Moleküler Biyoloji Derneği

MolBiyoKon'26

10th International Congress of the Molecular Biology Association of Turkey

Acıbadem University, İstanbul

17-19 September 2026

ACIBADEM UNIVERSITY



ABSTRACT BOOK

Dear Colleagues and Friends,

On behalf of the Molecular Biology Association, it is a great honor to invite you to the 10th International Congress of the Molecular Biology Association of Türkiye (MolBiyoKon'26), which will be held from September 17 to 19, 2026 at Acıbadem University in İstanbul.

Founded in 2011, the Molecular Biology Association aims to bring together the community of molecular biologists from Turkey and abroad to support the advancement of this rapidly evolving field. As a landmark 10th anniversary event, MolBiyoKon'26 will provide an international platform for researchers from various scientific backgrounds to disseminate research results, exchange innovative ideas, and inspire students to pursue excellence in life sciences.

The scientific program of MolBiyoKon'26 will encompass a broad spectrum of disciplines essential to modern biology, including cell biology, neuroscience, immunology, cancer biology, and developmental biology. Furthermore, the congress aims to highlight the integration of bioinformatics, biotechnology, and genetic engineering in addressing complex biological questions.

A central objective of this year's meeting is the fostering of critical synergies between basic and translational researchers. By bridging basic discoveries with health applications, the congress aims to showcase how molecular insights are translated into clinical impacts, such as new drug developments, personalized medicine, and advanced gene therapies.

Following our established tradition, the congress will feature keynote lectures from world-renowned speakers, selected oral presentations, and a vibrant poster session designed to foster collaborative discourse. Stay tuned in for the updates.

İstanbul, a city that uniquely bridges two continents, provides a magnificent and historic setting for this milestone event. As a global crossroads, İstanbul offers an inspiring atmosphere that fosters both academic collaboration and innovation. We hope you will take the time to explore and enjoy the historical and cultural beauty of our city during your visit.

We look forward to welcoming you to İstanbul for an inspiring and productive scientific exchange that will continue to promote the progress of life science research in Turkey.

Dr. Tolga Sütlü
Congress President
On behalf of the The Organizing Committee, 10th International Congress of the Molecular Biology Association of Turkey (MolBiyoKon'26)

Organizing Committee

Tolga Sütlü
(Congress President)
Acıbadem University
Umut İnci Onat
(Congress Secretary)
Acıbadem University
Eda Tahir Turanlı
Acıbadem University
Batu Erman
Acıbadem University
Nazan Saner
Acıbadem University
Zeynep Tokcaer Keskin
Acıbadem University
Nazlı Keskin
Acıbadem University
Ahmet Can Timuçin
Acıbadem University
Dilay Hazal Ayhan
Acıbadem University
Eda Süer
Acıbadem University
Neslihan Zohrap
Acıbadem University

Scientific Committee

Arzu Çelik
Boğaziçi University
İbrahim Yaman
Boğaziçi University
Güneş Özhan
İzmir Institute of Technology
Atakan Ekiz
İzmir Institute of Technology
Tolga Sütlü
Acıbadem University
Umut İnci Onat
Acıbadem University
Nuri Öztürk
Gebze Technical University
Umut Şahin
Sabancı University
Duygu Kuzuoğlu Öztürk
Sabancı University
Ceren Çıracı
İstanbul Technical University
Bahar Değirmenci Uzun
Bilkent University
Sreeparna Banerjee
Middle East Technical University

ORAL PRESENTATIONS ABSTRACTS

OP01
SS18L2 Is a Previously Unrecognized Component of the BAF Complex in Triple-Negative Breast Cancer

Erdem Ercan[1], Elif Ayça Guvener[1], Beril Esin[1], Ezgi Ozyerli-Goknar[2], H.T.Marc Timmers[2, 3], Ozlem Yedier-Bayram[1, 5], Tugba Bagci-Onder[1, 4, 5]

[1] Koç University Research Center for Translational Medicine (KUTTAM), Istanbul, Türkiye
[2] German Cancer Consortium (DKTK), a Partnership Between the DKFZ and Medical Center, University of Freiburg, Germany
[3] Department of Urology, Medical Center, University of Freiburg, Freiburg, Germany
[4] Koç University School of Medicine, Istanbul, Türkiye
[5] Correspondence: tuonder@ku.edu.tr, oyedier@ku.edu.tr

Background/Aim: Triple-negative breast cancer (TNBC) remains a major clinical challenge due to its aggressive, heterogeneous nature and the lack of targeted therapies. Through an epigenome-wide CRISPR/Cas9 knockout screen (EPIKOL), we identified SS18L2, a previously uncharacterized regulator of TNBC cell survival. SS18L2 shares strong structural homology with SS18 and SS18L1, two subunits of the SWI/SNF (BAF) chromatin-remodeling complex that is mutated in about 20% of human cancers, yet its function remains unexplored. In this study, we aimed to define the molecular mechanisms behind the essential role of SS18L2 in TNBC.
Materials and Methods: SS18L2 was knocked out in TNBC cells using CRISPR/Cas9, and rescue assays were performed using wild-type SS18L2, its paralogs, and engineered SS18L2 variants. Following assessment of their ability to restore the knockout phenotype, rescue-competent and rescue-deficient SS18L2 variants were characterized by immunoprecipitation coupled with mass spectrometry (IP-MS) to define their protein interactomes and by greenCUT&RUN to map genome-wide-chromatin occupancy.
Results: Knockout of SS18L2 impaired cell cycle progression, resulting in multinucleated, non-dividing giant cells. This phenotype was rescued only by re-expression of SS18L2, but not its paralogs, despite their shared N-terminal protein–protein interaction domain. IP-MS identified SS18L2 as a previously unrecognized component of the BAF complex through its interaction with both canonical and non-canonical BAF subunits and uncovered interaction partners unique to rescue-competent SS18L2 variants. Complementary greenCUT&RUN analyses revealed distinct genome-wide chromatin occupancy patterns associated with SS18L2.
Conclusion: These findings identify SS18L2 as a novel component of BAF chromatin-remodeling complex and establish it as a novel, non-redundant dependency in TNBC. Our ongoing studies aim to determine whether SS18L2 performs specialized functions within the BAF complex or mediates interactions with additional regulatory pathways.

This work was supported by Wellcome Trust Early Career Award (314358/Z/24/Z) and TÜBİTAK 1003-216S461 and 1001-221S419 grants. E.E. is supported by TÜBİTAK BİDEB 2211-A (1649B032200104)

Keywords: Epigenetics, CRISPR/Cas9, breast cancer, mass spectrometry, CUT&RUN

OP02
Development of a Humanized Knockin Mouse Model For Rahman Syndrome

Muhammed Kasım Diril[1, 2], Kerem Esmen[1, 3], Emre Kalay[1, 2], Nazlıcan Kesmik[1, 2], Imtiaz Nisar Lone[1, 4]

[1] Rare and Undiagnosed Diseases Platform – RUDIP, Izmir Biomedicine and Genome Center, Izmir, Türkiye
[2] Izmir International Biomedicine and Genome Institute, Dokuz Eylül University, Izmir, Türkiye
[3] Department of Medical Biology, Faculty of Medicine, Dokuz Eylül University, Izmir, Türkiye
[4] Present Address: Center for Genomics and Systems Biology, New York University Abu Dhabi, Abu Dhabi, UAE

Background/Aim: Rahman syndrome (RMNS) is a recently identified, rare genetic disorder characterized by overgrowth, intellectual disability, autism, and early aging. The disease is driven by de novo frameshift mutations in the HIST1H1E gene, which codes for the linker histone H1.4 (H1E). These mutations truncate the H1E C-terminal domain and introduce a novel 38-amino-acid acidic tail (RahCTD38) present in virtually all RMNS cases. This alters epigenetic charge dynamics, leading to aberrant chromatin organization. To investigate RMNS etiology in vivo, we aimed to develop a genetically humanized knockin (KI) mouse model.
Materials and Methods: The RMNS mouse model was generated utilizing the CRISPR-EZ technique. This highly efficient approach involves the direct electroporation of CRISPR-Cas9 ribonucleoprotein complexes, including specific guide RNAs and repair templates, into single-cell mouse embryos. This enabled the precise integration of the sequence encoding the human RahCTD38 tail directly into the endogenous mouse H1E locus. Modified embryos were subsequently transferred into pseudopregnant surrogates to generate founder knockin pups, which were bred to establish a stable colony.
Results: We successfully generated the targeted humanized KI mice expressing the RMNS-associated H1E-RahCTD38 mutant protein. Precise on-target integration of the KI allele was genetically characterized and confirmed by Sanger sequencing. Basic phenotypic characterization to assess overall viability, fertility and development was carried out. Furthermore, presence of the mutant H1E protein across tissues was analyzed and validated by western blotting and immunohistochemistry.
Conclusion: We established a novel humanized mouse model that accurately mirrors the genetic basis of RMNS, facilitating research on etiology and development of symptoms. This model will be further characterized by behavior tests, biochemical studies on mouse embryonic fibroblasts (MEF), comprehensive histological analysis to investigate the development of various RMNS-associated clinical phenotypes, and bioinformatics to investigate the underlying molecular mechanisms driving this rare disorder.

Keywords: Rahman Syndrome, Linker Histone H1E, Rare Genetic Disorders, Transgenic Mice, Epigenetics

OP03
In silico Characterization of a Rare Chromatin Disease

Büşra Yüksel[1, 3], Serhan Turunç[1, 2], Pınar Gençpınar[1, 4], Anastas Gospodinov[5], Stefan Dimitrov[5, 6], Seyit Kale[1, 3]

[1] İzmir Biomedicine and Genome Center, Dokuz Eylül University Health Campus, Balçova, İzmir, 35340, Türkiye
[2] İzmir Biomedicine and Genome Institute, Dokuz Eylül University Health Campus, Balçova, İzmir, 35340, Türkiye
[3] İzmir Katip Çelebi University, Faculty of Medicine, Department of Biophysics, Çiğli, İzmir, 35620, Türkiye
[4] İzmir Katip Çelebi University, Faculty of Medicine, Department of Pediatrics, Division of Pediatric Neurology, İzmir, Türkiye
[5] Roumen Tsanev Institute of Molecular Biology, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria
[6] Institute for Advanced Biosciences, UMR 5309, CNRS, Université Grenoble Alpes, Inserm U 1209, Grenoble, 38000, France

Background/Aim: Rahman Syndrome (RS) is a rare and severe genetic disease linked to frameshift mutations in the gene coding linker histone H1.4, a critical constituent of chromatin architecture. Pathogenic mutations are located in the carboxy-terminal domain (CTD) of H1.4, a domain that is responsible for the compaction of linker DNA and therefore the regulation of DNA-templated processes such as transcription and repair. The aim is to characterize the dynamics and interactions of pathogenic H1.4 variants with genomic DNA at the atomic level using in silico tools and to correlate these findings to the clinical profiles of RS patients.
Materials and Methods: Using tools of molecular modeling and high-performance computing, we characterized the dynamics of different frameshift RS H1.4 CTD mutations in the nucleosome, and calculated the affinity of histone H1.4 proteins to the DNA in the healthy and diseased cases. This metric was compared to the disease severity of RS patients.
Results: We find that the DNA affinity of the pathogenic H1.4 may be up 2-3 kcal/mol lower in comparison to the healthy individuals' protein. Our results suggested that electrostatically altered structure of RS H1.4 CTD reduces the affinity of histone H1.4 protein to the genomic DNA. Mutations more upstream in sequence display reduced affinities compared to mutations more downstream. Moreover, experimental validation (Fluorescence Recovery After Photobleaching, FRAP) demonstrates that frameshift mutations increase the H1.4 mobility, decreasing its ability to stabilize chromatin. Additionally, the protein affinity-clinical phenotype correlation revealed that earlier mutations with less affinity are associated with more severe clinical symptoms in RS patients.
Conclusion: The molecular etiology of the RS disease suggests that thermodynamic characterization of a pathological protein can be accurately linked to clinical outcomes in the context of a rare chromatin disease.

