ($p<0.001$) were risk factors for hyperlipidemia in ISR patients.

Conclusion: Our findings show thatECE-1b rs213045 polymorphism may be associated with the hyperlipidemia that is the leading cause of atherosclerosis and could be a valuable genetic marker for the detection of ISR development in CAD patients that have stent operations.

Keywords: Diabetes, endothelin converting enzyme-1b, hyperlipidemia, in-stent restenosis, variation.

OP-102

Investigation of the in vitro effects of mitochondrial-derived peptide (MOTS-c) on imatinib-resistant chronic myeloid leukemia model

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Objective: The mitochondrial short open reading frame (MOTS-c) of 12S rRNA type-c is one of the 16 amino acid MDPs encoded by the mitochondrial genome. There are studies of this biopeptides role in metabolic responses and cellular stress. However, MOTS-c's role on leukemia and drug resistance are unknown. Our aim is to investigate the possible contribution of exogenic MOTS-c exposure to cell viability and cell death in chronic myeloid leukemia (CML) and imatinib resistant-CML in vitro.

Methods: The ancestral K562 and K562-derived imatinib resistant cell line (K562-IR) is used for the leukemia model. Both cell lines was exposed to different doses and durations of MOTS-c (0.1, 1.0, and 10 µM) exogenously. We were evaluated MOTS-c function on these cell lines in terms of cell viability (MTT), cell death (Annexin-PI), and lipid peroxidation. Statistical analysis of the data was performed using IBM SPSS Statistics 24.0 with one-way ANOVA.

Results: MOTS-c significantly triggered cell death in both cell lines. Apoptosis in resistant cells was significant within the first 24 h at a concentration of 10 µM ($p<0.039$), and K562-IR induced significantly apoptosis at 72 h- 10 µM compared to ancestor cells ($p<0.046$). While the viability rate decreased in ancestral cells depending on the MOTS-c concentration, responses in the resistant group were found to be variable. However, in terms of lipid peroxidation, no significant difference was found between ancestral and resistant cell lines.

Conclusion: Cancer metabolism is a primary target for overcoming drug resistance, which develops over time despite the discovery of smart molecules. Our results are important as they show for the first time the effect of MOTS-c on viability and cell death in secondary drug resistance in cancer. Additionally, our results suggest that the cytotoxic effect of MOTS-c may vary depending on the concentration. More research is needed to understand MOTS-c and its functions.

Keywords: Chronic myeloid leukemia, drug resistance, imatinib, mitochondrial derived peptides, MOTS-c.

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OP-103

In vitro validation of newly discovered LIM kinase-targeted drug candidates for HCC treatment by protein domain-based in silico analysis

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Objective: LIM kinases (LIMK) play an important role in the regulation of cell motility, cell cycle progression and the dynamics of actin filaments. LIMKs phosphorylate the actin depolymerization protein Cofilin, making it inactive. Due to their important role in cell migration, LIMKs have become targets of cancer drug research. In this study, new LIMK inhibitors (LIMKi) were discovered by using "DRUIDom" (DRUg Interacting Domain prediction), a new virtual screening method targeting protein-compound connections designed for drug discovery, and by targeting the PI3K/AKT pathway. The syntheses and bioactivity analyzes of these molecules, as well as their in vitro effects, were studied experimentally in hepatocellular carcinoma cell lines.

Methods: The cytotoxic effects of LIMKi discovered by the DRUIdom virtual screening method were tested on HSK cell lines (Huh7 and Mahlavu) with Sulforhodamine B (SRB) and xCelligence SP Real-Time Cell Analysis System. The cytotoxicity of the molecules was measured and IC50 doses were determined to be used in other experiments. The inhibitory effects of the compounds on target proteins on the LIMK pathway were tested by Western Blot (WB) analysis. Finally, real-time cell migration assay and wound healing assay were performed to demonstrate how inhibitors affect the cellular migration, so that invasiveness, of cancer cells.

Results: It has been shown that the discovered LIMKi and its derivatives inhibit the phosphorylation of LIMK and Cofilin in its sub-pathway, thus proving the suppressive properties of the molecules towards their targets. An important feature of these inhibitors is that they are specific to cancer cells and do not have a cytotoxic effect on normal cells (HEK297). Real-time cell migration analysis and wound healing analysis results similarly significantly suppressed the migration feature in aggressive Mahlavu cell lines. This effect is very important to prevent cancer cell invasion.

Conclusion: The results found showed that DRUIDom can be used to identify drug candidate compounds for identified targets. In general, it is predicted that these discovered inhibitors may be a new approach for the treatment of hepatocellular cancer due to their cytotoxic effects on cancer cells and their invasion suppressing properties.

Keywords: Hepatocellular carcinoma, *in silico* drug discovery, LIM Kinase, small molecule inhibitors.

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OP-104

Evaluation of nonclonal chromosome anomalies in patients with chronic myeloid leukemia receiving tyrosine kinase inhibitor therapy

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Objective: While nonclonal chromosome anomalies (NCCAs) have been traditionally considered as stochastic events and background artifacts, recent research has suggested that NCCAs may contribute to chromosomal instability and play a role in cancer development, providing a new perspective. This study aimed to evaluate nonclonal chromosomal number and structural abnormalities in patients with chronic myeloid leukemia (CML) receiving tyrosine kinase inhibitor (TKI) therapy.

Methods: Cytogenetic results and clinical findings of 147 CML patients, including 95 males and 52 females, with a median age of 48 (range 16-83), were retrospectively analyzed. In the study, NCCAs observed to recur in cytogenetic analyses performed at different times during the follow-up of the cases were evaluated.

Results: Of the 147 cases examined, two groups consisting of 70 patients who were still being followed up with tyrosine kinase therapy and 30 patients who died were evaluated. Considering the initial samples, among the 70 patients who achieved complete responses, 15 (21%) had Ph(+), 5 (7%) had Ph(+)/(-), 47 (67%) had Ph(-), and 2 (3%) showed variant Ph. In this group, the number of recurrent NCCAs ranged from 2 to 27, with a higher frequency observed in Ph (-) cases. NCCAs were mainly chromosome loss, with the most common losses occurring in chromosomes 19, 20, 10, and 14. structural NCCAs were observed in 2 cases (3%). Among the 30 patients who did not survive, 24 (80%) had Ph(+), 1 (3%) had Ph(+)/(-), and 5 (16%) had Ph(-). In this group, the number of recurrent NCCAs ranged from 1 to 40. Similarly, NCCAs were predominantly chromosome losses, with the highest losses observed in chromosomes 19, 21, and 20. structural NCCAs were observed in 4 cases (13%). When comparing the two groups, the group achieving complete response showed a higher number of different chromosome losses during treatment, whereas the group with treatment-associated mortality exhibited more chromosome structural abnormalities. In both groups, some NCCAs were observed as clonal abnormalities in subsequent analyses.

Conclusion: Recent research suggests that NCCAs may serve as a fundamental mechanism for cancer development and establish a connection between increased NCCAs in cancer patients and shorter overall survival. In our patient group, the highest numerical and structural NCCA burden was observed in patients who experienced loss of response during treatment. Consequently, our study emphasizes that NCCAs, previously considered background artifacts, should be recognized as significant findings in cancer cytogenetics.

Keywords: Chronic myeloid leukemia, cytogenetics, nonclonal chromosome anomalies, tyrosine kinase inhibitor therapy.

OP-105

Progesterone receptor gene V660L associates with disease features in gliomas

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Objective: Gliomas, the most common type of primary CNS neoplasm, are derived from glial cells. Gliomas may be influenced by sex steroids. In teamwork with estrogen, progesterone affects neuronal excitability, learning, and neoplastic proliferation of glial cells. Low doses of progesterone stimulate glioma growth while higher doses act as antiproliferative. Progesterone receptors (PGR) are found in the brain and are responsible for progesterone effects. The PGR V660L (rs1042838) missense substitution in exon4 has not been extensively studied with glioma. We aimed to define whether the rs1042838 and PGR levels were associated with pathological features of gliomas.

Methods: PGR levels were measured by ELISA and rs1042838 was detected by allelic discrimination assay in 105 glioma patients.

Results: High-grade gliomas were observed in males (67.6%) compared to females (32.4%) (p=0.05, OR: 2.207, CI 95%: 0.971-5.015). CC genotypes (74.6%) were higher in high-grade gliomas than the rare-A-allele (25.4%) (p=0.183). High-grade gliomas were higher in the A-allele non-carrier (CC-genotype) males (84.4%) than in the A allele-carriers (AA+CA)(15.6%)(p=0.044, OR:0.289, CI 95%: 0.093-1.004). Females had higher PGR levels than males in glioma tissues (3.545±1.67 ng/ml vs.2.70±1.70 ng/ml; p=0.022). The A-allele carriers had higher PGR levels than non-carriers (CC)(3.73±1.54 vs 2.81±1.73; p=0.022). Higher PGR levels were observed in females with IDH-mutant than in IDH-Wild (p=0.032). High-grade gliomas had higher Ki67 than low-grades (p<0.001). High-grade females had higher Ki67 than low-grade females. The C-allele carrier females had Ki67<20% more than females with AA (p=0.034). Gliomas with p53-wild were higher in C-allele carriers than in AA (p=0.05, OR: 0.167, CI 95%: 0.028-0.997). Gliomas with IDH-mutation (p=0.020; OR: 8.167; CI 95%:1.419-4.702) and ATRX mutation were higher in females with A-allele than in with CC (p=0.022; OR: 13.333; CI 95%:1.280-138.845).

Conclusion: The rs1042838 rare A-allele, females, and IDH-mutant patients showed high PGR levels in gliomas. The A-allele carrier females had IDH and ATRX mutations, which were associated with good prognosis. High PGR levels may be associated with a good prognosis. Our findings suggested the rs1042838 rare A-allele may be a good prognosis factor for gliomas. It was the first and a preliminary study showing the importance of PGR rs1042838 in gliomas.

Keywords: Glioma, progesterone receptor gene, progesteron receptor levels, V660L variations.

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POSTER PRESENTATIONS

PP-1

Investigation of the relationship between androgen and death receptor ligand (PD-L1) in prostate cancer cells

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Objective: Androgen is important in the development of prostate cancer and causes cancer progression through its receptor. The androgen receptor is also expressed at different stages of prostate carcinogenesis, from organ-limited invasive prostate cancer to metastatic disease. The presence of PD-L1 proteins on the cell surface is one of the ways cancer cells escape in the immune system. PD-L1 suppresses the proliferation of PD-1 positive cells and reduces cytokine secretion. By reducing the host's immune response against tumor cells, PD-L1 activates the proliferation and survival pathways of cancer cells. For cancer cells, PD-L1 has a pro-tumorigenic effect. Considering all this information, cancer therapy targeting PD-1/PD-L1 can be considered as a promising treatment alternative for hormone-resistant prostate cancer patients. The aim of the study was to determine the changes in mRNA level in PD-L1 expression by administering dihydrotestosterone and enzalutamide separately or together to prostate cancer cell lines LNCaP and LNCaP AR++ cell lines. In the thesis project, it was aimed to investigate how PD-L1 behaves in these conditions by androgen stimulation or receptor blocking with enzalutamide in LNCaP and LNCaP-AR+ cell lines.

Methods: Cell viability was analyzed by flow cytometry with Annexin V/propidium iodide double staining. The expression of PD-L1 expression was assessed by RT-qPCR.

Results: It states that it has no effect on PD-L1 expression levels. According to the results of the study, it was shown that hormonal stimulation of the androgen pathway with DHT had no effect on PD-L1 expression in both cell lines. In our study, PD-L1 expression was significantly decreased in LNCap cells by suppressing the AR pathway with Enzulatamide; it was determined that it did not make a difference in LNCap-AR+ cells.