Keywords: Rahman Syndrome, overgrowth syndrome, linker histone H1.4, chromatin, molecular dynamics

OP04
Cholesterol-dependent Remodeling of Endothelial Membrane Signalling Reveals a Direct Vascular Mechanism of Tirzepatide

Ceylan Verda Bitirim[1], Dunya Aydos[1], Neslihan Başak[1]

[1] Ankara University Stem Cell Institute

Background/Aim: Endothelial dysfunction is the earliest pathological event in atherosclerosis, yet the mechanisms linking membrane cholesterol homeostasis to endothelial signaling remain incompletely understood. Cholesterol-rich caveolae coordinate β_2-adrenergic receptor (β_2AR), endothelial nitric oxide synthase (eNOS), and redox signaling. Our previous finding that the dual GIP/GLP-1 receptor agonist tirzepatide directly modulates β-adrenergic signaling in cardiomyocytes prompted us to investigate whether this mechanism also operates in endothelial cells, using isoproterenol as a pharmacological β-adrenergic reference.
Materials and Methods: Human umbilical vein endothelial cells (HUVECs) underwent membrane cholesterol depletion with methyl-β-cyclodextrin (MβCD) to disrupt cholesterol-rich membrane nanodomains. Cells were subsequently treated with tirzepatide or isoproterenol. Membrane order (NR12A), Caveolin-1 expression, β_2AR distribution, endothelin-1 (ET-1) localization, ROS production, mitochondrial membrane potential, intracellular Ca^{2+} signaling, PKA/PKC activation, and eNOS phosphorylation were assessed by fluorescence imaging, confocal microscopy, and immunoblotting.
Results: Membrane cholesterol depletion disrupted endothelial membrane signalling, causing loss of membrane order, reduced Caveolin-1 expression, β_2AR redistribution, mitochondrial depolarization, oxidative stress, intracellular Ca^{2+} accumulation, stress kinase activation, and ET-1 relocalization toward mitochondria. Tirzepatide restored membrane organization, normalized Caveolin-1 expression, improved mitochondrial function, and re-established endothelial signalling homeostasis. Restoration of membrane order coincided with normalization of downstream mitochondrial and endothelial signalling, supporting membrane organization as an upstream regulator of endothelial homeostasis. Importantly, these effects closely paralleled those induced by the β-adrenergic agonist isoproterenol, indicating that tirzepatide directly modulates endothelial β-adrenergic signalling beyond its established metabolic actions.
Conclusion: Our findings identify membrane cholesterol as a central regulator of endothelial signaling architecture and demonstrate that the disruption of cholesterol-rich membrane nanodomains triggers the coordinated remodeling of caveolae-associated signaling networks. Beyond its established metabolic actions, tirzepatide directly restores endothelial membrane signaling following cholesterol-dependent membrane disruption, revealing a previously unrecognized mechanism that may contribute to its cardiovascular protective effects. These findings provide a mechanistic framework for investigating endothelial dysfunction in chronic dyslipidaemic disorders, including familial hypercholesterolaemia.

Keywords: membrane order, beta adrenergic receptors, endothelial dysfunction , tirzepatide, caveola

OP05
Ochratoxin A Modulates Proteasome–Mediated Degradation of Neurodegeneration-Associated Protein Aggregates

Çağla ARSLAN[1], İrem ŞAHİN[1], İbrahim YAMAN[1]

[1] Department of Molecular Biology and Genetics, Bogazici University, 34342 Bebek, Istanbul, Turkiye

Background/Aim: Ochratoxin A (OTA) is a mycotoxin produced as a secondary metabolite by Aspergillus and Penicillium species. It contaminates a variety of food and feed products and has been associated with several adverse health effects. OTA was shown to activate the ubiquitin–proteasome system (UPS) in HK-2 and MEF cell lines. Many neurodegenerative diseases (NDDs) are characterized by the aggregation of misfolded or mutant proteins that may impair protein degradation pathways. In this study we aimed to investigate whether OTA-induced UPS activation promotes the clearance of NDD-associated protein aggregates formed by Ataxin-3(Q84) and truncated-NEK1 (tNEK1).
Materials and Methods: Stable SH-SY5Y cells expressing doxycycline-inducible EGFP-tagged Ataxin-3(Q84) were established. HEK293FT cells were transiently transfected with the same doxycycline-inducible EGFP-tagged tNEK1 construct. Protein aggregate formation was confirmed by confocal microscopy. A subtoxic concentration of OTA was determined using a cell viability assay. Following doxycycline induction, cells were treated with OTA in the presence or absence of the proteasome inhibitor epoxomicin. Aggregate clearance was evaluated by confocal microscopy and western blotting.
Results: EGFP-tagged Ataxin-3(Q84) and tNEK1 protein aggregates were formed by induction with 3 µg/mL doxycycline for 72 h. Western blot analysis demonstrated enhanced UPS activity in cells treated with 5 µM OTA compared to untreated controls, whereas this effect was abolished by the proteasome inhibitor epoxomicin. Consistently, confocal microscopy images showed that OTA reduced both the size and the number of Ataxin-3(Q84) and tNEK1 aggregates in inducible SH-SY5Y and HEK293FT cells, respectively.
Conclusion: These findings suggest that OTA-mediated proteasome activation may represent a potential strategy for promoting the clearance of neurodegeneration-associated protein aggregates. However, due to the known toxicity of OTA, further studies are needed to evaluate its safety and explore less toxic derivatives that retain its proteasome-activating properties.

Keywords: Ochratoxin A, protein aggregation, neurodegenerative diseases, proteasome degradation systems

OP06
A Cdc5-Dependent Phospho-Switch on Vac14 Sustains Mitotic PI(3,5)P2 Asymmetry

Ahmed Alsaftawi[1], Nurhan Ersoy[1], Maria Doukmak[1], Ayşe Koca Çaydaşı[1]

[1] Koç Üniversitesi

Background/Aim: Phosphatidylinositol 3,5-bisphosphate [PI(3,5)P2] is a conserved endolysosomal signaling lipid synthesized by the Fab1–Vac14–Fig4 complex. We previously showed that PI(3,5)P2 increases during budding yeast mitosis and preferentially accumulates on daughter vacuoles, but how global cell-cycle cues generate this spatially restricted signal remains unknown. Here, we investigated whether mitotic regulation of Vac14 converts cell-cycle timing into daughter-specific PI(3,5)P2 production.
Materials and Methods: Budding yeast were synchronized in G1, S and metaphase and telophase using alpha-factor, hydroxyurea and nocodazole, overexpression of nondegradable Clb2, or mitotic-exit temperature-sensitive mutants (cdc15-1, dbf2-2, mob1-67, cdc14-1, cdc5-10). Vac14 phosphorylation was assessed by mobility-shift and confirmed by λ-phosphatase treatment. Cdc5 activity was inhibited by shifting cdc5-10 cells to restrictive temperature or by repressing Gal1-Cdc5 expression with glucose. Phospho-site mutants were generated by site-directed-mutagenesis. Phosphorylation-dependent structural changes were modelled using AlphaFold3. Protein localization was analyzed by live-cell fluorescence microscopy. PI(3,5)P2 was visualized using the SnxA–GFP biosensor. Vph1–TdTomato served as vacuole marker.
Results: Vac14 displayed a mitosis-specific mobility-shift that was eliminated by λ-phosphatase and strongly reduced following Cdc5 inactivation. Loss of polo-like kinase Cdc5 activity prevented PI(3,5)P2 accumulation on the daughter-vacuole during late-anaphase. Alanine substitution of Cdc5-dependent phosphosites reduced the fraction of cells exhibiting daughter-vacuole PI(3,5)P2. In cells that established this signal, its persistence following vacuole segregation was shortened, indicating defective maintenance. AlphaFold3 predicted that phosphorylation yields a more compact Vac14 pentamer. In contrast, Cdc5 inactivation did not abolish hyperosmotic-stress-induced PI(3,5)P2 production, supporting a mitosis-specific regulatory mechanism.
Conclusion: Together, these findings identify a mitosis-specific Cdc5–Vac14 phospho-switch that translates cell-cycle timing into daughter-specific PI(3,5)P2 signaling. Preservation of the hyperosmotic response distinguishes this spatial mitotic mechanism from general pathway activation, suggesting that core cell-cycle machinery can program the signaling state of an inherited lysosome-like organelle.

Keywords: PI(3,5)P2, Vac14, Cdc5, Phosphoinositide signaling, Vacuole inheritance

OP07
Decoding Molecular Features Associated with High Translation Efficiency Using Machine Learning

Çağlan Turgut[1], Mehmet Tardu[2]

[1] Division of Biology, Institute of Science, Istanbul University, Istanbul, Turkey
[2] Department of Biology, Science Faculty, Istanbul University, Istanbul, Turkey

Background/Aim: Translational efficiency (TE) is a major determinant of cellular protein output. Although many factors influencing translation have been described, the molecular basis of high TE remains poorly understood. Here, we applied machine learning to identify molecular features associated with high TE.
Materials and Methods: Publicly available paired ribosome profiling (Ribo-seq) and RNA sequencing (RNA-seq) datasets were analyzed. TE values were z-score normalized within each dataset, and transcripts were classified into high-TE and low-TE groups. More than 1000 engineered molecular features describing sequence composition, RNA secondary structure, and peptide physicochemical properties were extracted. These features were then used to train and evaluate nine machine-learning models, including classical algorithms (random forest and support vector machine) and deep learning architectures.
Results: Machine-learning models achieved high classification performance, with AUROC values ranging from 0.83 to 0.90. Across all feature-based models, upstream AUG content in the 5′-untranslated region (UTR) was consistently identified as the strongest predictor of high TE. These transcripts contained substantially fewer upstream AUGs. They also had higher Kozak similarity scores, consistent with more efficient translation initiation. In addition, they exhibited shorter, GC-poor UTRs with fewer stems, hairpins, internal loops, and multiloops, a lower paired fraction, and less negative folding energy. At the ORF level, lower GC content, reduced base pairing, and higher Fickett scores were observed. At the protein level, peptides encoded by these transcripts were predicted to have greater stability.
Conclusion: Machine learning analysis identified a molecular signature of high TE spanning translation initiation, RNA structure, coding sequence composition, and peptide stability. These findings enhance our understanding of the molecular basis of efficient translation. They may also guide the development of synthetic therapeutic mRNAs with improved translational efficiency.

Keywords: Translation Efficiency, Ribosome profiling, Machine learning, mRNA

OP08

Integrated miRNA–mRNA Expression Analysis Reveals Candidate Regulatory Interactions in Autoinflammatory Diseases

Basak Sen[1], Mehmet Emre Ozkan[1], Tayfun Hilmi Akbaba[1], Seher Şener[2], Yeliz Z Akkaya-Ulum[1], Sabrina Fuehner[5], Federica Penco[4], Dirk Foell[5], Marco Gattorno[4], Isabella Ceccherini[3], Seza Ozen[2], Banu Balci-Peynircioglu[1]

[1] Department of Medical Biology, Hacettepe University Faculty of Medicine, Ankara, Türkiye
[2] Department of Pediatrics, Division of Rheumatology, Hacettepe University Faculty of Medicine, Ankara, Türkiye
[3] Unit of Genetics and Genomics of Rare Diseases , IRCCS Istituto Giannina Gaslini, Genova, Italy
[4] Unit of Rheumatology and Autoinfammatory Diseases, IRCCS Istituto Giannina Gaslini, Genova, Italy
[5] Department of Pediatric Rheumatology and Immunology, University of Muenster, Muenster, Germany

Background/Aim: This study was conducted within PerSAIDs (Personalised Medicine for Systemic Autoinflammatory Diseases), a European collaborative project to improve the diagnosis and management of autoinflammatory diseases through personalised medicine and multi-omics approaches. We aimed to identify microRNA (miRNA) patterns associated with disease, activity and genetic classification (monogenic-poligenic) and to explore candidate miRNA–gene networks.
Materials and Methods: Blood samples from 12 patients (six Turkish and six European) representing six autoinflammatory diseases (CAPS,FMF,DADA2,MKD,PFAPA,SURF) were analyzed by miRNA sequencing. 748 miRNAs were expressed in at least 80% of samples. Differential expression analysis was performed using edgeR with TMM normalization, $|\log2FC|>1$, and $p<0.05$. Matched RNA sequencing (RNA-seq; n=4) and NanoString (n=7) data were integrated with TargetScan 8.0 predictions for 69 shared genes. Platform-specific Spearman correlations with $|\rho|\geq0.6$ and $p<0.1$ were retained. miEAA was used for pathway enrichment.
Results: Principal component analysis and hierarchical clustering showed no clear grouping by disease, activity, or genetic classification. FMF versus other diseases showed the largest number of differentially expressed (DE) miRNAs (n = 131), whereas PFAPA vs. others showed the smallest (n = 33). Activity and genetic-class comparisons yielded 60 and 37 DE-miRNA, respectively. Overall, 278 unique miRNAs were identified; 209 generated 2,964 predicted interactions with 69 genes. Transcriptomic integration identified 123 (RNA-seq) and 153 (NanoString) negative correlations between miRNAs and their predicted targets. miEAA analysis of negatively correlated miRNAs suggested enrichment of mTOR signaling and CD3 and CD14-associated signatures in the RNA-seq dataset, and interleukin-1, cytokine–cytokine receptor and Toll-like receptor signaling in the NanoString dataset.
Conclusion: This study identified candidate miRNA–gene networks and immune-related enrichment patterns involved in autoinflammatory disease biology. The findings suggest the presence of distinct miRNA signatures associated with autoinflammatory diseases; however, validation in larger cohorts and functional studies is required. This project was supported by TÜBİTAK (ERA PerMed - 221N352).

Keywords: Autoinflammatory diseases, microRNA, gene regulation, RNA sequencing, transcriptomics

OP09

Platform-Agnostic Cell-Boundary Reconstruction for Nucleus-Resolved Whole-Transcriptome Spatial Data

Nuray Söğünmez Erdoğan[1]

[1] Kadir Has University, İstanbul, Türkiye

Background/Aim: Cell boundaries determine cellular morphology, direct cell–cell contacts, and the neighborhoods in which intercellular signaling occurs. Single-cell-resolved imaging-based in situ spatial transcriptomics (ST) platforms can provide computationally segmented cell territories; however, segmentation remains challenging in densely packed and morphologically heterogeneous tissues, where inaccurate borders may split or merge cells, misassign transcripts, and distort downstream communication analyses. Conversely, nucleus-resolved whole-transcriptome approaches provide broad molecular coverage and precise nuclear coordinates but lack information on cytoplasmic extent and membrane adjacency, reducing cells to point-like representations. We, therefore, developed CellBOUND (Cell-Boundary Optimization Using Nuclear and Donor Spatial Information), a platform-agnostic, cluster-constrained framework that transfers biologically compatible cell borders from in situ ST references to whole-transcriptome nuclei and reconstructs uncertainty-aware cellular territories.
Materials and Methods:
Mouse brain datasets comprising 130,870 cells profiled by an in situ ST platform and 839 nucleus-resolved ST were integrated. Candidate polygons were restricted to the matched cluster and ranked using polygon distance, local topology, expected cell-type-specific area, local density, and duplicate-assignment penalties. Model terms and weights were optimized by ablation, synthetic deformation, leave-one-out recovery, coordinate-jitter testing, and global one-to-one assignment. Translation-only, mild-scaling, and density-constrained territory fitting were compared using geometry, nuclear containment, retention, and overlap gates.
Results: In synthetic benchmarks, CellBOUND achieved 86.2% exact self-recovery, 97.5% true-template coverage, and 100% same-cluster recovery; held-out global one-to-one coverage remained 97% under perturbation. In real data, 770/839 assignments (91.8%) passed the biological assignment gate. Density-constrained fitting outperformed translation-only and mild-scaling, yielding a 90.9% hard-pass rate. It generated 700 accepted territories, representing 83.4% of all nuclei and 90.9% of gated nuclei. Cell-type-specific acceptance ranged from 84.6% to 100%.
Conclusion: CellBOUND reconstructs biologically plausible cell territories while preserving whole-transcriptome information. It enables more reliable analyses of cell morphology, physical contacts, spatial neighborhoods, and contact-dependent intercellular communication than nucleus-only representations.

Keywords: Spatial transcriptomics, Cell-boundary reconstruction, Nucleus-resolved transcriptomics, Multimodal data integration

OP10

BRD9 Inhibition Induces Selective Radiosensitivity in Glioblastoma Through MYC Pathway Modulation

Nareg Değirmenci[1], Serdar Aksel Çelikkol[1], Beyza Nur Köseoğlu[1], Adam P. Cribbs[2], Udo Oppermann[2], Uğur Selek[3], Tuğba Bağcı-Önder[1, 4]

[1] Brain Cancer Research and Therapy Lab, Koç University Research Center for Translational Medicine (KUTTAM), Istanbul, Türkiye
[2] Botnar Research Centre, University of Oxford, Oxford, United Kingdom
[3] Department of Radiation Oncology, Koç University School of Medicine, Istanbul, Türkiye
[4] Koç University School of Medicine, Istanbul, Türkiye.

Background/Aim: Radiotherapy is central to glioblastoma (GBM) treatment, yet resistance is nearly universal because tumour cells rapidly activate stress-adaptive survival programs. We aimed to determine whether the chromatin reader BRD9 gates this adaptive response and whether its inhibition can selectively sensitize GBM to radiation.
Materials and Methods: We performed an epigenetic drug screen under low-dose radiation and combined pharmacological inhibition (I-BRD9) and CRISPR-based genetic depletion of BRD9 with irradiation in GBM cell lines, non-malignant cells, and patient-derived models. DNA double-strand-break (DSB) kinetics (γH2AX/53BP1), apoptosis (Annexin V/propidium iodide), RNA sequencing, functional MYC rescue, and computational analysis of independent GBM patient cohorts were used to investigate the mechanism.
Results: BRD9 inhibition selectively enhanced radiation-induced lethality with minimal baseline cytotoxicity, delaying DSB resolution and increasing apoptosis after irradiation. The effect was selective for malignant and radiation-adapted GBM cells while sparing non-malignant cells. Transcriptomic profiling showed that BRD9 inhibition or depletion produced a coordinated, MYC-centered suppression of translational programs, including ribosome biogenesis, rRNA processing, tRNA aminoacylation, and translational initiation. Overexpression of MYC rescued the phenotype observed by BRD9 inhibition, confirming its regulatory role in BRD9-dependent radiation response. The BRD9–MYC program was conserved across independent clinical cohorts, with elevated BRD9 in recurrent versus primary tumours and coordinated BRD9–MYC co-expression in nine cohorts.
Conclusion: BRD9 is a context-dependent regulator of radiation-induced stress adaptation and a therapeutically actionable vulnerability. Combining BRD9 inhibition with radiotherapy may selectively improve treatment efficacy in glioblastoma.

Keywords: Glioblastoma, BRD9, MYC, Radiosensitization, Translational Control

OP11
Environmental Imprinting of Antitumor Memory: Emergence of KLRG1loCD62Lhi CD4⁺ T Cells in Triple-Negative Breast Cancer

Batuhan YAĞCIOĞLU[1, 2], Onur ETGÜ[1, 2], Haluk Barbaros ORAL[1, 2], Diğdem YÖYEN ERMİŞ[1, 2]

[1] Bursa Uludağ University, Faculty of Medicine, Department of Immunology
[2] Ursa Uludağ University, Faculty of Medicine, Laboratory Animal Breeding and Research Unit (DENHAB), Experimental and Molecular Immunology Research Group (ExPerIm)

Background/Aim: Antitumor immunity research has focused on immune cell composition, while the contribution of dynamic differentiation states to tumor control remains poorly understood. Memory T-cell repertoires are shaped by environmental conditions and accumulated antigenic exposure. We investigated whether distinct housing environments influence antitumor memory T-cell populations and alter tumor development and progression in a syngeneic triple-negative breast cancer (TNBC) model.
Materials and Methods: Female BALB/c mice, 6–8 weeks old, were maintained under two housing conditions: clean cage (CC) and dirty cage (DC). To establish the TNBC model, 4T1-conditioned medium (4T1-CM; 137 µg/mL) was injected into the mammary fat pad for 6 days, followed by syngeneic 4T1 cell injection on day 8. Control mice received culture medium alone or no treatment. Body weight and tumor growth were monitored for 21 days. At euthanasia, tumor, spleen, and thymus sizes and diameters were assessed. Memory T-cell populations were analyzed by flow cytometry using CD3,CD4,CD8,CD44,CD45.2,CD62L,CD69,CD103,CXCR3,CCR7, and KLRG1 markers.
Results: Our previous ex vivo findings showed that 4T1-CM stimulation induced a distinct CD4 memory precursor-like T-cell population characterized by a KLRG1^{lo}CD62L^{hi} phenotype. This population differed phenotypically from classical tissue-resident memory T cells and exhibited significant tumor-killing activity. In vivo preconditioning with 4T1-CM markedly suppressed tumor establishment and progression. Tumor formation was completely prevented in some animals, while developed tumors were smaller than those in the 4T1-only group. Differences between CC and DC groups further indicated that environmental conditions shape memory T-cell differentiation and influence the magnitude of antitumor immune responses.
Conclusion: These findings identify a distinct and functionally competent KLRG1^{lo}CD62L^{hi} CD4 memory precursor-like T-cell subset associated with improved tumor control. Further studies are needed to elucidate mechanisms regulating its differentiation and effector functions and to assess its translational potential for ex vivo expansion and adoptive T-cell transfer immunotherapy.

Keywords: Memory T cells, Triple-Negative Breast Cancer, Antitumor Immunity, Tumor Microenvironment, Adoptive Cell Transfer

OP12
Investigation of Protein Profile Changes in Glioblastoma Cells Treated With EDu

Öykü Bolulu[1], Evren Önay Uçar[2]

[1] Istanbul University
[2] Istanbul University

Background/Aim: Glioblastoma is an aggressive primary brain tumor with limited therapeutic options. 5-Ethynyl-2′-deoxyuridine (EdU), a synthetic thymidine analogue widely used to monitor DNA synthesis, has also been reported to exert cytotoxic effects. However, the proteomic mechanisms underlying EdU-induced cellular responses in glioblastoma remain poorly characterized. This study aimed to investigate the proteomic and molecular effects of EdU in glioblastoma cells and compare these responses with a non-malignant cell line.
Materials and Methods: U87-MG and LN-229 glioblastoma cells and HEK-293T cells were treated with EdU. Cytotoxicity was evaluated using the MTT assay, and 10 μM EdU was selected for subsequent analyses. Global protein profiles were characterized by liquid chromatography-tandem mass spectrometry (LC-MS/MS), followed by protein-protein interaction network analysis using STRING. Selected proteins associated with cellular stress, cell cycle regulation, autophagy, apoptosis, and immune response were validated by Western blotting.
Results: EdU exhibited dose- and time-dependent cytotoxicity, with U87-MG showing the highest sensitivity among the investigated cell lines. LC-MS/MS analysis revealed distinct proteomic profiles following EdU treatment, with protein networks associated with cellular stress, protein folding, epigenetic regulation, DNA damage response, and cell cycle control being prominent. HYOU1 and several heat shock proteins were uniquely detected in EdU-treated glioblastoma cells. Western blotting demonstrated increased HSF1, HSP70, HSP90, p53, and p21 expression and decreased CDK2 expression. Changes in ATG5, ATG7, and LAMP2A indicated modulation of autophagic pathways, while changes in the Bax/Bcl-2 ratio and Pro-caspase-3 levels were consistent with apoptotic signaling. PD-L1 expression was also increased in U87-MG cells.
Conclusion: These findings demonstrate that EdU induces broad molecular responses beyond its role as a DNA synthesis marker, involving cellular stress, cell cycle regulation, autophagy, apoptosis, and immune-related signaling. The results provide a proteomic framework for understanding EdU-induced responses in glioblastoma and support further investigation of its potential as an anticancer strategy.

Keywords: Glioblastoma, EdU, LC-MS/MS, Proteomics

OP13
Transposable Element Activities Drive Rapid Adaptation and Fitness Trade-Offs in a Fungal Pathogen

Dilay Hazal Ayhan[1, 2], Siyuan Wu[2], Serena Abbondante[3], Michaela Ellen Marshall[3], Eric Pearlman[3], Li-Jun Ma[2]

[1] Department of Molecular Biology and Genetics, Graduate School of Natural and Applied Sciences, Acibadem Mehmet Ali Aydinlar University, Istanbul
[2] Biochemistry and Molecular Biology, University of Massachusetts Amherst, Amherst
[3] Physiology and Biophysics and Ophthalmology, University of California Irvine, Irvine

Background/Aim: Fungal pathogens pose increasing threats to global food security and public health due to their capacity for rapid adaptation to novel environments. In the cross-kingdom pathogen Fusarium oxysporum, this adaptability is largely attributed to a "two-speed" genome architecture consisting of conserved core chromosomes (CCs) and rapidly evolving accessory chromosomes (ACs). While ACs are repeat-rich and enriched in host-specific virulence factors, the mechanisms that drive adaptation during niche transitions remain poorly characterized.
Materials and Methods: Using a short-term experimental evolution framework, we passaged a keratitis-causing F. oxysporum strain through mouse corneas, as well as in vitro in rich or minimal media at 28°C and under heat stress at 34°C. Through whole-genome resequencing and stress-response phenotyping of evolved populations, we identified genetic variations and used machine learning models to predict the top genes strongly associated with observed traits.
Results: Our results revealed that specialization in independent lineages resulted in an evolutionary cost, as seen in the loss of adaptation to ancestral hosts or niches when they were absent. Resequencing of the evolved populations identified transposable element (TE) insertions as the predominant driver of genetic variation, with the strain-specific SINE transposon, Foxy5, accounting for more than 90% of the new TE insertions. We also observed that the number of TE insertion events significantly increased at elevated temperatures. ACs served as hotspots for TE activity, with some transposition events possibly altering the expression of nearby genes, impacting heat tolerance and virulence.
Conclusion: These findings demonstrate that the F. oxysporum genome undergoes rapid remodeling to navigate environmental shifts, facilitated by TE activity and governed by inherent trade-offs that constrain fungal adaptation.