Conclusion: More studies are needed to reveal the potential for PD-L1/PD-1 targeted therapy for prostate cancer.

Keywords: Androgens, gene expression, PD-L1, prostate cancer.

PP-2

Investigation of the relationship between mitochondrial DNA mutation m.1555A>G and aminoglycoside ototoxicity in cystic fibrosis patients

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Objective: Cystic fibrosis (CF) is an autosomal recessive genetic disease seen in Caucasians and caused by mutations in the CFTR gene. Aminoglycosides used for treatment in CF patients cause hearing loss as a side effect. It is known that the m.1555A>G mutation in the MTRNR1 gene in mitochondrial DNA causes aminoglycoside-related hearing loss. For this reason, screening patients for mutations before administering aminoglycoside treatment is of great importance in CF patients. Our aim in this study is to investigate the frequency of the most common m.1555A>G mutation associated with aminoglycoside ototoxicity in MTRNR1 gene in CF patients.

Methods: 119 retrospectively selected patients with CF, with or without a history of aminoglycoside use, were included in our study. Patients were classified according to clinical features such as otorhinolaryngologic findings, audiologic findings, and aminoglycoside use. The m.1555A>G mutation was analyzed using the PCR-RFLP method. The 248 bp PCR products containing the mutation site were incubated with BsmAI restriction enzyme at 55 °C for 16 h.

Results: Aminoglycoside use was found in 97 of 119 patients, it was not found in 22 patients. Hearing loss was detected in 13 patients using aminoglycosides. High-frequency hearing loss (>8kHz) was observed in 11 patients. The hearing test of 106 patients was found to be normal. 119 patients were screened for the m.1555A>G mutation and no mutation was detected.

Conclusion: Despite aminoglycoside ototoxicity, MTRNR1 gene mutation screening is not routinely performed in our country. According to our research results, which we will continue by increasing the number of patients, mutation screening in CF patients before using aminoglycosides is planned to be included in patient diagnosis and monitoring guidelines. By evaluating mutation frequencies and hearing loss parameters together, a personalized treatment approach will be developed for CF patients.

Keywords: Aminoglycoside, *CFTR* gene, cystic fibrosis, *MTRNR1,* ototoxicity.

PP-3

Evaluation of kisspeptin and neurokinin receptor gene expressions in human exfoliated deciduous teeth pulp derived mesenchymal stem cells

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Objective: The aim of this study is to determine the expression levels of kisspeptin, neurokinin genes and receptors, which have a key role in puberty, in mesenchymal stem cells derived from deciduous teeth.

Methods: The characterization of mesenchymal stem cells cultured from decidous tooth whose exfoliation time has come was performed by flow cytometry. Expression levels of kisspeptin and neurokinin genes and receptors were determined by Real Time PCR in the 3rd, 4th and 5th passages of the cells that were confirmed to be mesenchymal stem cells.

Results: In our study, it was determined that gene expression levels of kisspeptin, neurokinin genes and their receptors increased with the number of passages in deciduous tooth derived mesenchymal stem cells.

Conclusion: Our results suggest that deciduous tooth derived mesenchymal stem cells may be an alternative source in the treatment of reproductive system diseases such as hypogonadism, since they express kisspeptin, neurokinin genes and their receptors which play a key role in puberty.

Keywords: Human exfoliated deciduous teeth stem cells, kisspeptin1, kisspeptin1 receptor, neurokinin1, neurokinin1 receptor.

PP-4

Investigation of the relationship between kisspeptin receptor gene expressions of mesenchymal stem cells obtained from rat bone marrow and the passages number

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Objective: Peptides which are fragments of a precursor polypeptide of 145 amino acids encoded by Kiss-1 gene (1q32) and it's receptor their effects via GPR54 receptor, constitute the family of neuropeptides termed as kisspeptins. Kisspeptin and its neurons are key factors in the release of gonadotropin-releasing hormone (GnRH), which plays a fundamental role in puberty. In this study, it was aimed to investigate whether bone marrow-derived mesenchymal stem cells could support the treatment of pubertal disorders.

Methods: In our study, firstly, the developmental stage of mesenchymal stem cells obtained from rat bone marrow was followed in the cell culture medium and the cells obtained until the 6th passage were passaged. In the next step, stem cell obtained by using CD90, CD45, CD29 antibodies in flow cytometry was determined to be mesenchymal stem cell. Finally, cDNA was obtained from RNA and Kiss-1 receptor gene expression was determined.

Results: It was determined that the more number of passages increased, the more gene expression increased for the kisspeptin receptor gene.

Conclusion: When examining gene expression of kisspeptin receptor gene, which plays an important role in puberty, it is necessary to pay attention to the number of passages.

Keywords: Gene expression, kisspeptin receptor gene, mesenchymal stem cell.

PP-5

Association of SRP9 gene expression with perineural invasion in breast cancer

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Objective: Breast cancer is the most common type of cancer among women worldwide and also the leading cause of death. It is known that not only mutations occurring in the cellular genome are effective in the formation of cancer, but also disruptions in post-transcriptional and translational mechanisms that regulate gene expression. Signal recognition particle (SRP) promotes co-translational translocation of the secretory or membrane proteins through or into the endoplasmic reticulum membrane besides, it has elongation arrest function. SRP9 is one of the six protein subunits of SRP and functions in elongation arrest activity by forming a heterodimeric structure with SRP14. It prevents the false modification and misfolding of proteins therefore, plays a critical role in the regulation of post-transcriptional and translational gene expression process. So, the aim of this study is to to investigate the SRP9 gene expression in normal and tumor tissues of patients with breast cancer and determine its prognostic significance.

Methods: The expression levels of SRP9 gene (mRNA) in tumor and corresponding adjacent normal tissue samples, obtained from 49 breast cancer patients, were analyzed by ''Quantitative Real Time-PCR'' and the comparison between the changes in SRP9 gene expression levels with clinopathological parameters were statistically analyzed by 'chi-square test' to investigate any possible relations.

Results: Though, there was not any significant difference in SRP9 gene expression between tumor and normal tissues (p=0.762), we found a significant relationship between the increase of SRP9 gene expression levels and perineural invasion (p=0.041*).

Conclusion: SRP9 dysfunction may play an important role in the development of metastasis and resistance to treatment of breast cancer cells by regulating the expression of secretory or membrane proteins; having or not any gene mutaions.

Keywords: Breast cancer, gene expression, perineural invasion, signal recognition particle 9.

PP-6

Association of single nucleotide polymorphisms in Monoamine Oxidase A and SLC6A4 genes with obsessive-compulsive disorder

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Objective: Obsessive-compulsive disorder (OCD) is a psychiatric condition characterized by uncontrollable, reoccurring thoughts (obsessions) and repetitive behaviors (compulsions). Serotonin is a chemical neurotransmitter commonly found in the human brain. Serotonin levels are controlled by intraneuronal monoamine oxidases (MAO). Monoamine oxidase A (MAO-A) regulates monoamine levels in the brain by metabolizing serotonin, noradrenaline, and dopamine. MAO-A enzyme has been associated with various other psychiatric disorders, including OCD. Solute carrier family 6 member 4 (SLC6A4) gene encodes the serotonin transporter protein and is a candidate gene for the development of OCD.

Methods: Single nucleotide polymorphism (SNP) analyses of rs909525 in MAO-A and rs16965628 in SLC6A4, which are among the candidate OCD-associated genes of serotonergic system genetics, were performed using real-time PCR. The study groups consisted of 95 patients diagnosed with OCD and 70 healthy volunteers. In the patient group, the severity of the disease was determined by the Yale-Brown Obsessive Compulsive Scale administered by an expert psychiatrist.

Results: The mean age of the patients was 32.92±10.01 with 56 females and 39 males. The control group consisted of 35 female and 35 male volunteers with a mean age of 31.73±9.08 years. A significant difference was found between the genotype distributions of rs909525 (C/T) in MAO-A in OCD patients and healthy controls ($p<0.001$). Likewise, a significant difference was obtained between the genotype distributions of rs16965628 (G/C) in SLC6A4 in the study groups ($p<0.001$).

Conclusion: Comparison of the polymorphic genotype distributions of MAO-A and SLC6A4 in the patient and control groups yielded significant data and showed that they may be associated with OCD. It is thought that this study will be helpful in understanding the effects of serotonergic system genetic mechanisms on the development of OCD.

Keywords: Monoamine oxidase A, obsessive-compulsive disorder, serotonin, single nucleotide polymorphism, SLC6A4.

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PP-7

The role of miR-770-5p on the KDM5B/HER2 axis in trastuzumab-resistant breast cancer cells

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Objective: Breast cancer is the most common malignancy in women and despite the development of various treatment strategies, it is still the second most common cause of cancer-related death in women after lung cancer. Overexpression of the ERBB2 gene occurs in the HER2 positive breast cancer subtype (HER2+), and HER2-targeted therapies have been used successfully in the treatment of HER2+ breast cancer. Although trastuzumab is used as a treatment option in HER2+ breast cancer, resistance to this drug is inevitable due to molecular interaction mechanisms in tumor cells. microRNAs (miRNAs) are short non-coding RNAs that regulate the expression of specific target genes and the role of miRNA expression modulation in drug resistance is known. In our previous data, miR-770-5p was the only miRNA that increased in response to trastuzumab and lapatinib in HER2+ breast cancer cell lines. In this study, we aimed to investigate the molecular characterization of the KDM5B-mediated effect of miR-770-5p on drug efficacy in trastuzumab-resistant HER2+ breast cancer cells.

Methods: A trastuzumab resistance model was generated by exposing the cells to increasing concentrations of trastuzumab and final clones selected with 10 mg/mL trastuzumab for this study. To investigate the effect of miR-770-5p on cancer cell proliferation in trastuzumab resistance state, trastuzumab sensivite and resistant cells were analyzed by Incucyte Live-Cell Analysis system. To understand the underlying role of miR-770-5p on KDM5B/HER2 axis in trastuzumab resistance, related protein expressions were analyzed by western blot.

Results: At first, real time cell proliferation analysis in trastuzumab sensitive and resistant BT-474 cells showed that trastuzumab sensitivity was restored in resistant cells in the presence of miR-770-5p. Additionally, the EMT process, which is a mechanism involved in drug resistance was examined and Vimentin, KDM5B and HER2 protein levels were significantly decreased upon miR-770-5p overexpression.

Conclusion: It was thought that miR-770-5p could sensitize cells to trastuzumab by targeting KDM5B, which is increased in trastuzumab resistance.

Keywords: HER2+ breast cancer, miR-770-5p, trastuzumab resistance.

Acknowledgment: This work was supported by Ankara University Scientific Research Projects Coordination Unit (DOSAP, Project number: TDS-2022-2401).

PP-8

Caspase-1 activity of macrophages promotes immune-cell infiltration via suppressing T helper cell function in oral squamous cell carcinoma

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Objective: A multi-step process initiated by various pathogens elicits a pro-inflammatory or immunosuppressive environment of oral squamous cell carcinoma (OSCC). One of the mechanisms that mediate the host response to microbial infection is the NOD-, LRR-, and pyrin domain-containing protein 3 (NLRP3) inflammasome complex. The NLRP3 carries out the proteolytic cleavage of inactive procaspase-1. However, the effect of NLRP3 and Caspase-1 on inflammatory cell traffic and immune-cell infiltration in OSCC is poorly understood. This study investigated the role of Caspase-1 activity of peripheral macrophages on population frequencies of macrophage, T helper cells, NLRP3 expressing tumor-associated macrophage (TAM) and neutrophile (TAN) infiltration, and OSCC tumor aggressiveness.

Methods: Eight OSCC patients and 8 controls were enrolled in the study. The caspase-1 activity of macrophages (CD14+, CD11b+) was determined using a Caspase-1 (active) Staining Kit (ab219935, Abcam, USA). The population of macrophages and T helper (CD3+, CD4+) cells was determined by flow cytometry. The NLRP3 expression of the TAM and TAN cells was observed by immunohistochemistry. An independent sample T-test analyzed the significance of the Caspase-1 activity of macrophages and the immune cell frequencies. A Pearson's correlation analysis determined the correlation between the Caspase-1 activity of peripheral cells and the TAM and TAN cells.

Results: The macrophage caspase-1 activity was increased compared to control ($p=0.002$). While the number of peripheral macrophages ($p<0.001$) was increased, CD4(+) T cells were decreased in OSCC patients. There was a positive correlation between the increased caspase-1 activity of peripheral macrophages and the NLRP3-expressed TAM and TAN cells in the tumor specimens of OSCC patients ($p<0.001$).

Conclusion: Macrophage-derived caspase-1 signaling may suppress the production of CD4(+) T cells and evoke NLRP3-expressed TAM and TAN infiltration to suppress anti-tumoral immune response and promote tumor aggressiveness.

Keywords: CASPASE-1, macrophages, oral squamous cell carcinoma.

Acknowledgment: This research was funded by Bursa Uludag University Scientific Research Projects Coordination Unit (TOA-2021-569).

PP-9

The combined treatment of tamoxifen and epibrassinolide elevated endoplasmic reticulum stress and apoptosis in breast cancer cells

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Objective: Epibrassinolide (EBR), a member of the brassinosteroids, is a plant growth regulator with a similar structure to the mammalian steroid hormones. Our recent data showed that EBR exerts anti-cancer effects in cancer cell lines or xenograft colon cancer mouse models without affecting healthy cells or mice. Endoplasmic reticulum (ER) stress is caused by the accumulation of misfolded/unfolded proteins in the ER lumen, which can form different responses based on the magnitude and duration of the stress. Tamoxifen (TAM) is a commonly used chemotherapeutic drug for the therapy of estrogen receptor-positive breast tumors. Resistance occurs in cancer cells due to both tumor heterogeneity and long-term use of TAM. The aim of the study was to demonstrate that ER stress and apoptosis can be elevated by TAM in the presence of EBR, especially in MDA-MB-231 triple negative and MCF-7 breast cancer cells.

Methods: MTT assay was performed for the detection of cell viability. Colony formation assay and hanging drop were used to show the capability of colony formation in 2D and 3D. Fluorescence microscopy was used to detect DiOC6, PI and DAPI positive breast cancer cells. Immunoblotting was performed to detect protein expression profiles.

Results: Combined treatment of EBR and TAM further induced cell viability loss compared to alone EBR or TAM exposure to MCF-7 and MDA-MB-231. Colony formation assay and hanging drop method also proved the enhanced inhibitory effect of TAM with the combination of EBR. We also determined that DNA condensation and cell death rate also were augmented with the combined treatment. Finally, the expression profiles of ER stress and apoptosis biomarkers were found to increase with both drug exposure.

Conclusion: Our results indicated that EBR might be an effective agent which elevates TAM response, especially in drug-resistant breast cancer cells.

Keywords: Drug resistance, endoplasmic reticulum stress, epibrassinolide, tamoxifen.

PP-10

Tamoxifen resistance was prevented by epibrassinolide via endoplasmic reticulum stress in MCF-7 and MDA-MB-231 breast cancer cells

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Objective: Hormonal therapy is one of the suggested therapeutic strategies to slow down or cease the growth of hormone-related breast cancer. Tamoxifen (TAM) is a therapeutic agent preferred for hormone-positive breast cancer to impede estrogen receptor activation. TAM resistance may occur because of several reasons, including an increase in drug efflux, anti-apoptotic protein expressions, drug metabolism, etc. Epibrassinolide (EBR) is described as an anti-cancer agent via endoplasmic reticulum (ER) stress induction by our group. This study aims to show that ER stress contributes to overcome drug resistance in breast cancer.

Methods: MTT cell viability assay was performed to show the effect of TAM and/or EBR treatment in the presence of tauroursodeoxycholic acid (TUDCA). Trypan blue dye exclusion assay was used to detect the survival rates after drug treatments. Fluorescence cell sorting (FACS) was utilized to show the subG1 populations and finally immunoblotting was performed to show the expression profiles of proteins having a role in drug resistance and ER stress.

Results: EBR treatment in the presence of TAM further induced cell viability loss and apoptosis in breast cancer cells. This induction was prevented by TUDCA, a well-known ER stress inhibitor. EBR and TAM treatment increased the subG1 population ratio in MCF-7 and MDA-MB-231 cells. The exposure of cells to the combined treatment elevated ER stress levels which can be prevented by TUDCA, and also decreased the expression of drug resistance proteins MRP1 and MDR1 in breast cancer cells.

Conclusion: We proposed that the combination of TAM with EBR induces ER stress to increase TAM sensitivity and decrease drug resistance. These findings might shed light on research and the mechanism of TAM with EBR via ER stress and the application of combination therapy in breast cancer.

Keywords: Drug resistance, endoplasmic reticulum stress, epibrassinolide, tamoxifen.

PP-11

Tamoxifen in combination with epibrassinolide induced autophagy in breast cancer cells which was prevented with 3-methyladenine

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Objective: The heterogenic nature of breast cancer cells at the molecular level can influence treatment options, usually remains unsatisfactory especially in drug-resistant tumors. Tamoxifen (TAM), a selective estrogen receptor modulator, is primarily used for estrogen receptor-positive breast cancer patients. The long-term usage of TAM results in the development of resistance. Endoplasmic reticulum (ER) stress, a type of cellular stress response, is one of the proposed mechanisms to combat against drug resistance. The level of ER stress is strongly correlated with cell fate through either apoptosis or autophagy. Autophagy, is an evolutionary conserved process, takes role in the degradation also recycling of the damaged organelles, misfolded proteins through lysosomes in response to stress conditions. Epibrassinolide (EBR) is a member of brassinosteoids, plant growth regulatory family. EBR has been suggested as an apoptotic or autophagic inducer depending on cell type and stress level. We aimed to investigate the autophagic effect of EBR alone or in combination with TAM through ER stress in distinct type of breast cancer cells, MCF7 and MDA-MB-231.

Methods: To evaluate the autophagic effect of drugs we performed MTT assay, monodansylcadaverine (MDC) staning assay, immunoblotting techniques and we also used 3-Methyladenine (3-MA) as an autophagy inhibitor in all steps of experiments.

Results: We found that the combination of EBR and TAM resulted in decreased cell viability through the autophagic flux. 3-MA inhibited autophagic induction induced by EBR and TAM combination therapy which was detected with decreased number of MDC-stained in breast cancer cells, as well as the increase expression profiles of autophagic markers, including ATGs, p62 and Beclin-1.

Conclusion: EBR has crucial effect of onto autophagic flux. It can take its place as a drug of choice in breast cancer treatments in the future, as it helps to break the resistance of tamoxifen.

Keywords: Autophagy, drug resistance, epibrassinolide, tamoxifen.

PP-12

IKBKE involvement in ulcerative colitis and colitis associated carcinoma

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Objective: Colorectal cancer (CRC) is one of the most common cancer types worldwide with one of the highest cancer-related mortality rates. Ulcerative colitis (UC) not only increases the rate of CRC development 4-5 times later in life, but also decreases the life quality of patients. IKBKE (Inhibitor of nuclear B kappa kinaz subunit epsilon) is a component of interferon signaling, which is disrupted in UC and CRC patients. IKBKE is involved in pro-inflammatory signaling in various pathologies including cancer, thus it is thought to be a target for anti-inflammatory therapies. However, the role of IKBKE in UC and colitis-associated colorectal cancer (CAC) has not been fully investigated.

Methods: IKBKE was pharmacologically inhibited in acute UC and CAC mouse models. The effect of this pharmacological inhibition on intestinal damage was investigated clinically, histopathologically, and biochemically.

Results: Pharmacological inhibition of IKBKE led to worse clinical and histopathological outcomes in both UC and CAC mouse models at the acute phase. This outcome was observed to be more significant in acute CAC mouse models compared to acute UC mouse models. Moreover, a significant change in the expression level of certain pro-inflammatory and anti-inflammatory cytokines in the colon microenvironment was observed upon pharmacological inhibition of IKBKE, which can explain the increased inflammation upon inhibition.

Conclusion: Our findings suggest that IKBKE does not act as a pro-inflammatory gene in UC and CAC at the acute phase *in vivo*. Therefore, strategies aiming to inhibit IKBKE to treat inflammatory conditions may instead cause exacerbation of the inflammation. The molecular pathways leading to this phenomenon should be further investigated.

Keywords: Colorectal cancer, IKBKE, mouse model.

PP-13

Examining the effect of mitochondrial-derived peptide (MOTS-c) on viability in breast cancer cells

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Objective: Mitochondrial ORF of The 12S rRNA Type-c (MOTS-c) is one of the mitochondrial derived peptides that has 16 amino acids. It was first discovered in 2015. Its major target tissue is the skeletal muscle, where it increases glucose uptake and insulin sensitivity. Research highlights that in cells exposed to metabolic stress, MOTS-c triggers the AMPK signaling and is rapidly transferred to the nucleus, where it induces expression of antioxidant response genes like NFR2. Cancer cells are known to have metabolic flexibility to survive. There are limited studies showing the relationship between MOTS-c and cancer cell survival. Moreover, there is evidence that AMPK and NFR2, which are involved in the MOTS-c mechanism of action, can have an oncogenic or tumor suppressive effect during the tumorigenesis process. In this context, our study aimed to contribute to the investigation of MOTS-c on cell survival in different types of breast cancer in vitro.

Methods: MDA-MB-231 and MCF-7 cell lines were treated with increasing concentration of MOTS-c for 48h, 72h and assessed with cell viability assays. p-values were calculated using one way ANOVA.

Results: MOTS-c does not seem trigger cell viability in MDA-MB- 231 and MCF-7 breast cancer cells. But contrary to cell viability assays, flow cytometric measurement results showed that MOTS-c induced cell death in estrogen receptor positive MCF-7 cells treated for 72 hours. ($p<0.000$)

Conclusion: The results of our study show that the effect of MOTS-c on the different breast cancer cell viability. Our data suggest that MOTS-c's effect may differ depending on the biological subtype of the tumor. In addition, it may have a proliferative effect on resistant cells. Cancer metabolism is a complicated subject, with cells in different metabolic states within the same tumor. More detailed research is needed to elucidate the effect of MOTS-c has on metabolic pathways in cancer cells.

Keywords: Breast cancer, cancer metabolism, mitochondrial derived peptides, MOTS-c.

PP-14

Diagnostic significance and prognostic role of the GTSE1 gene in cancer

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Objective: Our study aimed to elucidate the prognostic value of the GTSE1 gene, which regulates genes in the G2 and S phases of the cell cycle and plays a role in carcinogenesis, including its mechanisms in cancer development.

Methods: We compared GTSE1 expression levels in tumor and normal tissues across various TCGA cancers. Survival analyses were performed using TIMER2.0 and GEPIA2, and GTSE1 genetic alterations were studied with cBioPortal. Validation was done for cancers showing significant results using independent patient datasets from GEO, including correlation with cancer progression and survival. LinkedOmics identified pathways associated with genes positively correlated with GTSE1 in cancer RNA-seq data, and String was used to identify interacting proteins with GTSE1.