Keywords: Fungal pathogens, Genomics, Transposable Elements, Adaptation, Evolution

OP14
The Paradoxical Origin of Small Molecule Plant Hormones, Cytokinins in Mailman Systems

Muhammad Naseem[1, 2]

[1] Zayed University, Abu Dhabi
[2] University of Wuerzburg, Germany

Background/Aim: Cytokinins (CKs) are adenine-derived metabolites traditionally recognized as plant hormones, yet their occurrence, origins, and functions in mammals remain poorly understood. Here, **Materials and Methods:** we integrated metabolomics, microbiome profiling, and metagenomic analyses to characterize CKs in mammals and investigate their dietary and microbial determinants.
Results: Serum profiling across five animal species consistently detected multiple CK derivatives, with zeatin-O-glucoside predominating, followed by trans-zeatin and kinetin, at concentrations substantially lower than those in plant tissues. Species-specific differences suggested divergent regulatory patterns. In mice, CKs were detected in vascular tissues of the kidney, heart, and liver, indicating systemic distribution. Starvation markedly reduced CK abundance in serum, colon, feces, and urine, supporting diet as a major contributor to the mammalian CK pool. Meta-omics identified CK-related genes across diverse gut microbial taxa, while germ-free mice exhibited substantially lower CK levels than conventionally raised mice, demonstrating a microbiome-dependent contribution. We further investigated the microbiota–cytokinin axis in allergic contact dermatitis (ACD). TNCB exposure increased ear swelling and IgE levels, and disrupted gut microbial structure, which was restored by kinetin application.
Conclusion: Collectively, our findings establish CKs as diet- and microbiome-modulated metabolites in mammals and suggest a potential microbiota–cytokinin axis in inflammatory disease, highlighting kinetin as a candidate modulator of host–microbiome interactions and the importance of dose optimization.

Keywords: Cytokinins , Gut-Food-Microbiomes Nexus, Oxidative Stress, TRIT1

OP15
Genomic Investigation of Central Asian Related Ancestry in Present-day Turkish Population

Seher Elif Mekik[1, 2], Ercan Arıcan[3]

[1] Dept. Of Molecular Biotechnology and Genetics, Institute of Graduate Studies in Sciences, Istanbul University, Istanbul
[2] Dept. Of Biotechnology, Institute of Nanotechnology and Biotechnology, Istanbul University-Cerrahpaşa, Istanbul
[3] Dept. Of Molecular Biology and Genetics, Faculty of Science, Istanbul University, Istanbul

Background/Aim: Anatolia has long been a crossroad of population movements between Europe, the Near East, and Asia. This study aimed to investigate the presence, magnitude, and timing of Central/Inner Asian related genetic ancestry in present-day Turkish populations using ancient and modern genomic data.
Materials and Methods: Genome-wide data from the Allen Ancient DNA Resource (AADR) were analyzed, including Iron Age, Roman, Byzantine, Ottoman, and modern Anatolian groups together with reference populations from Central Asia, East Asia, Siberia, and the Eurasian Steppe. Population structure and genetic affinities were assessed using Principal Component Analysis (PCA), ADMIXTURE, and f-statistics. Admixture models were tested with qpAdm and admixture dates were estimated using DATES.
Results: Pre-1071 Anatolian groups predominantly showed a West Eurasian genetic profile, whereas present-day Turks and some Ottoman-period groups displayed additional Central/East Eurasian affinity. In present-day Turks, 94 qpAdm models met all acceptance criteria, with a mean Central/Inner Asian related ancestry estimate of 13.7%. In the Ottoman-period Çapalıbağ group, accepted models indicated 27.8-39.5% Central/Inner Asian-related ancestry, with a mean of 34.2%. In contrast, no comparably consistent signal was detected in post-1071 Muğla and Gaziantep groups or in the general Ottoman sample. DATES estimated the admixture in present-day Turks at approximately 32 generations ago, centered around the 11th century CE, while the Çapalıbağ group yielded estimates spanning between the 11th-12th centuries CE.
Conclusion: These findings support a predominantly local Anatolian–West Eurasian genetic background in present-day Turkish population, with a significant but heterogeneous Central/Inner Asian contribution associated with medieval Turkic migrations.

Keywords: Ancient DNA, Population Genomics, Turkish Population, Central Asia

OP16
Identification and Functional Engineering of T Cell Receptors from Tumor-Infiltrating Lymphocytes into Natural Killer Cells (TCR-NK)

Elif Çelik[1], Betül Çakıcı[2], Beyza Nur Uysak[2], Enes Kardeş[3], Mehmet Karaçay[4], Batuhan Yağcıoğlu[5], Serra Özarı[3], Serkan Yazıcı[6], Süleyman Çeçen[7], Diğdem Yöyen Ermiş[8], Haluk Barbaros Oral[8], Tolga Sütlü[1, 2, 9]

[1] Acıbadem Mehmet Ali Aydınlar University, Graduate School of Health Sciences, Department of Translational Medicine
[2] Acıbadem Mehmet Ali Aydınlar University, Graduate School of Natural and Applied Sciences, Department of Molecular Biology and Genetics
[3] Boğaziçi University, Faculty of Sciences, Department of Molecular Biology and Genetics
[4] Van Yüzüncü Yıl University, Faculty of Medicine, Department of Internal Medicine, Division of Medical Genetics
[5] Bursa Uludağ University, Institute of Health Science, Department of Medical-Immunology
[6] Bursa Uludağ University, Faculty of Medicine, Department of Dermatology and Venereal Diseases
[7] Bursa Uludağ University, Faculty of Medicine, Department of Plastic, Reconstructive and Aesthetic Surgery
[8] Bursa Uludağ University, Faculty of Medicine, Department of Immunology
[9] Acıbadem Mehmet Ali Aydınlar University, Faculty of Engineering and Natural Sciences, Department of Molecular Biology and Genetics

Background/Aim: T-cell receptor (TCR)-based immunotherapies occupy a unique and promising niche for targeting intracellular tumor-specific antigens that would otherwise be inaccessible to therapeutic approaches such as monoclonal antibodies, bispecific engagers or CAR-T cells. As T cells already express endogenous TCRs, transferring tumor-specific TCRs into T cells is inefficient and risks TCR chain mispairing. NK cells lack endogenous TCR expression and avoid this risk, making them better suited for off-the-shelf production of TCR-based therapies. This study aimed to identify tumor-specific TCRs via single-cell sequencing of melanoma-derived Tumor-infiltrating-lymphocytes (TILs), and to engineer them into NK cells using the TCR-NK platform.
Materials and Methods: TIL cultures from eleven melanoma patients were evaluated and TILs from three patients were subjected to single-cell RNA and paired TCRα/β sequencing. Clonally expanded, potentially tumor-reactive TCR clonotypes were used to design eleven candidate TCRs and introduced into NK-92 cells modified to express CD3 chains of the TCR complex. Surface TCR/CD3 assembly was confirmed by flow cytometry and antitumor activity was assessed by CD107a degranulation assays and live-cell imaging against a panel of 11 cancer cell lines. K562-based feeder cells were used for GMP-compatible primary NK cell expansion, and five lead TCR constructs were transduced into NK cells from healthy-donor PBMCs.
Results: These analyses revealed expanded, tumor-reactive TCR repertoires from melanoma TILs. Tetramer staining together with degranulation and live-cell imaging assays confirmed that reconstitution of the CD3 complex enables functional, antigen-specific TCR expression and cytotoxicity in NK cells. The engineered feeder platform supported robust NK cell expansion, and five lead TCRs were successfully transduced into primary NK cells, confirming feasibility.
Conclusion: This study establishes a translational pipeline for discovering and validating tumor-specific TCRs and engineering them into an off-the-shelf TCR-NK platform for melanoma.
Supported by TÜBİTAK 1003 (Project No: 218S897) and TÜBİTAK 2247-A (Project No: 123C511).

Keywords: TCR-NK Cells, Tumor-infiltrating lymphocytes, Melanoma, Single-cell sequencing

OP17
Engineering Lipid Nanoparticle Coronas to Overcome Hepatic Sequestration and Enable Extrahepatic mRNA Delivery

Alisan Kayabolen[1, 2, 3, 4, 5, 6], Cian Schmitt-Ulms[1, 3, 4, 2], Alexander Elsener[1, 3, 5], Francesca Ferraresso[1, 3, 2], Keira Donnelly[1, 3, 2, 4], Angela Xinyi Nan[1, 3, 2], Ian Harris[1, 3, 2], Samantha Sgrizzi[1, 3, 2, 4], Aroosa Anwer[1, 2, 3], Sabrina Pia Nuccio[1, 2, 3], Patrick T. Paine[1, 2, 3, 4], Sanah Langer[1, 3, 2], Christopher Fell[1, 2, 3, 4], Jonathan S. Gootenberg[1, 2, 3, 4], Omar O. Abudayyeh[1, 2, 3, 4]

[1] Division of Engineering in Medicine, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, 02115 MA, USA
[2] Gene and Cell Therapy Institute, Mass General Brigham, Cambridge, 02139 MA, USA
[3] Center for Virology and Vaccine Research, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, 02115 MA, USA
[4] McGovern Institute for Brain Research, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
[5] Translational Advanced Research on Gene Engineering & Therapeutics (TARGET) Lab, Koç University School of Medicine, 34450 Istanbul, Turkey
[6] Koç University Research Center for Translational Medicine (KUTTAM), 34450 Istanbul, Turkey

Background/Aim: Systemically administered lipid nanoparticles (LNPs) predominantly accumulate in the liver due to the spontaneous adsorption of serum Apolipoprotein E (ApoE), which mediates hepatic uptake via low-density lipoprotein receptors (LDLR). This pronounced liver tropism severely restricts extrahepatic nucleic acid delivery and clinical translational potential. Here, we aimed to develop a modular and programmable platform that simultaneously bypasses hepatic sequestration and retargets LNPs to specific extrahepatic cell types in vivo.
Materials and Methods: LNPs encapsulating reporter (fLuc/GFP) or therapeutic mRNAs were formulated using microfluidics/T-tube mixing with ionizable lipids, DSPC, cholesterol, PEG-lipids, and DSPE-PEG-maleimide. To achieve liver detargeting, we engineered LDLR-binding-defective ApoE mutants (dApoE) that retain lipid-binding capacity, or utilized hyperactive PCSK9 (haPCSK9) to downregulate surface LDLR. For active retargeting, targeting antibodies functionalized via SATA-maleimide chemistry were conjugated to LNPs and purified by size-exclusion chromatography. LNP dynamics, protein binding, cell-specific delivery, and in vivo bio-distribution were systematically evaluated.
Results: Pre-coating LNPs with dApoE or cellular pretreatment with haPCSK9 significantly reduced LDLR-mediated hepatic uptake in vitro and reduced in vivo liver transduction by up to 90%. Integrating this detargeting system with antibody conjugation redirected LNPs precisely to T cells, brain tissue, and pulmonary endothelial cells. Furthermore, systemic administration of CD5-targeted, dApoE-coated LNPs successfully generated functional CAR-T cells in vivo with robust cytotoxic activity against CD19+ target cells while minimizing hepatic exposure.
Conclusion: This dual-action platform combines efficient liver detargeting with antibody-mediated retargeting, expanding the therapeutic scope of LNP-based mRNA therapeutics. Building upon these findings, our laboratory at Koç University focuses on adapting this modular framework for targeted delivery across the blood-brain barrier and engineered cell therapies.