Results: When examining 33 cancer types in the TCGA database, GTSE1 showed statistically significant overexpression in 24 types compared to normal tissues and exhibited substantial changes in 17 types at various cancer stages. GTSE1 was found to have a significant negative impact on prognosis in 10 cancer types and was associated with poor disease-free survival in 10 different cancers. CBioPortal found that GTSE1 amplification is high in cancers, with the highest alteration rate at 5.26% in lung cancer. Our analyses obtained significant results for glioma, liver, lung, pancreas, and kidney cancers, which are public health concerns regarding incidence and mortality. We also analyzed independent patient datasets for these cancers, confirming the association between high GTSE1 expression and poor prognosis. LinkedOmics analyses revealed that GTSE1 triggers carcinogenesis in these cancers through cell cycle pathways.

Conclusion: In our study, it has been observed that GTSE1 is highly expressed in many cancer types, affects negatively patients' prognosis. Our findings also suggest that the increase in GTSE1 expression levels may have potential as a novel therapeutic and prognostic target.

Keywords: Bioinformatic analyses, cancer, G2 and S-phase expressed gene, prognostic biomarker.

PP-15

Effect of prunus laurocerasus in n chemotherapy-induced secondary infertility and DNA methylation changes

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Objective: Chemotherapy-induced toxicity and the effectiveness of antioxidant agents in protecting against reproductive damage, in an important research area. During spermatogenesis, DNA methylation plays a pivotal role in mammalian sperm maturation. This study aims to investigate how the anticancer agent Doxorubicin (DXR) treatment affects global DNA methylation in the testes and assess the protective effects of *Prunus laurocerasus* extract (PE) from an epigenetic perspective.

Methods: Sprague-Dawley rats were randomly divided into four groups. As the control group, Group 1 received no treatment. Group 2 and Group 3 were administered PE at doses of 500 mg/kg/day and 1000 mg/kg/day, respectively, for 14 days. Group 4, without any additional treatment until the 13th day. On the 13th day, intraperitoneal DXR was administered to Groups 2, 3, and 4. On the 15th day, the animals were euthanized under anesthesia, and tissue samples were collected**.** To evaluate spermatogenesis, sperm count was conducted from the caudal epididymis. Global DNA methylation analysis and testicular Dnmt1 and Dnmt3a levels were evaluated using ELISA-based methods.

Results: DXR significantly reduced epididymal sperm count, leading to a substantial decrease in reproductive toxicity. DXR toxicity markedly decreased global DNA methylation levels in the testes compared to the control group ($p<0.05$). Regardless of sperm parameters, methylation levels in Group 3 resembled those in the control group ($p>0.05$). In contrast, in Group 4, methylation levels dropped to levels similar to DXR alone ($p>0.05$). While there were no correlation between methylation status and Dnmt1 levels ($p>0.05$) a remarkable correlation was observed between Dnmt3a levels and methylation status ($r=0.527$, $p<0.05$).

Conclusion: PE plays a protective role in chemotherapy-induced testicular damage. The DNA methylation process, which likely involves not only Dnmts but also other proteins, appears to be crucial in both causing damage and protection. Therefore, the selection of appropriate dosages and a comprehensive investigation of gene interactions through epigenetic approaches are of paramount importance in fertility therapies.

Keywords: DNA methylation, Dnmt1 and Dnmt3a levels, infertility.

PP-16

Three-dimensional modeling of pyrin protein using homology modeling method

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Objective: Pyrin protein, encoded by the MEFV gene, is a biomolecule playing a role in the pathology of autoinflammatory diseases. Particularly, the M694V variant associated with Familial Mediterranean Fever (FMF) is directly linked to the disease's poor prognosis. This study aims to model the three-dimensional structures of Pyrin protein and the M694V pathogenic variant using bioinformatics tools and evaluate their physicochemical properties. It is known that the M694V variant is associated with severe symptoms, as well as serious issues such as kidney pathologies and acute kidney failure. This research aims to provide fundamental information to understand molecular-level changes in the protein, elucidate the mechanism of the disease, and contribute to the development of treatment approaches for autoinflammatory diseases with a genetic basis.

Methods: DNA sequences for Pyrin protein and the M694V variant, obtained from the NCBI database, were uploaded to the Swiss Model database for three-dimensional homology modeling. Subsequently, the three-dimensional structure of Pyrin protein and the M694V pathogenic variant was modeled. Physicochemical properties were calculated using the ProtParam database. Finally, the obtained three-dimensional structures were visualized using the Chimera program.

Results: Homology modeling using the Swiss Model database successfully generated three-dimensional structures for Pyrin protein and the M694V pathogenic variant. The ProtParam database determined physicochemical properties, including molecular weight, isoelectric point, and amino acid composition, while the Chimera program facilitated the visualization of the created three-dimensional structures. Alongside these data, the study examined the relationship between structural similarities and differences of the variant and kidney anomalies observed in Familial Mediterranean Fever (FMF) pathology.

Conclusion: This study contributes to our understanding of the complex relationship between three-dimensional structure, protein folding, and the physicochemical properties of molecules, providing a crucial foundation for shaping future-oriented targeted drug design and treatments to improve poor prognoses. In this context, the acquired information is expected to shed light on future research focused on comprehending biological processes and developing effective therapeutic approaches targeting specific molecular goals.

Keywords: Bioinformatics, computational biology, homology modeling, pyrin protein.

PP-17

Investigation of therapeutic targets of orlistat in PC3 prostate cancer and PNT1A prostate epithelial cells lacking LKB1 or AMPK expression

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Objective: In this study, the objective is to partially define the LKB-independent molecular targets of orlistat, a lipase inhibitor, by using CRISPR to delete LKB expression in LKB1-deficient PC3 prostate cancer and PNT1A prostate epithelial cells, and to elucidate the roles of key molecules in lipid metabolism that interact with LKB1/AMPK in the cellular response mechanism.

Methods: In the study, CRISPR/Cas9 gene editing technology was utilized to generate AMPK or LKB1 deficient PC3 prostate cancer and PNT1A prostate epithelial cells. MTT and colony formation assays were used to determine the optimal orlistat concentration for PC3 and PNT1A cells. Proliferation assay was performed to observe the time dependent effect of Orlistat on prostate cancer and prostate epithelial cells. To define the cellular effects of Orlistat, fluorescent staining techniques were applied.

Results: In the study, LKB1 or AMPKα1 deficient PC3 prostate cancer and PNT1A prostate epithelial cell lines were generated by CRISPR/Cas9 gene editing technology. The optimal dose of Orlistat was determined as 30 μM by MTT cell viability and colony formation assays. It was found that, Orlistat, shows an anti-proliferative effect and triggers cell death on prostate cancer cells for both dose and time dependent manner. Orlistat causes reduced membrane potential and increased reactive oxygen species (ROS) correlated with cell death on especially AMPKα1 or LKB1 deficient prostate cancer cells.

Conclusion: This study explores the potential of orlistat, an anti-obesity drug, as a promising combinatorial treatment option for prostate cancer. Through the manipulation of LKB and AMPK genes using CRISPR/Cas9, it was demonstrated that orlistat exhibits antiproliferative and anticancer effects in prostate cancer cells through the inhibition of cell proliferation, induction of cell death, and the generation of cellular stress through changes in membrane potential and increased reactive oxygen species. Orlistat, when used in conjunction with molecular biology techniques, could serve as a valuable addition to the arsenal of treatments for prostate cancer, addressing a significant public health concern.

Keywords: Cell death, cell metabolism, clustered regularly interspaced short palindromic repeats, prostate cancer.

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PP-18

Investigation of expression levels of genes in miRNA formation pathway in thyroid cancers

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Objective: Thyroid cancer is the most common endocrine cancer and its incidence is increasing worldwide. Therefore, it is important to reveal the specific molecular genetic changes underlying it. For this purpose, in our study, we thought that *DICER1, DROSHA, DGCR8, TARBP2, AGO1* genes, which are genes in the formation pathway of microRNAs (miRNAs), which play a role in the regulation of gene expression in the cell and play a role in all developmental, differentiation and physiological functions, may affect the formation and development of thyroid tumor tissues. For this purpose, it is planned to investigate the expression levels.

Methods: In our study, 42 thyroid cancer (17 papillary thyroid cancer, 18 follicular thyroid cancer, 7 medullary thyroid cancer) individual samples and 42 healthy control samples were taken from patient tissue samples diagnosed with thyroid cancer, and total RNA isolation, followed by cDNA synthesis was performed. Expression analyzes of the identified genes were performed using the comparative CT method in Real Time PCR.

Results: Expression levels were statistically evaluated using the Shapiro-Wilk statistic and the Mann-Whitney U test. As a result of the evaluation, no statistically significant difference was observed in the expression levels of *DICER1, DROSHA, DGCR8, TARBP2, AGO1* genes in patient samples from three types of thyroid cancer compared to controls.

Conclusion: It has been reported in the studies that miRNAs and the disruptions that may occur in their formation pathways, by affecting many pathways, pave the way for cancer and affect its onset and course. It is suggested that miRNA expression data on thyroid cancer may contribute to loss of differentiation, progression of tumorigenesis, and identification of clinically relevant subclasses in papillary thyroid cancer. Since irregular expressions of miRNAs were observed in all types of thyroid cancers in our study, it is necessary to study with larger and more homogeneous patient groups in all subtypes according to clinical data.

Keywords: *DICER1, DROSHA*, expression, microRNA, thyroid cancer.

Acknowledgment: This study was supported by Mersin University Scientific Research Projects Unit as project number 2019-3-TP2-3790.

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PP-19

Investigation of the relation between DHRS2 gene and advanced glycation end products in breast cancer

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Objective: Advanced glycation end products (AGEs) are formed by glucose-mediated modification of lipids, proteins, and nucleic acids in the cell. Their accumulation in the cell can activate many signaling pathways, such as oxidative stress pathway and cytokine release. DHRS2 is an enzyme with dehydrogenase/reductase activity and has been shown to protect cells from oxidative stress. In this study, we examined how the amount of AGEs in cells changed in the presence of increased and suppressed expression of DHRS2.

Methods: DHRS2 expression was manipulated separately in MCF10A, MCF7, T47D, and MDA MB 231 cell lines by gene-specific siRNA (suppression) and lentiviral vectors (enhancement) via liposomal agent-mediated transfection. The highest DHRS2 suppression and enhancement rates in the cells were determined by qRT-PCR, and the groups with the best rates were used for further analysis. AGE determination was performed by the ELISA method. Samples taken from cell media after the transfections were used and it measured colorimetrically.

Results: Changes in DHRS2 expression were found to alter the amount of AGEs in cells dramatically. A more pronounced increase was observed in MCF7 cells with suppressed gene expression compared to other cell groups. In the non-carcinoma MCF10A cell line, the amount of AGEs changed minimally in both conditions. However, when DHRS2 expression in the cells was increased, AGE amounts in the cells decreased approximately 1.5-fold.

Conclusion: DHRS2 is a protein with enzymatic activity and has the ability to reduce metabolites and reactive oxygen species in the cell. Based on these properties, this study showed that advanced glycation end products are affected by DHRS2 expression. The increased DHRS2 expression in the cell supports its ability to protect cells from oxidative stress. We suggest that DHRS2 may be an important marker for metabolic stresses in further studies.

Keywords: AGEs, breast cancer, DHRS2, oxidative stress.

PP-20

Virtual crossmatching and epitope mapping for better donor match in kidney transplantation

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Objective: Human Leukocyte Antigen (HLA) disparity between donors and recipients is the primary driver of Donor Specific Antibodies (DSA) formation and graft rejection after transplantation. The enormous amount of identified HLA alleles reflects the highly polymorphic character of the HLA system. Nowadays, 35,823 different HLA alleles have been described, which illustrates that these genes are highly polymorphic, related to their primary function in the immune response. We aimed to predict the DSA by finding the HLA antigen mismatches, searching the eplets of antigens that bind to the recipient's anti-HLA antibodies, calculating the number of shared eplets between the mismatched donor HLA antigens and the recipient's pre-transplantation anti-HLA antibody-bound antigens.

Methods: We have included a total of 8 kidney transplantation recipient-donor paired data already resulting in Istanbul Faculty of Medicine, Tissue Typing Laboratory as a retrospective study database. We used two Algorithms; Algorithm 1: To find mismatched antigens, we compared HLA Typing results and searched the corresponding eplets with Luminex SAB for the recipient's pre-transplant anti-HLA antibodies. To find the maximum number of shares, we calculated the shared eplets between the donor's mismatched HLA antigens and the recipient's pre-transplant anti-HLA antibodies. We then listed Antibodies containing Common Shared Eplets between Donor Antigens and Patient Pre-Transplant Antibodies (PreTxAb) as the most likely DSAs. Algorithm 2: To find mismatched antigens, we compared HLA Typing results and searched the corresponding eplets with Luminex SAB for the recipient's pre-transplant anti-HLA antibodies. To find the highest number of shares, we calculated the mismatched eplets between the donor's mismatched HLA antigens and the recipient's mismatched HLA antigens. We then listed Antibodies containing Incompatible Epletes between Donor Antigens and Patient Antigens as the most likely DSAs.

Results: Predicted Donor Specific Antibodies found within Post Transplant Antibody Detection Test Results for HLA Class I; Pair 1- 25, 10, 9 of 10, 8 of 10, Pair 2- 3, 7, 7 of 7, 7 of 7, Pair 3- 3, 26, 24 of 26, 24 of 26, Pair 4- 3, 2, 2 of 2, 1 of 2, Pair 5- 12, 12, 12 of 12, 12 of 12, Pair 6- 4, 4, 4 of 4, 4 of 4, Pair 7- 17, 14, 14 of 14, 9 of 14, Pair 8- 7, 18, 17 of 18, 17 of 18 (HLA-ABC) (Number of PreTxAb, Number of PostTxAb, Algorithm 1, Algorithm 2, respectively) and predicted Donor Specific Antibodies found within Post Transplant Antibody Detection Test Results forHLA Class II (HLA-DRB1 & HLA-DQB1); Pair 1- 19, 6, 6 of 6, 6 of 6, Pair 2- 6, 6, 6 of 6, 6 of 6, Pair 3- 0, 15, 14 of 15, 14 of 15, Pair 4- 3, 3, 3 of 3, 3 of 3, Pair 5- 19, 14, 13 of 14, 13 of 14, Pair 6- 6, 14,14 of 14, 14 of 14, Pair 7- 12, 9, 9 of 9, 9 of 9, Pair 8- 5, 9, 9 of 9, 9 of (Number of PreTxAb, Number of PostTxAb, Algorithm 1, Algorithm 2, respectively).

Conclusion: Various factors can determine the strength of these responses, whether measured as Mean Fluorescence Intensity (MFI) or the actual titer of the antibody. Antibody reactivity against a particular epitope can yield significantly different MFI values in a single antigen bead assay. Another factor that may affect antibody binding ability is the peptide and tissue-specific reactivity presented within the HLA molecule. This is why we did not choose the MFI value of the recipient's anti-HLA antibody as a criterion during analysis.

Keywords: Antibody prediction, donor specific antibody, epitope mapping, kidney transplantation.

PP-21

Effect of long non-coding RNA linc00968 on apoptosis in in-vitro Alzheimer's model

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Objective: Substantial evidence indicates that various Long Non-Coding RNAs (LncRNAs) are commonly involved in different neurodegenerative disorders. This study aimed to investigate the regulatory effect of LncRNA linc00968 on apoptosis in an in vitro model of Alzheimer's disease (AD).

Methods: Ab 25-35 fragment was used in SH-SY5Y cells to mimic AD neurotoxicity. Cell viability was determined by MTT analysis and the inhibition concentration (IC50) value of Ab 25-35 was calculated as 40 µM. Expression of linc00968 in cells treated and untreated (control) with 40 µM Ab 25-35 was examined by quantitative real-time polymerase chain reaction (qRT-PCR). Then, linc00968 was transfected with siRNA into SH-SY5Y cells with and without Ab 25-35 to determine its cellular role on apoptosis, and its expression was confirmed by qRT-PCR. To examine their effects on apoptotic pathways, BCL-2, BAX, CYC-S and internal control GAPDH gene expressions were analyzed by qRT-PCR. In order to evaluate post-transcriptional effects, Bcl-2, Bax, Cyc-s and Beta actin levels were analyzed by western blot method.

Results: Our results showed that LncRNA linc00968 deregulation is involved in apoptotic processes in neuroblastoma cells induced Alzheimer's neurotoxicity by Ab 25-35. Linc00968 silencing increased anti-apoptotic Bcl-2 expression, while decreased pro-apoptotic Bax and Cyc-s expressions and reversed Ab 25-35-induced neurotoxicity in SH-SY5Y cells.

Conclusion: LncRNA linc00968 is involved in the cellular functions and biological processes of neuroblastoma cells in the in vitro AD model, and the diagnostic value and therapeutic potential of Linc00989 are highlighted in our study.

Keywords: Amyloid beta, apoptosis, long non-coding RNA.

PP-22

Investigation of the photodynamic therapy efficacy of a novel protoporphine IX with organometallic character against lung cancer cells

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Objective: According to World Health Organization, the prevalence of lung cancer worldwide is 11.4% and the mortality rate is 18%. Although many methods are used in the treatment of lung cancer, alternative methods are still being investigated. One of these alternative and promising methods is photodynamic therapy (PDT). PDT changes the molecular systematics of the cell by generating reactive oxygen species (ROS) through interactions between light, photosensitizer and molecular oxygen. PDT is a invasive method and can be used alone or with other clinical treatments. An organic compound, Protoporphyrin IX (PpIX), forms aggregates at physiological pH yet, its light-sensitive property makes it attractive for PDT research. The fact that PpIX and the Schiff-based boron element we added to it can target the tumor area, show low dark toxicity, contribute to ROS production and can be easily detected in the tissue in which it is localized, has made this molecule the most researched PDT agent. The aim of this study is to investigate the PDT effectiveness of Schiff-based boron element PpIX, which has organometallic properties and has newly synthesized by our team, on the lung cancer cell line.

Methods: In this study, the synthesized PpIX molecule with organometallic properties was treated with A549 lung cancer cells at different concentrations and stimulated with white LED light. As a result of the PDT analysis, the effect of the molecule on cell viability as a result of stimulation with light was examined by MTT analysis. The observed effects were also confirmed under a fluorescence microscope using the acridine orange/ethidium bromide staining method. These effects were also compared with the viability observed in cells treated with the same molecule without light.

Results: It was shown that the molecule had an anti-proliferative effect on lung cancer cells as a result of PDT application and this effect was not observed in without light.

Conclusion: The results indicate that the new Protoporphin IX molecule, which has organometallic properties synthesized as a photosensitizer, may be a PDT agent with antiproliferative effects that can be used in lung cancer cells, and further analyzes are recommended in this direction.

Keywords: Lung cancer, photodynamic therapy, protoporphyrin IX.

PP-23

Investigation of the anti-cancer effects of mumio in cancer cell lines

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Objective: Mumio or Shilajit is a traditional drug that has been used for years. It has been narrated that it also was used by Alexander the Great. Recently, it has been marketed as the "elixir of life". Mumio is used to treat many diseases such as neuralgia, radiculitis, Alzheimer`s disease, diabetes, tuberculosis and asthma. Cancer is also one of the most common diseases. Therefore there are limited studies describing the effects of mumio on cancer. Thus, we aimed to evaluate the anti-cancer effects of mumio on cancer cells.

Methods: Mumio solution was prepared from commercially available pills. Pills were dissolved in medium and centrifuged. The supernatant was filtered and the stock solution (100 mg/ml) was diluted to obtain the various concentrations (0.5, 1, 2, 5, 10, 20 and 40 mg/ml). HEK (epithel), HUVEC (endothel), HEp2 (larynx cancer) and Saos2 (osteasarcoma) cells were treated with mumio for 48h. Cytotoxicity of mumio was analysed using XTT assay. Morphological analysis of the cells were also performed by light microscope.

Results: Cell viability was significantly changed by mumio compared to control cells in a dose-dependent. There were no significant differences in cells viability after 48h at 0.5, 1 and 2 mg/ml; but significant and dramatic reduce was noticed at 20 mg/ml in all cell lines ($p<0.05$). However, at 10 mg/ml, the viability of cancer cell lines decreased to 43% in average while the viability of normal cell lines decreased to 93% ($p<0.01$).

Conclusion: The data clearly indicate that mumio has a cytotoxic activity against cancer cell lines but at the same concentration (10 mg/ml), it caused a little damage to normal cells. Thus, we suggest more interest should be given to the investigation of mumio in cancer.

Keywords: Cancer, cytotoxicity, mumio, shilajit.

PP-24

Investigation of synthetic lethal interaction between ARID1A and EZH2 in acute lymphoblastic leukemia

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Objective: Genes encoding SWI/SNF chromatin remodeling complex proteins are frequently mutated in common human cancers. ARID1A, an important subunit of this complex, binds to DNA and targets SWI/SNF complexes to the chromatin location that needs to be remodeled. Genome-wide sequencing studies have revealed ARID1A mutations in various cancer types. In addition, studies in ARID1Amt cancers have led to the identification of some vulnerable genes. Targeting these vulnerable genes in ARID1Amt cancers can typically lead to synthetic lethality. EZH2, which represses histone methylation and transcription, is the catalytic subunit of Polycomb Repressive Complex 2 (PRC2). Defects in SWI/SNF function through mtARID1A facilitate Polycomb activity on chromatin, which may sensitize cells to EZH2 inhibition. This study aims to investigate the possible synthetic lethality effects of EZH2 inhibition using the Jurkat cell line harboring a frameshift mutation in ARID1A.

Methods: Inhibition of EZH2 in the Jurkat cell line was achieved with GSK2816126. WST-1 and Annexin V/7AAD assays were performed to measure cell viability and apoptosis and necrosis, respectively. ARID1A, EZH2, H3k27me3, total H3 and GAPDH protein levels were visualized by WB analysis.

Results: Inhibition of EZH2 by GSK2816126 treatment in Jurkat cells was detected by decreased protein levels of H3K27me3. EZH2 inhibition in Jurkat cells decreased cell viability and induced apoptosis in a time- and dose-dependent manner. 10 µM GSK2816126 treatment decreased cell viability by an average of 60% after 120 hours and induced apoptosis.

Conclusion: In *ARID1Amt* Jurkat cells, inhibition of EZH2 by GSK2816126 led to synthetic lethal effect via apoptosis. These results suggest that EZH2 may be a therapeutic target in ARID1A defective acute lymphoblastic leukemia.

Keywords: ARID1A, EZH2,jurkat,synthetic lethality.

PP-25

Pilot study of chimeric recombinant antibody production from Anti-ASCT2 monoclonal antibody

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Objective: Biological treatments developed using antibodies are today's most innovative drugs. It is especially common in cancer treatments and inflammatory diseases. Metabolic targeting of cancer cells is also an intensive area studied and contributes to new treatment approaches. Glutamine metabolism is an important pathway for cancer survival and energy production. ASCT2 (Alanine-serine-cysteine transporter 2) is responsible for transporting glutamine into the cell and is highly expressed in cancer cells. Although intracellular glutamine metabolism is a well-studied subject, its modification with antibodies is not well understood.