Keywords: lipid nanoparticles, mRNA delivery, protein corona, targeted therapy, cell engineering

OP18

De Novo Microbial Biosynthesis of 4-Vinylcatechol and Evaluation of Its Selective Anticancer Effects

Esra Kaynak[1], Arzu Aysan[1], Hüseyin Balcı[1], Merve Seven[2], Hülya Akdemir Koç[1]

[1] Gebze Technical University, Faculty of Science, Molecular Biology and Genetics, 41400, Kocaeli, Türkiye
[2] Yeditepe University, Faculty of Engineering and Natural Sciences, Department of Genetics and Bioengineering, 34755, Istanbul, Türkiye

Background/Aim: 4-Vinylcatechol (VCAT) is a high-value phenolic compound with potent antioxidant and potential anticancer properties. Its broader application is hindered by extraction limitations and chemical synthesis costs. This study aims to achieve the de novo microbial biosynthesis of VCAT in engineered Escherichia coli and evaluate its metabolic and molecular effects on human cancer cells.
Materials and Methods: The phenolic acid decarboxylase (pad) genes from different Bacillus subtilis strains were cloned and co-expressed with a caffeic acid biosynthesis module in E. coli rpoA14(DE3) and BL21(DE3) hosts. VCAT production was quantified via high-performance liquid chromatography (HPLC). Cytotoxicity was assessed using the MTT assay on lung carcinoma (H292), and healthy cells. Reactive oxygen species (ROS) generation, apoptosis, cell cycle arrest, and gene expression were analyzed using flow cytometry and quantitative real-time PCR (RT-qPCR).
Results: The engineered rpoA14(DE3) strain expressing the pad variants successfully yielded high VCAT titers in flask cultures without exogenous precursor feeding. VCAT demonstrated selective cytotoxicity against cancer cells compared to healthy cell lines. Molecular analyses revealed that VCAT acted as a pro-oxidant in glioblastoma cells, significantly increasing ROS levels and inducing cell cycle arrest alongside apoptosis. Despite the compensatory activation of the Nuclear factor erythroid 2-related factor 2 (Nrf2) antioxidant pathway that marked by upregulated target gene expressions, the cancer cells ultimately succumbed to the severe redox imbalance.
Conclusion: A sustainable, high-yield microbial platform for de novo VCAT production was successfully established using alternative enzyme sources. VCAT exhibited significant and selective anticancer activity by disrupting cellular redox homeostasis, positioning it as a promising therapeutic candidate for further oncological studies.
Funding: This study was financially supported by the TÜSEB under Grant No. 42263 and GTÜ BAP Grant No. 2026-102-3. Esra Kaynak was financially supported by the BİDEB 2211-A Program.

Keywords: 4-Vinylcatechol, Microbial Production, Phenolic Acid Decarboxylase, Reactive Oxygen Species, Anticancer Agent

OP19

Receptor-interacting Protein Kinase 4 (RIPK4) Deficiency Modulates the Long Mon-coding RNAs (lncRNAs) Expression During Keratinocyte Differentiation

Ümit UZUN[1], Gökhan YILDIZ[2], Ersan KALAY[2], Tuba DİNÇER[1, 2]

[1] Karadeniz Technical University, Drug and Pharmaceutical Technology Application and Research Center, Trabzon, Türkiye
[2] Karadeniz Technical University, Faculty of Medicine, Department of Medical Biology, Trabzon, Türkiye

Background/Aim: The epidermal barrier is established through the tightly regulated differentiation of basal keratinocytes into stratified epidermal layers. RIPK4 is an essential regulator of keratinocyte differentiation. Increasing evidence indicates that keratinocyte differentiation is also regulated by epigenetic mechanisms, with lncRNAs emerging as key regulators of this process. Although RIPK4 signaling has been extensively studied, the lncRNA landscape associated with RIPK4-mediated keratinocyte differentiation has not yet been systematically characterized. This study aimed to characterize dynamic changes in the lncRNA transcriptome induced by RIPK4 deficiency during *in vitro* keratinocyte differentiation.
Materials and Methods: RIPK4 knockout (KO) HaCaT cells were generated using CRISPR/Cas9 system. Wild-type and RIPK4-KO cells were subjected to calcium-induced differentiation *in vitro*. Cells collected at differentiation days 0, 7, and 14 were analyzed by RNA sequencing. Differentially expressed lncRNAs between wild-type and RIPK4-KO cells at each differentiation stage were identified using DESeq2. Annotation and filtering were performed using Ensembl (GRCh38.p14), followed by BioMart-assisted characterization of genomic features.
Results: Consistent with our previous findings, KO cells maintained a stem cell-like phenotype throughout the differentiation process, whereas control HaCaT cells underwent progressive differentiation. Differential lncRNA expression between KO and control cells increased over the course of differentiation, with 21, 39, and 70 transcripts detected on days 0, 7, and 14, respectively. Across all differentiation stages, structural and genomic characterization showed that differentially expressed lncRNAs were distributed across all chromosomes, predominantly contained two exons, and were 500–1000 bp in length. Among differentially expressed lncRNAs SALRNA1 (log2FoldChange $\geq$1,5; adj p$\leq$ $5x10^{-2}$), and DANT1 (log2FoldChange$\leq$-7; adj p$\leq$$1x10^{-10}$) were of particular interest, as they have previously been reported as a negative regulator of cellular senescence and a stem cell-state-associated lncRNA, respectively.
Conclusion: Together, our findings suggest that stem cell state- and senescence-associated lncRNAs may contribute to the impaired differentiation observed in RIPK4-KO keratinocytes.

This work was supported by TÜBİTAK-1001 (Project No:118Z124)

Keywords: Keratinocyte differentiation, LncRNA, RIPK4, RNAseq, Transcriptomics

OP20
Evaluation of Maternal Plasma Growth Factors in Major Fetal Congenital Cardiac Anomalies

Sibel Bulgurcuoğlu Kuran[1], Sena Öner[2], Birsen Karaman[1]

[1] Department of Medical Genetics, Istanbul Faculty of Medicine, Istanbul University, Istanbul
[2] Medical Laboratory Techniques Program, Department of Medical Services and Techniques, Vocational School, Biruni University, Istanbul
[3] Department of Medical Genetics, Istanbul Faculty of Medicine, Istanbul University, Istanbul

Background/Aim: Major fetal congenital cardiac anomalies (CCA) are a major cause of perinatal morbidity and mortality. Vascular endothelial growth factor-C (VEGF-C) and fibroblast growth factor-19 (FGF-19), involved in angiogenesis and embryonic development, may contribute to fetal cardiac development. This study investigated the association between maternal plasma VEGF-C and FGF-19 levels and major fetal CCA.
Materials and Methods: Forty-two pregnancies with major fetal CCA and 21 healthy pregnancies were included. Maternal plasma samples were collected before invasive prenatal diagnostic procedures and stored at −80°C until analysis. VEGF-C and FGF-19 were measured using commercial ELISA kits. Group comparisons were performed using the Mann–Whitney U test. Analysis of covariance (ANCOVA) adjusted for maternal and gestational age. Spearman correlation and receiver operating characteristic (ROC) analyses were performed.
Results: Gestational age was higher in the CCA group than in controls (23.1 ± 5.2 vs. 20.0 ± 5.1 weeks, p=0.028), whereas maternal age, smoking status, and fetal sex were similar. Median (IQR) VEGF-C levels were 577 (526–720) vs. 559 (523–630) pg/mL (p=0.67), and FGF-19 levels were 82 (61–169) vs. 72 (55–279) ng/L (p=0.65) in the CCA and control groups, respectively. ANCOVA confirmed no significant differences (VEGF-C, p=0.55; FGF-19, p=0.95). FGF-19 showed a weak negative correlation with maternal age (r=−0.25, p=0.046). ROC analysis showed poor discrimination (AUC: 0.53 for VEGF-C and 0.54 for FGF-19).
Conclusion: Maternal plasma VEGF-C and FGF-19 levels were not associated with major fetal CCA and demonstrated poor diagnostic performance. The limited sample size and heterogeneous nature of CCA may have reduced the ability to detect small effects. Larger studies with more homogeneous populations are needed to clarify the clinical value of these biomarkers in prenatal diagnosis.

Keywords: Prenatal diagnosis, Maternal plasma, VEGF-C, FGF-19, Congenital cardiac anomalies

OP21
Molecular Mechanism and Functional Consequences of the SETD3–BRD2 Interaction in Mouse Embryonic Stem Cells

Dersu SEZGİNMERT[1], Nihal TERZİ ÇİZMECİOĞLU[1]

[1] Middle East Technical University

Background/Aim: Embryonic stem cell differentiation requires coordinated epigenetic regulation of chromatin and gene expression. SET domain-containing protein 3 (SETD3) has previously been implicated in endodermal differentiation of mouse embryonic stem cells (mESCs); however, the mechanisms linking SETD3 to chromatin-dependent transcription remain unclear. This study aimed to characterize the functional interaction between SETD3 and bromodomain-containing protein 2 (BRD2), a chromatin reader involved in developmental gene regulation.
Materials and Methods: The SETD3–BRD2 interaction was investigated using co-immunoprecipitation, chromatin fractionation, and streptavidin pull-down approaches. BRD2 chromatin occupancy in wild-type (WT) and setd3Δ mESCs was examined by chromatin immunoprecipitation sequencing (ChIP-seq). SETD3 occupancy was assessed by cleavage under targets and release using nuclease sequencing (CUT&RUN-seq). These datasets were integrated with histone modification profiles and previously generated RNA sequencing data to evaluate transcriptional consequences.
Results: SETD3 and BRD2 physically interact in the nucleus, with evidence supporting a chromatin-associated interaction. Loss of SETD3 caused a marked reduction in BRD2 occupancy around transcription start sites. Genes associated with SETD3-dependent BRD2 binding showed reduced levels of active chromatin marks, including H3K4me3, H3K36me3, and histone H4 acetylation. These chromatin alterations were accompanied by reduced expression of associated genes, including genes involved in pluripotency, signaling, and differentiation-related processes.
Conclusion: Our findings identify SETD3 as an important regulator of BRD2 chromatin occupancy in mESCs. The SETD3–BRD2 interaction supports active chromatin states and transcription at developmentally relevant genes, providing a potential mechanism through which SETD3 contributes to early lineage commitment. This work was supported by TÜBİTAK 121Z497 and TEZ-D-108-2022-10818 projects.

Keywords: SETD3, BRD2, embryonic stem cells, chromatin, differentiation

OP22
CD25 Overexpression as a Novel Approach to Enhance NK Cell-Based Immunotherapies

Betül Çakıcı[1], Elif Çelik[2], Beyza Nur Uysak[1], Tolga Sütlü[1, 2, 3]

[1] Acıbadem Mehmet Ali Aydınlar University, Graduate School of Natural and Applied Sciences, Department of Molecular Biology and Genetics, İstanbul
[2] Acıbadem Mehmet Ali Aydınlar University, Graduate School of Health Sciences, Department of Translational Medicine, İstanbul
[3] Acıbadem Mehmet Ali Aydınlar University, Faculty of Engineering and Natural Sciences, Department of Molecular Biology and Genetics, İstanbul

Background/Aim: Natural killer (NK) cells are promising candidates for cancer immunotherapy; however, their therapeutic applications can be limited by poor in vivo persistence. Interleukin-2 (IL-2) is an important cytokine for NK cell activity that is not only used for ex vivo NK cell expansion but also as in vivo support after infusion of cells to a patient. IL-2 receptor α chain (CD25) enables the formation of the high-affinity IL-2 receptor, but its expression is lacking in the majority of NK cells. This study aimed to investigate whether CD25 overexpression could enhance NK -cell survival under low-dose IL-2 conditions and provide a selective approach for enriching genetically modified NK cells without compromising cytotoxic function.
Materials and Methods: NK-92 cells were genetically modified using lentiviral vectors encoding CD25 together with fluorescent reporters. To evaluate the effect of CD25 overexpression on IL-2 dependency and selective expansion, control and CD25-expressing cells were subjected to different IL-2 concentrations and monitored longitudinally by cell counting and flow cytometry. Cytotoxic function was assessed by measuring intracellular perforin and granzyme B levels and degranulation against K562 target cells.
Results: CD25 overexpression enhanced NK-92 cell survival and competitive expansion under low-dose IL-2 conditions. CD25-expressing cells became selectively enriched over 90% at 10 and 30 U/mL IL-2, whereas no substantial enrichment was observed in control cells or at higher IL-2 doses. CD25-overexpressing cells also showed modest increases in perforin and granzyme B levels, while CD107a-based degranulation against K562 cells remained largely comparable to control cells, indicating preserved cytotoxic function.
Conclusion: CD25 overexpression reduced the IL-2 dependence of NK-92 cells and enabled selective enrichment under low-dose IL-2 conditions without compromising their cytotoxic function. These findings support CD25 as a potential selection marker for genetically modified NK-cell products and provide a rationale for further evaluation in primary NK cells and therapeutic models.