Methods: It was aimed to produce a chimeric humanized recombinant antibody of anti-ASCT2 monoclonal antibody. For this purpose, hybridoma was performed and monoclonal antibodies were produced. The properties of the antibodies obtained were determined by antigen-antibody binding-based ELISA and western blotting and were purified with Protein G. Its anti-proliferative effect has been demonstrated in colon (HT29) and breast (MCF7) cancer cell lines. The variable regions of both the heavy chain and light chain of the anti-ASCT2 monoclonal antibody were isolated and cloned into the vector containing the sequence of human immunoglobulin constant regions by a system based on homology of short regions.

Results: The plasmid that will produce the chimeric anti-ASCT2 antibody that we obtained was transfected into embryonic kidney (HEK293T) cell lines and produced.

Conclusion: Our most important goal is to humanize monoclonal antibodies that can be used in the treatment of diseases and to carry out pilot production for human use. With the recombinant antibody we have successfully developed, similar systems will be created in Türkiye and a step will be taken to develop innovative, internationally competitive products at the national level, reducing foreign dependency. In this regard, The humanized recombinant antibody against ASCT2 will become a market-competitive molecule.

Keywords: ASCT2, cancer, glutamine metabolism, recombinant antibody.

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PP-26

Evaluation of relapse with IKZF1 plus in pediatric Ph-negative acute lymphoblastic leukemia cases

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Objective: Leukemias constitute 25-30% of childhood malignancies. Genetic factors play an important role in the development of acute leukemia. Recently, cure rates for pediatric B ALL had improved to greater than %80, however relapse and treatment-related toxicities are still observed in 10% of the cases. Therefore, current treatment approaches aim to make the process short and effective. At least 50% of relapses occur in the standard or intermediate risk group. Cases classified as high risk group mostly receive long-term treatment with chemotherapy. One of the negative prognostic factors in treatment plans is the presence of IKZF1 gene abnormalities, which occur together with a high MRD level, especially at the end of induction therapy. The association of IKZF1 deletion and MRD has been described in B-ALL. JAK-STAT signal activation can increase the negative prognostic effect caused by IKZF1 deletions, while ERG deletions can reduce it. International working groups have included IKZF1 deletion status when determining high-risk treatment protocols for B-ALL cases. When the prognostic effect of IKZF1^{plus} is compared with MRD classifications; The 5-year EFS for MRD-SR IKZF1^{plus} patients was 94±5%, while it was 40±10% in MRD-IR and 30±14% in MRD-HR. The aim of the study is to evaluate the prognosis of Ph-negative patients by comparing MRD risk groups with IKZF1^{plus}.

Methods: DNA isolation was performed from the bone marrow diagnostic material of 27 patients. MLPA analysis was performed with the isolated DNAs in accordance with the protocol using the SALSA MLPA Probemix P335 ALL-IKZF1 and SALSA MLPA Probemix P202 IKZF1-ERG kits.

Results: IKZF1 heterozygous deletion was found in 19% of 27 patients, IKZF1 heterozygous duplication in 11%, CDKN2A-2B heterozygous deletion in 15%, and CDKN2A homozygous deletion in 7%. Prognoses were evaluated by comparing MLPA analysis results, MRD findings and treatment class. According to the results of MLPA analysis performed with P335 and P202 probes, patients determined to be Normal (%19) are in the low risk group, while patients in the high risk class according to the Berlin Frankfurt Munich (BFM) classification have IKZF1 heterozygous deletion and CDKN2A/2B heterozygous deletion.

Conclusion: IKZF1^{plus} is described as a novel and powerful prognostic profile for pediatric ALL cases treated with clinical protocols based on BFM therapy. The findings indicate the need to improve current treatment protocols in order to identify patients at highest risk of relapse in B-ALL earlier.

Keywords: Multiple ligation-dependent probe amplification (MLPA) analysis, pediatric, ph-negative acute lymphoblastic leukemia.

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PP-27

Does HLA heterozygosity influence the gut microbiota?

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Objective: The aim of this study was to determine the relationship between homozygous or heterozygous HLA alleles and microbial diversity in the intestine.

Methods: Thirty-three healthy individuals were included in the study. After nucleic acid isolation from stool samples, the samples were sequenced using the Next Generation Sequencing (NGS) (ZymoBiomics DNA Kits, USA) method.

Results: The mean age was 45.27±12.37 years (27-67). The gender distribution was M/F:17/16. Most common alleles were HLA-A*24:02, HLA-B*35:01, HLA-C*04:01, HLA-DQB1*03:01, HLA-DRB1*13:01. The alpha-diversities of the gut microbiomes of people that are homozygous or heterozygous for five different HLA types (HLA-A,-B,-C, DRB1, DQB1) were compared using a Wilcoxon Rank Sum test. Diversities were calculated on the genus level. A significant difference could only be observed between HLA-A homozygous and HLA-A heterozygous individuals' microbial diversities, when Shannon or Inverse Simpson diversity indices are considered. HLA-A heterozygous individuals seem to have higher gut microbiome alpha-diversity compared to HLA-A homozygous individuals. In HLA-A group some of significantly different groups based on p-values are; *Actinobacteria, Negativicutes, Lachnospirales.* In HLA-B group some of significantly different groups based on p-values are; S*treptomyces sp, Oscillibacter, Lachnospiraceae UCG-003.* In HLA-C group some of significantly different groups based on p-values are; *Candidatus Soleaferrea, Bacteroides caccae, Lactobacillus delbrueckii.* In HLA-DBQ1 group some of significantly different groups based on p-values are; *Verrucomicrobiae, Clostridia UCG-014, Lachnospirales.* In HLA-DQB1 group some of significantly different groups based on p-values are; *Oscillibacter, Lachnospira.* The KEGG database showed that HLA-A was closely related to Amino acid metabolism and Protein digestion and absorption pathways.

Conclusion: It is thought that there is a relationship between HLA heterozygosity and intestinal flora due to antigen presentation in individuals with HLA heterozygosity compared to individuals with HLA homozygosity. In other words, a significant abundance of species such as *Anaerostipes hadrus, Clostridiaceae bacterium, Streptomyces sp* was found in HLA-B homozygotes.

Keyword: Heterozygous, homozygous, human leukocyte antigen, microbiota.

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PP-28

Effects of exercise, metformin, pioglitazone and exenatide treatments on insulin receptor substrate-2 gene expresion in obese animal model

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Objective: The purpose of this study; To examine the destruction of the insulin receptor substrate-2 (IRS-2) molecule, which is a cytoplasmic signaling molecule that can cause insulin resistance in obesity, and also mediates the effects of insulin-like growth factor 1 and other cytokines, and its reducing effect on this destruction. For this purpose, the effects of metformin, exenatide, pioglitazone and exercise treatments, which are used clinicaly in the treatment of diabetes and obesity, on IRS-2 ubiquitination in liver tissue were investigated in obese animal model.

Methods: Obese rat model was used in this study. This model is characterised by obesity, diabetes and insulin resistance. IRS-2 gene expression were analzed in the liver tissue of obese rats treated with metformin, exenatide, pioglitazone and exercise. Real Time PCR method was used with Fluidigm BioMark HD device to analyze IRS-2 gene expression.

Results: Gene expression analysis results were evaluated, the decrease in the IRS-2 gene in the liver tissue of rats administered pioglitazone was found to be statistically significant ($p>0.05$).

Conclusion: Our findings showed that some drugs used in the treatment of obesity and diabetes can alter the proteasomal degradation of IRS-2 protein in liver tissue. However, more comprehensive studies are required to show the contribution of ubiquitination in the destruction of IRS-2 and which drugs are effective on this mechanism.

Keywords: Animal model, insuline receptor substrat 2, obesity.

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PP-29

Effect of vitamin-D on liver regeneration in type 2 diabetic rats induced by high dose fructose/low dose streptozotocin

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Objective: In this study, the effects of Vitamin-D (Vit D) on the regeneration mechanism against liver damage in a modified-experimental diabetes model were investigated.

Methods: A modified type 2 diabetes model was established in Sprague-Dawley rats by long-term administration of high dose fructose (10%) and low/single dose STZ (40 mg/kg). The experiment was divided into 4 groups. 1) Diabetic group (D). 2) Diabetic group treated with Vit D (170 IU/week) (D+Vit D). 3) Vit D group (Vit D), 4) Control group (C). At the end of the 9th week, the experimental animals were sacrificed and liver tissues were prepared for histological examination. Body weight, blood glucose (BG) levels and calorie intake were measured. H+E, Van Gieson and PAS staining and immunohistochemical (IHC) staining with TGF-β1, α-SMA, Ki-67, SIRT-1, YAP/TAZ and OV-6 antibodies were performed on the tissue sections. Results were evaluated by statistical analyses.

Results: BG levels were significantly higher in the diabetic group compared to the other groups ($p<0.001$). In D-group, collagen and glycogen accumulation and vacuolisation in hepatocytes were observed. In D+Vit D, a decrease in BG, a decrease in degeneration signs, and a decrease in the expression levels of α-SMA and TGF-β1, which are increased in diabetics, were observed with Vit D administration ($p<0.001$). The number of Ki-67 immunopositive cells was higher in the Vit D-treated diabetic group compared to the C and D groups ($p<0.001$). YAP/TAZ and SIRT-1 immunopositive cells were increased in the periportal area, bile duct epithelial cells and cells in the periphery of bile ducts in D+Vit D compared to the diabetic group ($p<0.001$). In the D+Vit D group, the number of OV-6 positive-oval cells localised in the portal area increased. The number of OV-6-positive cells in the bile ducts was significantly higher in the D+Vit D group compared to the D group ($p<0.01$).

Conclusion: Vit D, which was found to have a significant effect on the prevention of diabetic liver damage by stimulating regeneration mechanisms in the diabetes model used, may be used as a useful option in treatment.

Keywords: Fructose, liver, regeneration, streptozotocin, vitamin D

PP-30

Investigation of the cytotoxic effect of quercetin and RXR agonist CD3254 on breast and colon cancer cells

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Objective: Quercetin is a flavonoid known to have the ability to inhibit the growth of cancer cells. Various biological effects of quercetin have been reported, including antioxidant, anticarcinogenic, anti-inflammatory, anti-diabetic, and antimicrobial activities. RXR retinol or retinol (RA), the most important bioactive metabolite of vitamin A, plays a role in cell growth, differentiation and embryonic development. RXR modulators have therapeutic potential for cancer treatment. CD3254 is a synthetic RXR agonist obtained by converting the 30-methyl group to a pentoxy group. The study aimed to investigate the cytotoxic effects of quercetin and CD3254 on MCF-7 breast cancer cells and colon cancer cells (HCT-116).

Methods: In this study, quercetin and CD3254 were applied to MCF-7 and HCT-116 cells. The CCK8 method was used to determine the antiproliferative effect on both MCF-7 and HCT-116 cells. The IC50 (50% inhibitor) values were determined using the GraphPad 6 software.

Results: The IC50 values of quercetin in the MCF-7 cell line were determined to be 68.3 at 24 hours and 97.74 at 48 hours. However, no cytotoxic effect of quercetin was observed in HCT116 cells. No cytotoxic effect of CD3254 was observed on either cell line.

Conclusion: Our study observed that quercetin has a cytotoxic effect on MCF-7 cells, but not on HCT116 cells. According to our results, quercetin may be used in non-metastatic breast cancer. Further studies are needed to use quercetin as an adjuvant agent in the treatment of breast cancer.

Keywords: CD3254, cytotoxicity, HCT-116, MCF-7, quercetin.

Acknowledgment: This work was supported by the Research Fund of Istanbul University-Cerrahpasa (Project ID number 37223).

PP-31

In vitro analysis of markers associated with obesity and breast cancer in 3T3-L1 cell lines differentiated to adipocytes on transcriptional and translational level

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Objective: The aim of study is to analyze the changes in expression levels of selected markers associated with obesity and breast cancer, based on the specialized differentiation of adipocyte cells, a significant component of adipose tissue.

Methods: The 3T3-L1 preadipocyte cell line was utilized in *in vitro* cell culture experiments in the study. One group, proliferated without differentiation, served as the control, while another group was differentiated into fully matured adipocyte cells to mimic obesity. The transcriptional and translational expression levels of obesity-associated markers (LPL, FAS, and PPAR-γ) and breast cancer-associated markers (BRCA1, BRCA2, and HER2/erbB-2) were analyzed in both undifferentiated and differentiated cells using Real-time PCR and ELISA methods.

Results: The Lpl's expression levels at both transcriptional and translational levels increased in differentiated cells compared to controls. However, while this increase was significant at the mRNA level (p=0.01), it was not significant at the protein level (p=0.12). Expression levels of Fas and Ppar-γ were increased in differentiated cells compared to controls. However, this increase was not significant at the transcription level (p=0.11 and p=0.27, respectively), but was significant at the translation level (p=0.0004 and p=1.09x10-7). It was determined that there was a significant increase in both mRNA expression levels (p=0.05 and p=0.0007, respectively) and protein expression levels of Brca1 and Brca2 in differentiated cells compared to the control group (p=0.0001 and p=7.9x10-5, respectively). The mRNA and protein expressions of erbB-2 significantly increased in differentiated cells compared to controls (p=0.04 and p=0.006, respectively).

Conclusion: This study, in order to elucidate the mechanisms underlying the relationship between obesity and breast cancer, it creates an infrastructure for studies in which methods such as co-culture and conditioned medium, which include breast cancer cell lines as well as adipocyte cell lines.

Keywords: Brca1, breast cancer, erbB-2, Fas, Lpl, Ppar-γ.

PP-32

Evaluation of cytogenetic results in patients with prediagnosis of abortion

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Objective: Spontaneous abortion (SA) is defined as fetal loss before 20 weeks of gestation. Clinically, 10% to 15% of pregnancies result in SA and total pregnancy loss is estimated to be 30% to 50% of all pregnancies. The most common cause of SA is fetal chromosomal abnormalities. The frequency of chromosome imbalance in SA is at least 50% in the first trimester and 20% in the second trimester. The main aim of this study was to evaluate miscarriages in couples with SA in terms of cytogenetic abnormalities.

Methods: The study group consisted of 100 couples with at least 2 miscarriages who applied to the Genetic Diagnostic Laboratory of Dicle University, Department of Medical Biology-Genetics in 2023. Karyotype analysis was performed by applying GTG (Trypsin-Giemsa) banding to the chromosomes obtained after peripheral lymphocyte culture of venous blood samples obtained from the patients in the study group.

Results: Normal chromosome structure was observed in 80% of the male patients, while heterochromatin increase was observed in 13%, inversion in 3%, satellite increase in 2% and translocation in 2%. In the female patient group, 76% had normal chromosome structure, 18% had increased heterochromatin, 3% had increased satellites, 2% had mosaic anoploidy and 1% had deletion. In 5% of the couples, chromosomal abnormalities were detected in both partners.

Conclusion: Our study is important in terms of determining the frequency of chromosomal abnormalities in couples with spontaneous abortion. Cytogenetic examination will provide useful information for the diagnosis and genetic counseling of SA patients in order to be careful for subsequent pregnancies.

Keywords: Abortion, chromosomal abnormality, cytogenetics.

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Molecular and in silico analysis of monoglyceride lipase (MGLL) gene and associated miRNAs in lung cancer

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Objective: Lung cancer is one of the leading causes of cancer-related death. Altered lipid metabolism in cancer cells is among the cellular processes that attract attention. Monoglyceride lipase (MGLL) is a lipolytic enzyme that converts monoacylglycerides into fatty acids and glycerol. Changes in the expression of genes lie behind the biological events that take place in cells. MicroRNAs (miRNA), one of the crucial epigenetic regulators of gene expression in cells, are considered potential biomarkers in the diagnosis, treatment and prognosis of lung cancer. The study aimed to determine the expression levels of the MGLL gene and miR-302b-5p, miR-190a-3p and miR-450a-2-3p miRNAs, one of the target gene of which is MGLL, and to perform in silico analysis.

Methods: Expression levels of genes were determined by the real-time PCR and, gene ontology (GO) and pathway analyses of genes interacting with the MGLL gene and miRNAs, of which one of the target genes is MGLL, were analyzed in silico.

Results: We determined that the expression levels of the MGLL gene and miR-302b-5p, miR-190a-3p and miR-450a-2-3p miRNAs were significantly decreased in lung cancer tissues ($p<0.05$). 37 genes with which the MGLL gene interacts were determined. Genes that may be targeted for lung cancer included methionine sulfoxide reductase B3 (MSRB3), Fc epsilon receptor immunoglobulin (FCER1G), 5-hydroxytryptamine receptor 4 (HTR4) and leukotriene B4 receptor 2 (LTB4R2) genes. Although miRNAs whose expression levels have been investigated are involved in various biological processes and signalling pathways known to play a role in cancer pathogenesis, it has been observed that the prominent signalling pathway is the transforming growth factor beta (TGF-β) signalling pathway.

Conclusion: MGLL gene and miR-302b-5p, miR-190a-3p and miR-450a-2-3p miRNAs were tumour suppressive activity in lung cancer. MSRB3, FCER1G and LTB4R2 genes, especially the HTR4 gene, may be potential target genes for lung cancer.

Keywords: Gene expression, in silico, lung cancer, MGLL, miRNA.

PP-34

The relationship between long non-coding RNA NEAT1, miR-139-5p, miR-129-5p, TGF-β1, and collagen type I with pelvic organ prolapse

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Objective: Pelvic Organ Prolapse (POP) is a gynecological benign condition characterized by the downward movement of pelvic organs due to weakening of the pelvic support tissue. Although its molecular mechanism is not fully understood, previous studies in the literature have shown that Transforming Growth Factor-Beta 1 (TGF-β1) and collagen type I levels are altered in POP. MicroRNAs (miRNAs/miRs) and long non-coding RNAs (lncRNAs), which play important roles in the regulation of gene expression, have been found to provide significant benefits in the diagnosis, prevention, treatment, and prognosis of diseases. The aim of our study is to investigate the effects of nuclear-enriched abundant transcript 1 (NEAT1), miR-139-5p, and miR-129-5p expression levels, known to be involved in the regulation of TGF-β1 and collagen type I, on POP.

Methods: A total of 34 patients diagnosed with POP and 30 healthy control individuals were included in our study. RNA was extracted from fascial tissues of the sample group, and the expression levels of NEAT1, miR-139-5p, and miR-129-5p were analyzed using the real-time quantitative polymerase chain reaction (RT-qPCR) method. The protein levels of TGF-β1 and collagen type I were evaluated using the enzyme-linked immunosorbent assay (ELISA) method.

Results: As a result, the expression level of miR-129-5p was found to be significantly higher in the POP group compared to the control group (p=0.011). Additionally, the TGF-β1 protein level was higher in the POP group compared to the control group (p=0.000).

Conclusion: The obtained data indicate the potential involvement of miR-129-5p in the mechanism of POP. In further studies, lncRNAs regulating miR-129-5p and other genes regulated by miR-129-5p should be identified to investigate their relationship with POP.

Keywords: Collagen, lncRNA, miRNA, pelvic organ prolapse, TGF-β1.

PP-35

Changes of miR-139-5p, TGFB1, and COL1A1 in the placental tissue of cases with gestational diabetes mellitus

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Objective: There are structural changes in the placenta of cases with Gestational Diabetes Mellitus (GDM). TGF-β and collagen pathways have crucial roles in tissue remodelling and TGF-β1 and COL1A1 are important genes in these signalling respectively. Also, lncRNA NEAT1 and miRNA hsa-miR-139-5p and hsa-miR-129-5p have regulatory effects on TGF-β1 and COL1A1. Here we aimed to assess their expressions in the placenta tissue of GDM cases.

Methods: 30 patients with GDM and 30 healthy pregnant women participated in the study. Placental tissues taken during normal or cesarean delivery were used and total RNA was isolated from the tissues. mRNA levels were determined by qPCR and protein levels were determined by ELISA methods. An in silico analysis was done to elucidate the possible relation of TGF-β1 and COL1A1 gene networks with GDM.

Results: We determined that NEAT1 and miR-129-5p expression levels did not differ between GDM and healthy control groups (p=0.697 and 0.412, respectively). But, miR-139-5p mRNA level, TGFB1 and COL1A1 protein levels significantly differ between the GDM and control groups (p=0.000, p=0.000 and p=0.001, respectively). The in silico analysis revealed that the TGFB1 and COL1A1 genes network may have an important role in the GDM with their variety of members and regulatory molecules NEAT1, hsa-miR-139-5p, and hsa-miR-129-5p can control their functions.

Conclusion: The expression of TGFB1, COL1A1 and miR-139-5p is changed in placenta tissue of GDM cases and many genes in the interacting networks of TGFB1 and COL1A1 could contribute to the pathogenicity of GDM.

Keywords: COL1A1, gestational diabetes mellitus, in silico, TGF-β.

PP-36

Investigation of the role of TAU in the pathophysiology of Hereditary spastic paraplegia-4

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Objective: Hereditary Spastic Paraplegia (HSP) is a genetically and clinically heterogeneous neurodegenerative disease characterized by degeneration of the corticospinal tract. Mutations of *SPG4*, which encodes Spastin, a microtubule-severing protein, are the commonest type of the disease. Spastin has two major isoforms but only the M1 isoform is associated with HSP-SPG4. It has been shown that HSP-SPG4 pathophysiology is caused by the gain of function due to the accumulation of mutant M1 Spastin in mice expressing human-mutant Spastin. In our previous study, we showed that mice with *knock-out* pf one allele of *Spast* with a human Spastin carrying C448Y mutation exhibit FAT (Fast Axonal Transport) defect is mediated by a decrease in acetylated tubulin, which has been linked to increased activity of Histone Deacetylase 6 (HDAC6). Here, we investigated the relationship between the toxic effects of Spastin and Tau, another regulator of axonal transport and a substrate of HDAC6. In Tau-related disease, phosphorylation and deacetylation of Tau decrease the affinity of Tau on microtubules leading to detachment of Tau from the microtubules resulting in microtubule destabilization. As a result, on the one hand, Tau oligomers are formed, and on the other hand, destabilized microtubules cause FAT. Although some patients with HSP-SPG4 have Tau lesions, the link between Tau and HSP-SPG4 pathology is yet to be investigated. Our goal is to elucidate the relationship between the toxic effect of mutant Spastin and Tau.

Methods: To understand the role of Tau in HSP-SPG4, cervical and lumbar levels and motor cortex were dissected from wild-type (wt), *knock-out* of one-allele of *Spast* (KO-Het), carrying-human-C448Y mutation (Het) and, KO-Het mouse were crossbred with a Het mouse (dHet). After protein isolation, the level of total Tau protein was investigated by Western Blot.

Results: Here, we showed that the protein levels of 70 kDA and 50 kDA Tau are increased in the Het and dHet mice compared to wt and KO-het mice. Also, Big Tau is only expressed in the cervical and lumbar of Het and dHet mice.

Conclusion: Taken together, our findings reveal a relationship between the toxic effect of mutant Spastin and Tau pathology and promise a new perspective on understanding the pathophysiology of HSP-SPG4.

Keywords: HSP-SPG4, microtubule, spastin, tau.