Keywords: CD25, interleukin-2, NK cells, cancer immunotherapy, lentiviral vectors

OP23

Induced Pluripotent Stem Cell-Derived Monocytes Model Dysregulated Cell Migration in Mevalonate Kinase Deficiency and Familial Mediterranean Fever

Başak Şen[1], Mehmet Emre Özkan[1], Tayfun Hilmi Akbaba[1], Yeliz Z. Akkaya-Ulum[1], Müşerref Kasap Cüceoğlu[2], Fatima Aerts-Kaya[3], Ömer Karadağ[4], Seza Özen[5], Banu Balcı-Peynircioğlu[1]

[1] Hacettepe University, Faculty of Medicine, Department of Medical Biology
[2] Konya City Hospital, Department of Pediatrics, Pediatric Rheumatology Division
[3] Hacettepe University Center for Stem Cell Research and Development
[4] Hacettepe University, Faculty of Medicine, Department of Rheumatology
[5] Hacettepe University, Faculty of Medicine, Department of Pediatric Rheumatology

Background/Aim: Mevalonate Kinase Deficiency (MKD) and Familial Mediterranean fever (FMF) are two autoinflammatory diseases linked to the pyrin inflammasome. Activation of the pyrin inflammasome has been linked to increased inflammatory cell migration in patient monocytes and neutrophils. However, the rarity of these diseases and the short life of blood-derived cells makes access to primary cell-based models difficult. We aimed to create a new induced pluripotent stem cell-derived monocyte model for these diseases to investigate inflammatory cell migration.
Materials and Methods: Skin biopsies from 7 patients [3 MKD (MVK: V377I +/+, V377I +/+, M300V+/+), 3 FMF (MEFV: M694V +/+, M694V +/+, V726A +/+), 1 healthy control] were used to establish primary fibroblast cultures. Epi5 Episomal iPSC Reprogramming Kit was used to generate iPSC colonies, which were tested for pluripotency markers by qRT-PCR, immunofluorescence (IF) staining and STEMDiff Trilineage Kit. Suitable clones were used for monocyte differentiation with the STEMDiff Monocyte Kit. Resulting cells were characterized with flow cytometry and subjected to in vitro chemotaxis assay with MCP-1. Migrated cells were quantified with flow cytometry.
Results: Patient-specific iPSCs were shown to express pluripotency markers SOX2, OCT3/4, NANOG and TRA-1-81 with qRT-PCR and IF. Functional pluripotency was demonstrated with IF staining against germ layer-specific proteins. iPSC-derived monocytes (iPSDMs) were generated and confirmed with flow cytometry to be CD45+CD11b+CD14+. Chemotaxis assays revealed migration was increased in FMF cells, and decreased in MKD cells compared to control.
Conclusion: This study demonstrates a cell migration defect in MKD-iPSDMs, compared to FMF and control-iPSDMs. This finding is consistent with previous research in human monocytes and THP-1 models. The mechanisms leading to this defect remain unclear. Our study presents iPSDMs as a viable alternative to model rare autoinflammatory diseases, introducing iPSCs containing novel mutations to the literature.
This project was supported by TÜBİTAK (SBAG-1001-122S379) and Hacettepe University Scientific Coordination Unit (THD-2024-21311).

Keywords: autoinflammation, cell migration, induced pluripotent stem cells

OP24

TLR7/8 Stimulation Drives a Disease-Specific Cytokine and Gut-Homing Signature in Crohn's Disease Eosinophils Underpinned by a NOD2–CARD9 Axis

Elif Yaprak Sarac[1], Orhan Tat[1], Ece Dervis[1], Sude Kara[1], Edanur Barutcu[1], Onur Olgac Karagulle[2], Ceren Cıracı[1]

[1] Department of Molecular Biology and Genetics, Istanbul Technical University, Istanbul, Türkiye
[2] Department of General Surgery, Istanbul Training and Research Hospital, University of Health Sciences, Istanbul, Türkiye

Background/Aim: Crohn's disease (CD) is a chronic inflammatory bowel disease genetically linked to nucleotide-binding oligomerization domain-containing protein 2 (NOD2) and its adaptor caspase recruitment domain-containing protein 9 (CARD9). Eosinophils are increasingly implicated in antiviral immunity, yet their Toll-like receptor 7/8 (TLR7/8)-driven responses in CD remain undefined. We characterized the cytokine, gut-homing, and degranulation phenotype of primary blood eosinophils from CD patients versus healthy donors under viral-mimicking TLR7/8 stimulation, and dissected the NOD2–CARD9 axis in the EoL-1 eosinophilic cell line.
Materials and Methods: Eosinophils from CD patients and healthy donors were isolated by negative selection and cultured 24 h with TLR7/8 agonists R848 or ssRNA40. We assessed viability (7-AAD), degranulation (CD63), and gut-homing markers (CCR9, α4β7 integrin) by flow cytometer; measured extracellular IFN-α2, IL-1α, IL-1Ra, IL-4, and IL-10 by ELISA; measured NOD2/CARD9 mRNA by qRT-PCR. In EoL-1 cells, NOD2 or CARD9 were silenced by siRNA before TLR7/8 stimulation, with mRNA analyzed by qRT-PCR, protein by immunocytochemistry, and cytokines by ELISA.
Results: Viability (>90%) and CD63 were not affected. CD eosinophils had higher CCR9 and α4β7 expressions ($p<0.05$) than healthy donors, and α4β7 further induced by R848 ($p<0.001$). Cytokines were largely undetectable in healthy donors but measurable in CD eosinophils: ssRNA40 selectively induced IFN-α2 ($p<0.05$), R848 induced IL-1Ra ($p<0.05$), and ssRNA40 suppressed IL-10 ($p<0.05$), whereas IL-1α and IL-4 were not differentially expressed. NOD2 mRNA was much higher in CD ($p<0.05$) than healthy donors, while ssRNA40 induced NOD2 and CARD9 in healthy donors. In parallel, siRNAs downregulated NOD2/CARD9 mRNAs in EoL-1 cells; and NOD2 silencing decreased CARD9, indicating a unidirectional NOD2-to-CARD9 axis while IL-1β secretion did not change.
Conclusion: CD eosinophils display a disease-specific, TLR7/8-driven gut-homing and cytokine phenotype supported by a NOD2-dependent CARD9 axis, and may contribute to altered antiviral responses and enhanced intestinal migration in CD.

Keywords: Crohn's disease, eosinophils, TLR7/8, NOD2/CARD9, cytokine secretion

OP25
Investigation of Multiple Sclerosis Associated LAMA5 Variants on Endothelial Barrier Function and Immune-Cell Transmigration

Umut Voyvoda[1], Kerem Cotuk[2], Hanbike Efe[1], Tugba Ustu[1], Irmak Komurcu[2], Abdullah Alper Bulbul[3], Ahmet Can Timucin[4], Umut Inci Onat[4], Eda Tahir Turanli[4]

[1] Department of Molecular Biology and Genetics, Graduate School of Natural and Applied Sciences, Acibadem University, Istanbul, Türkiye
[2] Department of Molecular Biology and Genetics, Faculty of Engineering and Natural Sciences, Acibadem University, Istanbul, Türkiye
[3] Department of Biostatistics and Bioinformatics, Graduate School of Health Sciences, Acibadem University, Istanbul, Türkiye
[4] Graduate School of Natural and Applied Sciences, Acibadem University, Istanbul, Türkiye

Background/Aim: Multiple sclerosis (MS) involves blood–brain barrier (BBB) breakdown and immune-cell infiltration into the CNS. Laminin alpha-5 (LAMA5), a principal endothelial basement-membrane component, regulates barrier integrity and restricts leukocyte passage. Whole-exome sequencing of Turkish MS families identified candidate LAMA5 variants of unknown functional consequence. We therefore investigated how these variants impair endothelial barrier function and permit immune-cell infiltration, and whether they act through shared or domain-dependent mechanisms.
Materials and Methods: Three missense variants (p.Lys812Thr, p.Arg1369Gln, p.Thr3126Ile) and a LAMA5 knockout (LAMA5-KO) were engineered in HUVECs by CRISPR base and prime editing, then characterized by FoldX stability prediction, qPCR, bulk RNA sequencing with gene-set variation analysis (GSVA), Western blotting, transendothelial electrical resistance (TEER), Transwell PBMC transmigration, flow cytometry, and MIP-1alpha/IFN-gamma ELISA.
Results: Each LAMA5 variant elicited a distinct endothelial phenotype. The EGF-like-domain variants were destabilizing (ΔΔG +1.44 and +0.94 kcal/mol) but acted passively: p.Lys812Thr upregulated ZO-1 (~2.3-fold, $p<0.05$) with concomitant VE-cadherin loss and increased transmigration (~1.4-fold, $p<0.01$) despite preserved TEER, whereas p.Arg1369Gln reduced TEER ($p<0.05$) with minimal inflammatory activation. In contrast, the integrin-binding variant p.Thr3126Ile (ΔΔG −0.43 kcal/mol) drove an active, pro-inflammatory phenotype: ICAM1 induction (~3.7-fold, $p<0.01$), increased transmigration (~1.4-fold, $p<0.01$), and elevated MIP-1alpha and IFN-gamma ($p<0.01$), all with preserved TEER. NK cells were enriched in the transmigrated fraction (~2–3-fold of CD45+) across all conditions. LAMA5-KO produced the broadest transcriptional response, downregulated VE-cadherin (~0.4-fold, $p<0.01$), upregulated ITGB1 (~1.45-fold, $p<0.05$), and showed the greatest barrier loss (~50% TEER reduction, $p<0.01$), with a compensatory interferon/innate-immune signature by GSVA. Transcriptomic clustering separated p.Thr3126Ile from the co-clustering EGF-like variants.
Conclusion: MS-associated LAMA5 variants do not converge on a uniform loss of function but segregate into distinct, domain-dependent programs: passive destabilization (EGF-like) versus active pro-inflammatory, gain-of-function signaling with an intact barrier (LG). These findings support a contribution of basement-membrane genetic variation to MS pathogenesis.

Keywords: Multiple Sclerosis, LAMA5, Blood Brain Barrier, CRISPR, Immune Cell Infiltration

OP26

HPat Knockdown Reveals Synaptic and Molecular Defects in a Drosophila Model of PATL1-Related Neurodevelopmental Disorders

İlayda Yenen[1, 2, 3], Berfin Dağ[1, 2], Abdullah Sezer[4], Gülsüm Kayhan[5], Ferda Perçin[5], Arzu Çelik Fuss[1, 2, 3]

[1] Department of Molecular Biology and Genetics, Bogazici University, 34342 Bebek, Istanbul, Turkiye
[2] Center for Life Sciences and Technologies, Bogazici University, 34342 Bebek, Istanbul, Turkiye
[3] Center for Targeted Therapy Technologies (CT3), Bogazici University, Kandilli Campus, Cengelkoy, Istanbul 34685, Turkiye
[4] Ankara Etlik Hastanesi, Ankara, Turkiye
[5] Gazi University Medical School, Ankara, Turkiye

Background/Aim: PATL1 is a conserved RNA-regulatory protein best known for roles in processing bodies (P-bodies) and cytoplasmic mRNA regulation. Pat1 proteins have also been implicated in nuclear pre-mRNA processing. Rare truncating PATL1 variants are associated with neurodevelopmental phenotypes, including speech delay and motor impairment. We investigated how downregulation of the Drosophila ortholog HPat affects nervous-system development, synaptic function, and RNA regulation.
Materials and Methods: HPat was downregulated by tissue-specific RNA interference in Drosophila. Brain and neuromuscular junction (NMJ) morphology, and larval locomotion were examined using immunohistochemistry and behavioral assays. Synaptic vesicle cycling was assessed using FM1-43 uptake and release. Human wild-type and C-terminally truncated PATL1 were tested for rescue experiments. Whole-larval RNA-seq assessed differential expression and alternative splicing. In parallel, miniTurbo-based proximity labeling following mass spectrometry in Drosophila S2 cells characterized the HPat protein-proximity network and C-terminal dependence.
Results: HPat was broadly expressed in the nervous system, including mushroom body (MB) Kenyon cells. Tissue-specific HPat knockdown caused structural abnormalities in MB and ellipsoid body (EB), regions involved in learning/memory and spatial orientation, respectively. Neuronal HPat knockdown impaired larval locomotion and caused NMJ overgrowth. FM1-43 uptake did not significantly change, whereas dye release was markedly reduced, consistent with impaired synaptic vesicle exocytosis. Wild-type human PATL1 rescued selected HPat-knockdown phenotypes, supporting functional conservation, whereas the truncated variant showed reduced rescue capacity. Transcriptomics revealed widespread expression and splicing alterations in neuronal genes, including increased CaMKII transcripts lacking the association-domain-encoding region. Proximity proteomics identified HPat-enriched proteins associated with RNA regulation and vesicle trafficking and revealed C-terminal-dependent changes in the HPat-proximal network.
Conclusion: Our findings identify HPat as an important regulator of nervous-system development and synaptic function. The impaired rescue ability of C-terminally truncated PATL1, together with morphological, functional, transcriptomic, and proteomic alterations, provides insight into the cellular and molecular mechanisms underlying PATL1-related neurodevelopmental disorders.