PP-37

Chromosome aberrations in peripheral blood lymphocyte cultures of reproductive-age women exposed to sodium hypoclorite

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Objective: With the spread of COVID-19, the increased use of sodium hypochlorite (NaOCI) as a disinfectant may cause serious harm to human health and ecosystems in the future. Several recent studies suggest that sodium hypochlorite can cause eye irritation, asthma, oxidative damage, central nervous system dysfunction and reproductive disorders in humans. Due to its cytotoxic and genotoxic effect, NaOCl can be considered as an important risk factor in many diseases such as reproductive disorders. The aim of this case-controlled study was to determine whether NaOCl has an effect on chromosomal damages causing reproductive failure in women and to detect structural and numerical chromosomal abnormalities.

Methods: Karyotypes obtained by routine karyotype analysis from peripheral blood for cytogenetic diagnosis were divided into 3 groups; group I included 104 women exposed to sodium hypochlorite (≥5 liters bleach/weight for ≥10 years), group II included 51 women not exposed to sodium hypochlorite. Group III consisted of 15 women selected from group I who underwent repeated chromosome analysis three months after stopping NaOCl use. 6800 metaphases of 156 patients obtained by peripheral lymphocyte tissue culture technique were analyzed by G-banding.

Results: 943 (13.86%, p=0.002) of 6800 metaphases showed chromosomal abnormalities. Chromosomal abnormalities were 34.4% (p=0.001) in group I, 0.66% (p=0.000) in group II and 5.77% (p=0.001) in group III).

Conclusion: We suggest that prolonged and continuous exposure to NaOCl may adversely affect chromosomes, trigger chromosomal abnormalities and cause adverse effects on the reproductive system.

Keywords: Chromosomal abnormalities, reproductive, sodium hypochlorite.

PP-38

Prevalence of HLA-B27 in patients with Ankylosing spondylitis in southeastern anatolia region

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Objective: Ankylosing spondylitis (AS), one of the pathologies in which immune repertoire studies can have a significant impact, is a disease characterized by inflammation of the peripheral joints and periarticular tissues of the spine. Human leukocyte antigen (HLA-B27) is a genetic marker of AS. Therefore, AS is also called HLA-B27-related disease. Our study aims to contribute to the understanding of the prevalence and impact of HLA-B27 in the local population in patients diagnosed with AS in Diyarbakır between 2022-2023.

Methods: Between 2022 and 2023, 280 patients (143 males, 137 females) who presented to Dicle University Faculty of Medicine, Physical Therapy Clinic with AS symptoms were referred to Dicle University Department of Medical Biology and Genetics. 50 unrelated individuals (25 males, 25 females) who applied to our laboratory to become bone marrow donors were selected as the control group. DNA was isolated from peripheral blood using salt precipitation method in both groups. Rotar Gene Q device was used for PCR analysis. The prevalences of the variables were analyzed. Mann-Whitney U test was used for statistical analysis and Spearman rho correlation statistics were used as a method.

Results: Radiologic examinations and laboratory tests were performed in 280 patients with suspected AS. The prevalence of those with a definitive diagnosis of AS was 22.5 (95% CI: 0.75-0.9325). The prevalence of HLA-B27 was 90% in 63 of 70 patients diagnosed with AS as a result of radiologic examinations and laboratory tests.

Conclusion: We believe that our study will contribute to the literature in the evaluation of HLA-B27 positivity in AS patients in the Southeastern Anatolia Region. The prevalence of HLA-B27 in our region was higher than the prevalence in Türkiye.

Keywords: Ankylosing spondylitis, human leukocyte antigen-B27, prevalence.

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Functional characterization of MAP7D1 gene mutation in Shwachman-Diamond syndrome

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Objective: Shwachman-Diamond Syndrome (SDS) is an autosomal recessive disease characterized by bone marrow failure, pancreatic failure and skeletal disorders. SDS is associated with genes involved in ribosome biogenesis, and it is known that mutations observed in SBDS, one of these genes, cause bone marrow failure due to mitotic spindle instability. In a genetic study conducted with the SDS cohort, while no mutation was observed in SDS-related genes in a patient with SDS, the MAP7D1:c.601C>T, p.Arg201Trp variant was noted in the gene encoding the MAP7D1 protein. The mutation in the MAP7D1 protein, which is a member of the microtubule-related protein family and plays a role in the microtubule re-organization and transport of organelles/vesicles by binding to microtubules and kinesins through its microtubule and kinesin binding sites, was examined by in silico analysis, and it was observed that the mutation hit the microtubule binding site of MAP7D1 and changed the 3-dimensional structure. Considering the region where the mutation hits and the importance of mitotic spindle stability in the etiology of SDS, it is aimed to examine the possibility that the MAP7D1:p.Arg201Trp mutation may be responsible for SDS by disrupting the microtubule-MAP7D1 relationship with functional studies.

Methods: Microtubule-MAP7D1 interaction due to MAP7D1:p.Arg201Trp mutation was examined both in HEK293T cells by pull-down experiment and in control/patient primary fibroblast cells and T98G cells by immunocytochemistry experiment. Then, the effect of MAP7D1:p.Arg201Trp on mitotic spindle stability was investigated by examining the mitotic spindle structures in control/patient fibroblast cells by immunocytochemistry method.

Results: It has been determined that the MAP7D1:p.Arg201Trp mutation significantly disrupts the microtubule-MAP7D1 interaction. Moreover, it has been observed that MAP7D1 proteins, which normally co-localize with microtubules and enable the formation of filament microtubule structure, have decreased co-localization with microtubules due to the p.Arg201Trp mutation and microtubules have lost their stable structure in the form of bundled filaments. In addition, it was found that control fibroblast cells had a stable mitotic spindle structure between two centrosomes and the chromosomes were aligned in the mitotic spindle, whereas patient fibroblast cells formed an unstable mitotic spindle between more than two centrosomes and the chromosomes could not be aligned.

Conclusion: This study has preliminary data indicating that mitotic spindle changes, which were previously observed by a limited number of researchers, actually have an important role in the etiology of SDS, and is essential in that it may reveal the possibility of a new causal gene associated with SDS.

Keywords: MAP7D1, microtubule, mitotic spindle.

Acknowledgment: This study is founded by TÜBİTAK-COST project (121Z610).

PP-40

Molecular and cytogenetic case report in a patient with prediagnosed azoospermia

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Objective: In men with azoosporemia or oligozoosporemia, testing for deletions of the AZFa, AZFb, AZFc regions of the Y chromosome is extremely useful in choosing between diagnostic testicular biopsy, cryo-TESE and TESE/ICSI. The diagnosis of Y-deletions not only identifies the etiology of the defect in spermatogenesis, but also provides valuable information for the appropriate clinical follow-up of the infertile man and the male child. Numerical and structural chromosomal abnormalities are a leading cause of azoospermia and infertility. In addition to molecular genetic examinations, cytogenic and, if necessary, molecular cytogenetic examinations should be performed in patients with a preliminary diagnosis of infertility in order to ensure a healthy diagnosis and treatment.

Methods: DNA was isolated from peripheral blood using salt precipitation method. Panels suitable for RT-PCR were prepared to examine microdeletions in the AZF-a AZF-b AZF-c region of the Y chromosome. SRY and ZFY were used as controls. The results were analyzed with Qiagen rotor gene Q. For cytogenetic analyses, chromosomes were obtained from peripheral blood using whole blood cell culture method and the metaphases obtained were karyotyped and a report was prepared.

Results: Molecular analysis of the AZF-a AZF-b AZF-c region of the Y chromosome revealed microdeletion in AZF-b (sY127-sY134) and AZF-c (sY254-sY255) regions. As a result of chromosome analysis, 46,XY,inv(9)(p13;q13) chromosome organization was detected in the patient.

Conclusion: Detection of microdeletions in the analyzed regions is consistent with the patient's history of azoospermia and it is recommended that this result of the patient be evaluated together with clinical and laboratory findings. Sequence analysis for other possible mutations and genetic counseling are recommended in the case with positive clinical and laboratory findings.

Keywords: Azoospermia, karyotype, Y microdeletion.

PP-41

Investigation of the effect of meclofenamic acid on the proteome of LNCaP cells reveals changes in alternative polyadenylation and splicing machinery

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Objective: Prostate cancer is the most common type of cancer among men, and there is still no definitively effective drug treatment. Thus, the search for novel drug agents that may be used for the effective treatment continues. Meclofenamic acid (MA), a non-steroidal anti-inflammatory drug, with anti-tumor effects in various types of cancers was used to investigate its effects on LNCaP cells, a prostate cancer cell line, at the proteome level.

Methods: The cells were treated with 80 µM MA for 24 h and a comparative proteomic analysis was performed with their untreated control cells. Proteins were extracted from the cells and then were subjected to two-dimensional gel electrophoresis. Protein spots displaying changes in their regulation ratios for more than two-fold were excised from the gels and identified with MALDI-TOF/TOF mass spectrometry.

Results: Bioinformatics analysis of the differentially regulated proteins that we identified showed that they were all associated with and took part in related pathways. Glycolytic pathway, cytoskeletal formation, transport activity, protein metabolism, and most notably an mRNA processing pathway (especially RBM4, RBM4B, CSTF1, SFRS2 and SFRS3 proteins that play roles in splicing and alternative polyadenylation) were affected by the MA treatment.

Conclusion: In addition to presenting a detailed information for what is happening inside the cells upon MA treatment, the proteins affected by MA treatment hold the potential to be novel targets for prostate cancer treatment provided that further in vivo experiments are carried out.

Keywords: LNCaP cells, MALDI-TOF/TOF, meclofenamic acid, prostate cancer, proteomics.

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Investigation of the association of circulating miR-146a-5p relative expression levels and MIR146A rs2910164 G>C genetic polymorphism with relapsing-remitting multiple sclerosis

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Objective: Multiple Sclerosis (MS) is an inflammatory, autoimmune, neurodegenerative central nervous system disease. Relapsing-remitting MS (RRMS) continues with recovery after attacks. microRNAs (miRNA) are a class of non-coding RNAs involved in post-transcriptional regulation. miR-146a-5p has a role in the regulation of immune responses and inflammation. There are several single nucleotide polymorphisms (SNPs) in miRNA genes. The rs2910164 G>C SNP is an intronic variation of the MIR146A gene and can alter its expression. The Multiple Sclerosis Severity Score (MSSS) predicts disease progression over time, where >5 is the marker of fast progression status. In this study, association of the circulating miR-146a-5p relative expression levels and rs2910164 SNP with MS risk and progression was investigated.

Methods: Blood samples were collected from 364 patients with RRMS and 364 healthy controls (HC) by Ankara City Hospital Neurology Clinic. DNA isolation was performed, and rs2910164 G>C genotypes were determined using the TaqMan genotyping method. miR-146a-5p plasma expression levels of 27 naïve patients with RRMS, 26 patients treated with fingolimod, and 28 HC were determined using the qRT-PCR and fold-change was calculated using the 2 ΔΔCt method.

Results: There was no significant difference between groups in the relative expression level of circulating miR-146a-5p (p=0.750).The frequency of the polymorphic C allele was 0.268 in patients and 0.228 in HC (OR=1.24, p=0.08). Genotype distribution was significantly different between patients and HC in dominant model (CC+GC vs. GG) (OR=1.35, CI=1.01-1.82, p=0.045). Frequency of patients with MSSS>5 was significantly different between genotypes in both the co-dominant model (p=0.03) and dominant model (p=0.01).

Conclusion: According to the findings obtained in this study, MIR146A rs2910164 G>C SNP is associated with MS risk; additionally, there appears to be a relationship between this SNP and disease progression in the dominant model.

Keywords: Gene expression, microRNAs, multiple sclerosis, single nucleotide polymorphism