Keywords: PATL1, neurodevelopmental disorders, alternative splicing, synaptic function, Drosophila melanogaster

OP27
Environmental Enrichment Drives AP-1 Mediated Chromatin Remodeling and Epigenetic Canalization in the Mouse Hippocampus

Zeynep Tansu Atasavum[1, 2], Gerd Kempermann[1, 3]

[1] Deutsches Zentrum Für Neurodegenerative Erkrankungen E.V. Dresden
[2] Technische Universität Dresden, Dresden
[3] CRTD Zentrum Für Regenerative Therapien TU Dresden

Background/Aim: Genetically identical mice housed in an enriched environment (ENR) develop stable, divergent behavioral phenotypes, yet the epigenomic basis of this individualization remains unknown. We asked whether ENR increases or decreases inter-individual variability of hippocampal chromatin accessibility, and whether molecular individuality corresponds to behavioral individuality.
Materials and Methods: Female C57BL/6 mice were housed for approximately eleven weeks under enriched (n = 40) or standard (n = 40) conditions. Enriched animals were continuously tracked with a radio-frequency identification based system, yielding longitudinal behavioral metrics including roaming entropy. Hippocampi were profiled by parallel bulk assay for transposase-accessible chromatin sequencing (ATAC-seq) and RNA sequencing (RNA-seq). Differential accessibility and expression were assessed with DESeq2, complemented by motif enrichment, transcription factor (TF) footprinting, gene set enrichment analysis, and coefficient of variation based individuality analysis.
Results: ENR produced a broad and coordinated shift in hippocampal chromatin state, with several thousand differentially accessible regions in both directions and consistently modest effect sizes, indicating system-level rather than locus-specific remodeling. Motif enrichment and TF footprinting independently identified activator protein 1 (AP-1)/bZIP family members, including JUND, FOS and BATF, as the dominant regulatory signature of enrichment. Unexpectedly, ENR reduced rather than increased inter-individual variability: the large majority of analyzed TF motifs showed lower variability in enriched mice, a pattern we term epigenetic canalization. RNA-seq revealed extensive differential expression, with synaptic translation as the leading enriched pathway, and a comparable but weaker stabilization of individuality. Chromatin accessibility profiles were unrelated to any behavioral measure, whereas the principal axis of transcriptional variation correlated modestly with locomotor activity.
Conclusion: Environmental enrichment drives AP-1 mediated chromatin remodeling in the hippocampus while paradoxically canalizing the epigenome across individuals. This epigenetic canalization is largely uncoupled from behavioral individuality, indicating a multi-layered and partially independent architecture of molecular and behavioral individuality.

Keywords: environmental enrichment, individuality, AP-1, ATAC-seq, hippocampus

POSTER PRESENTATIONS ABSTRACTS

PP001
IRF4 in Heterochromatin and Mobile Genetic Element Regulation in Melanoma Cells

Başak Nisa Kurt[1], Ali Ergül[1], Ekinsu Gülten[1], Ilgın Karaoğlu[1], Ulduz Sobhiafshar[1], N.C. Tolga Emre[1]

[1] Department of Molecular Biology and Genetics, Boğaziçi University, Istanbul, Turkey

Background/Aim: Interferon regulatory factor 4 (IRF4) is a transcription factor with established roles in immune cell differentiation and B-cell malignancies, and is also highly expressed in melanoma, where we previously showed that it regulates DNA and H3K27 methylation, implicated in gene silencing and heterochromatin formation. Given that epigenetic silencing normally represses mobile genetic elements (MGEs), we hypothesized that IRF4 controls MGEs, through additional chromatin modifications such as H3K9 methylation via the histone methyltransferase SETDB1, and that its loss de-represses endogenous retroviruses (ERVs), triggering a viral mimicry response. This study aimed to test this dual hypothesis using IRF4 loss- and gain-of-function models in melanoma cells.
Materials and Methods: Doxycycline-inducible shRNA (knockdown) and overexpression lentiviral systems targeting IRF4 were used in melanoma cell lines with high (SK-MEL-28, G361, MALME-3M) or low/absent (MEL-ST, A375) basal IRF4 expression. IRF4, SETDB1, and H3K9me3 levels were assessed by immunoblotting and RT-qPCR over time-course treatments. In parallel, IRF4 knockdown effects on ERV and interferon stimulated gene (ISG) expression were examined in SK-MEL-28 and MALME-3M by RT-qPCR and by immunofluorescence for double-stranded RNA (dsRNA) accumulation using a J2 antibody.
Results: Ongoing experiments show that IRF4 knockdown progressively reduces SETDB1 and H3K9me3 levels at both protein and mRNA levels while IRF4 overexpression produces a transient increase in SETDB1 and H3K9me3. Additionally, IRF4 knockdown increases dsRNA immunofluorescence signal, indicating de-repression of MGEs, and elevated expression of viral RNA sensors (MDA5, RIG-I) and downstream ISGs (IFIT2, IFI6).
Conclusion: These findings support a model in which IRF4 maintains heterochromatic silencing in melanoma via SETDB1-dependent H3K9 methylation, and its loss activates ERV-driven viral mimicry and innate immune signaling. Further studies will test the generalizability of these findings across melanoma models and flesh outs its potential for enhancing immunotherapy strategies.

Keywords: IRF4, SETDB1, H3K9 methylation, endogenous retrovirus, melanoma

PP003
Epigenetic Analysis of Monozygotic Twins Discordant for Autoinflammatory Phenotypes

Irmak Kömürcü[1], Özdeyiş İşgördü[1, 2], Alper Bülbül[3], Sevim Naz Karışık[2], Cansu Şahin[4], Serdal Uğurlu[5], Eda Tahir Turanlı[1, 2]

[1] Department of Molecular Biology and Genetics, Faculty of Engineering and Natural Sciences, Acibadem Mehmet Ali Aydinlar University, Istanbul, Turkey.
[2] Department of Molecular Biology and Genetics, Graduate School of Natural and Applied Sciences, Acibadem Mehmet Ali Aydinlar University, Istanbul, Turkey
[3] Depertmant of Biostatistics and Bioinformatics, Graduate School of Health Sciences, Acibadem Mehmet Ali Aydinlar University, Istanbul, Turkey.
[4] Cerrahpasa Medical Faculty, Istanbul University-Cerrahpasa, Istanbul, Turkey.
[5] Division of Rheumatology, Department of Internal Medicine, Cerrahpasa Medical Faculty, Istanbul University-Cerrahpasa, Istanbul, Turkey.

Background/Aim: Familial Mediterranean Fever (FMF) is an autoinflammatory disease caused by pathogenic MEFV variants. This study investigates twins of unknown zygosity with clinically suspected FMF but no pathogenic MEFV variants, exhibiting markedly divergent phenotypes, to identify the molecular and epigenetic mechanisms underlying this heterogeneity. One twin showed more severe inflammatory attacks and significantly higher IL-1β production than the co-twin, who had a milder disease course.
Materials and Methods: Zygosity was determined using targeted Sanger sequencing of 10 highly polymorphic SNP loci within PANX1, ST8SIA2, AP2A1, IL1RAPL2, IQSEQ3, F5, HSD11B1-AS1, DSC3, IL36, and ADA2. Genome-wide DNA methylation profiling was performed using Oxford Nanopore Reduced Representation Methylation Sequencing (RRMS), with differentially methylated regions (DMRs) identified relative to an age- and gender-matched healthy control. Bulk RNA sequencing was performed on PBMCs to identify differentially expressed genes, which were integrated with the RRMS dataset to identify genes with concordant epigenetic and transcriptional alterations.
Results: Zygosity testing showed 100% allelic concordance, confirming monozygotic status. Genome-wide DMR analysis identified 230 differentially methylated genes between the twins, distributed across multiple chromosomes, with functional enrichment in inflammatory regulation pathways. Integration of RNA sequencing and RRMS datasets identified genes with concordant epigenetic and transcriptional alterations, among which the long non-coding RNA, NEAT1 emerged as the most prominent candidate, prompting further isoform-specific characterization.
Conclusion: These findings suggest epigenetic mechanisms may contribute to divergent inflammatory phenotypes in monozygotic twins with clinically suspected autoinflammatory disease lacking pathogenic MEFV variants. Identification of NEAT1 as both differentially methylated and differentially expressed highlights its potential role in regulating inflammatory responses. Functional studies are ongoing to investigate the mechanistic role of NEAT1 isoform 2 in inflammatory regulation.

Keywords: oxford nanopore, monozygotic twins, Familial Mediterranean Fever, epigenetics, zygosity testing

PP004
Identification and Validation of Novel MBD2-targeting Inhibitor Candidates

Gizem Kugu[1], Fatma Özdemir[1], Merve Tuzlakoğlu Öztürk[1], Uygar Halis Tazebay[1], Zihni Onur Çalışkaner[2]

[1] Gebze Technical University
[2] Biruni University

Background/Aim: Methyl-CpG binding domain 2 (MBD2) is an epigenetic reader belonging to the MBD family. It recognizes methylated CpG dinucleotides and contributes to transcriptional silencing by coordinating DNA methylation and histone deacetylation. MBD2 has been associated with many cellular pathways and diseases, especially cancer, yet KCC-07 is the only commercially available inhibitor reported to reverse MBD2-mediated repression of tumor suppressor genes such as BRCA1, GSTP1, and BAI1. This study aimed to evaluate and validate the in vitro efficacy of novel MBD2 inhibitor candidates previously identified through in silico analyses and to compare their activities with KCC-07.
Materials and Methods: Three candidate molecules (Compounds 1, 2, and 3) identified by in silico screening were synthesized and structurally verified, whereas KCC-07 was purchased commercially. Extracellular inhibition of MBD2 binding to CpG-methylated DNA was assessed by electrophoretic mobility shift assay (EMSA) using a methylated BRCA1 promoter probe containing CpG sites. Intracellular chromatin-binding effects were investigated by chromatin immunoprecipitation (ChIP) analyses targeting the BRCA1 and GSTP1 promoter regions. Direct interaction between MBD2 and the compounds was examined using cellular thermal shift assay (CETSA), which detects ligand-induced thermal stabilization of proteins.
Results: EMSA demonstrated that KCC-07 and all candidate compounds interfered with MBD2 binding to methylated CpG-containing DNA. ChIP analyses confirmed the expected inhibitory activity of KCC-07, while the remaining compounds produced variable effects depending on compound identity and concentration. CETSA findings showed direct target engagement, with Compound 2 stabilizing MBD2 at levels comparable to or stronger than KCC-07 within the 46.8-54.4°C temperature range. Signalling pathway analyses are ongoing to distinguish on-target from off-target effects.
Conclusion: These findings indicate that the synthesized candidates, particularly Compound 2, directly interact with MBD2 and modulate its association with methylated chromatin, highlighting their promise as epigenetic inhibitor candidates. Further validation will be performed through siRNA-mediated silencing experiments and downstream pathway analyses.

Keywords: Epigenetics, MBD2, CETSA, EMSA, ChIP

PP005
Identification of Candidate piRNAs Potentially Targeting de novo DNA Methylation Genes by Sequence-Based Computational Analysis

Ece Gumusoglu-Acar[1]

[1] Istanbul University, Faculty of Science, Molecular Biology and Genetics Department, Istanbul, Turkiye.

Background/Aim: PIWI-interacting RNAs (piRNAs) are small non-coding RNAs that participate in transposon silencing and epigenetic regulation. The piRNA pathway contributes to de novo DNA methylation during germline development; however, the potential interactions between human piRNAs and genes involved in de novo DNA methylation remain poorly characterized. This study aimed to identify candidate human piRNAs with the potential to target genes associated with de novo DNA methylation using a sequence-based computational approach.
Materials and Methods: A curated list of genes involved in de novo DNA methylation was compiled from Gene Ontology, KEGG pathway annotations, and manually curated literature. Gene symbols were standardized according to HGNC nomenclature, and duplicates were removed. Human piRNA sequences were obtained from piRBase (v3.0); duplicate sequences, entries containing ambiguous nucleotides, and piRNAs outside the 18–45 nt range were excluded. Canonical transcript sequences for the curated genes were retrieved via the Ensembl REST API. Potential piRNA–transcript interactions were predicted using a custom Python pipeline, in which each piRNA was aligned to transcript sequences using a reverse-complement sliding-window approach. Candidate binding sites were evaluated based on seed-region complementarity (positions 2–11), total mismatch number, mismatch distribution, and predicted binding-site count. Interactions were ranked by interaction score, and the highest-scoring piRNAs were selected for further evaluation.
Results: Sequence-based screening prioritized 14 piRNAs with comparatively high predicted interaction scores against transcripts associated with de novo DNA methylation. The two highest-ranked candidates were piR-hsa-40348 and piR-hsa-59793, followed by piR-hsa-4726, piR-hsa-58589, piR-hsa-37416, piR-hsa-54785, piR-hsa-9229, piR-hsa-52469, piR-hsa-35335, piR-hsa-45855, piR-hsa-54469, piR-hsa-49015, piR-hsa-53139, and piR-hsa-48393. These candidates were prioritized for subsequent functional and experimental investigation.
Conclusion: This analysis identified a set of candidate piRNAs with predicted interactions involving genes associated with de novo DNA methylation. The prioritized candidates provide a basis for subsequent experimental studies investigating the potential contribution of piRNAs to epigenetic regulation.

Keywords: piRNA, de-novo DNA methylation, epigenetics, target prediction, bioinformatics

PP006
The Role of IL-6 and SOCS3 Gene Polymorphisms in Type 2 Diabetes Risk, Insulin Resistance, and Response to Metformin Therapy

Feryal SHAWA[1], Emel SAGLAM[2], Hazal ERDEM[2], Deniz KIRAC[3], Gulsah KOC[4]

[1] Institute of Graduate Studies, Molecular Medicine Program, Istanbul Aydin University, Istanbul, Turkey
[2] Department of Internal Medicine, University of Health Sciences, Bagcilar Training and Research Hospital, Istanbul, Turkey
[3] Department of Medical Biology, Faculty of Medicine, Yeditepe University, Istanbul, Turkey
[4] Department of Medical Biology, Faculty of Medicine, Istanbul Aydin University, Istanbul, Turkey

Background/Aim: Type 2 diabetes (T2D) is a multifactorial metabolic disease characterized by insulin resistance and progressive β-cell dysfunction. Chronic low-grade inflammation promotes interleukin-6 (IL-6)-mediated activation of the JAK/STAT3 pathway, increasing suppressor of cytokine signaling 3 (SOCS3) expression and impairing insulin signaling. This study investigated associations of IL-6 rs1800795, IL-6 rs1800796, and SOCS3 rs4969170 polymorphisms with T2D susceptibility, insulin resistance, and response to metformin therapy.
Materials and Methods: A total of 172 individuals (86 controls and 86 patients with prediabetes or T2D) were included. Genomic DNA was isolated from peripheral blood using a spin-column kit. IL-6 (rs1800795 and rs1800796) and SOCS3 (rs4969170) polymorphisms were genotyped by real-time PCR using TaqMan assays.
Results: IL-6 rs1800795 showed no significant association with T2D risk. In contrast, IL-6 rs1800796 and SOCS3 rs4969170 were significantly associated with T2D susceptibility ($p = 0.001$ and $p = 0.021$, respectively). None of the polymorphisms was associated with response to metformin monotherapy compared with combination therapy. For rs1800796, the C allele was identified as the risk allele ($p = 0.002$), with GC and CC genotypes associated with increased T2D risk. Moreover, carriers of mutant alleles had lower HDL cholesterol levels and a higher TG/HDL ratio, suggesting greater insulin resistance. For SOCS3 rs4969170, the AA genotype was associated with increased T2D risk, whereas the AG genotype reduced disease risk by approximately 60%. Additionally, individuals with the AA genotype had higher fasting glucose levels, supporting a potential role of SOCS3 in impaired glucose homeostasis.
Conclusion: The IL-6 rs1800796 C allele (GC/CC genotypes) and SOCS3 rs4969170 AA genotype were associated with increased T2D risk in the Turkish population. These variants may contribute to T2D susceptibility through adverse effects on glycemic control and lipid metabolism. Larger studies are needed to further investigate the role of these variants in T2D susceptibility and their potential clinical relevance.

Keywords: IL-6 , SOCS3 , T2D, Polymorphism, Insulin Resistance

PP007
Association Between Telomere Length and Childhood Interstitial Lung Disease

Ali Samet KARA[1], Halime NAYIR-BÜYÜKŞAHİN[2], Burcu ÇAPRAZ-YAVUZ[2], Raziye ATAN[2], Umay KAVGACI[2], Nagehan EMiRALİOĞLU-ORDUKAYA[2], Uğur ÖZÇELİK[2], Deniz DOĞRU[2], Nural KİPER[2], Ebru YALÇIN[2], Didem DAYANGAÇ-ERDEN[1]

[1] Hacettepe University, Faculty of Medicine, Department of Medical Biology, Ankara, Türkiye
[2] Hacettepe University, Faculty of Medicine, Department of Pediatric Pulmonology, Ankara, Türkiye

Background/Aim: Childhood interstitial lung disease (chILD) comprises a heterogeneous group of rare disorders characterized by progressive inflammation and fibrosis that disrupt the alveolar–capillary unit and impair gas exchange. Telomere maintenance is essential for genomic stability and replicative potential. In the lung, telomere attrition may limit alveolar type II cell self-renewal and epithelial repair, thereby contributing to the pathobiology of interstitial lung disease. However, evidence regarding telomere length in chILD remains very limited. Therefore, we aimed to evaluate leukocyte telomere length in patients with chILD and investigate its relationship with telomere biology–related disease phenotype.
Materials and Methods: A total of 8 patients diagnosed with chILD and 7 age-matched controls were enrolled in this study. The chILD cohort comprised patients with hypersensitivity pneumonitis (n=2), nonspecific interstitial pneumonia (n=1), and fibrotic interstitial lung disease (n=5). Peripheral blood samples were collected, and genomic DNA was isolated from leukocytes. Telomere length was quantified using SYBR Green–based quantitative polymerase chain reaction (qPCR) and reported as the relative telomere-to-single-copy gene (T/S) ratio using the $2^{-\Delta\Delta Ct}$ method. The albumin (ALB) gene was used as the single-copy reference gene.
Results: The median telomere length T/S ratio was lower in the chILD group compared with the control group (median [IQR]: 0.966 [0.734–1.185] vs. 1.594 [1.467–1.658]). The median T/S ratio in the chILD group was approximately 0.61-fold that of the control group. This corresponded to a 39.4% lower median T/S ratio in the chILD group.
Conclusion: Our preliminary findings suggest that telomere length is reduced in children with chILD compared with age-matched controls. Future studies with a larger patient cohort are ongoing and will determine the relationship between telomere length and disease severity to evaluate the potential of telomere length as a biomarker across different chILD subtypes.

This study is funded by Hacettepe University Scientific Research Projects Coordination Unit(Project ID: TSA-2024-20787)

Keywords: Telomere, Childhood Interstitial Lung Disease, Telomere Length

PP009
Effects of Autologous Mitochondrial Transfer and mitoQ on Mitochondrial Homeostasis in an in vitro Model of Diabetic Retinopathy

Candan Altuntaş[1, 2], Ahmet Öztürk[1, 2, 3], Gökhan Duruksu[1, 2], Kamil Can Kılıç[1, 2, 3], Meltem Göksel[1, 2], Yusufhan Yazır[1, 2, 3]

[1] Department of Stem Cell, Institute of Health Sciences, Kocaeli University, Izmit, Kocaeli, Turkey
[2] Center for Stem Cell and Gene Therapies Research and Practice, Kocaeli University, Izmit, Kocaeli, Turkey
[3] Department of Histology and Embryology, School of Medicine, Kocaeli University, Izmit, Kocaeli, Turkey

Background/Aim: Diabetic retinopathy is one of the major microvascular complications of diabetes associated with mitochondrial dysfunction, oxidative stress, and impaired mitochondrial quality control mechanisms [1]. Advanced glycation end products (AGEs) trigger oxidative stress and mitochondrial damage in retinal cells [2]. MitoQ, a mitochondria-targeted antioxidant, is effective in reducing mitochondrial oxidative damage. The aim of this study is to investigate the effects of autologous mitochondrial transfer and MitoQ administration on mitochondrial function, mitochondrial integrity, and mitophagy in an in vitro diabetic retinopathy model induced by AGEs.
Materials and Methods: A model of diabetic retinopathy was established in ARPE-19 cells, retinal pigment epithelium cells, using AGEs. Through optimization experiments the optimal working concentrations of AGEs, isolated autologous mitochondria, and the mitochondria-targeted antioxidant MitoQ were determined. The experimental groups were established as control, AGE, AGE+autologous mitochondrial transfer, AGE+MitoQ, and AGE+MitoQ+autologous mitochondrial transfer. Mitochondrial membrane potential was assessed using JC-1 staining, mitochondrial structure and integrity were evaluated via immunoreactivity assessment of TOM20 and COX4 proteins, and mitophagy activity was assessed using laser scanned confocal microscopy with Mitophagy Dye.
Results: In our research, changes in mitochondrial function and integrity, and mitophagy in a cellular damage model induced by AGEs were evaluated using JC-1, TOMM20, COXIV, and Mitophagy Dye assays. Additionally, the potential effects of autologous mitochondrial transfer and MitoQ treatment on AGE-induced changes were examined in a comparative analysis.
Conclusion: This study is expected to provide new insights into the role of autologous mitochondrial transfer in maintaining mitochondrial homeostasis in diabetic retinopathy and to contribute to mitochondrial-targeted therapeutic approaches.

REFERENCE
1. Malaviya, P. and R.A. Kowluru, *Homocysteine and mitochondrial quality control in diabetic retinopathy.* Eye and Vision, 2024. **11**(1): p. 5.
2. Toma, C., et al., *Oxidative Stress in Diabetic Retinopathy: Pathogenic Mechanisms, Biomarkers and Clinical Implications.* Antioxidants (Basel), 2026. **15**(4).

Keywords: Diabetic retinopathy, autologous mitochondrial transfer, mitochondrial dysfunction

PP010
Impact of ETI Treatment on miR-449c-5p Expression and its Candidate Target Genes in Pediatric Cystic Fibrosis Patients

Ayberk Mustafaoğlu[1], Birce Sunman[2], Rüveyda Çöklü[1], Nagehan Emiralioğlu[2], Uğur Özçelik[2], Deniz Doğru[2], Ebru Yalçın[2], Didem Dayangaç-Erden[1]

[1] Hacettepe University, Faculty of Medicine, Department of Medical Biology, Ankara, Türkiye
[2] Hacettepe University, Faculty of Medicine, Department of Pediatric Pulmonology, Ankara, Türkiye

Background/Aim: Cystic fibrosis (CF) is a common autosomal recessive disease caused by mutations in the CF transmembrane conductance regulator (CFTR) gene. It is characterized by chronic progressive lung disease and multisystem involvement. Modulator drugs, such as Elexacaftor/Tezacaftor/Ivacaftor (ETI), have been shown to rescue defects caused by specific CFTR mutations. In Türkiye, mutations are heterogeneous and patients with the same genotype may show variability in clinical severity and prognosis of the disease. miRNAs, which regulate gene expression, may contribute to prognosis by regulating disease-related pathways. It was shown that miR-449c-5p was upregulated in severe compared to mild CF patients. In this study, we compared miR-449c-5p expression between ETI-treated and untreated CF patients.
Materials and Methods: Total RNA was isolated from nasal epithelial cells collected from 19 pediatric CF patients (11 ETI-treated, 8 untreated). miR-449c-5p expression was analyzed by qRT-PCR. To detect miR-449c-5p target genes, bioinformatic analyses were conducted using miRWalk 3.0, TargetScan 8.0, miRDB, and miRTarBase databases. Gene ontology and pathway analysis of the target genes was performed using Enrichr.
Results: miR-449c-5p expression was significantly downregulated 2.72 fold in ETI-treated compared to untreated CF patients. miR-449c-5p target genes were intersected with the dataset obtained from a pediatric airway transcriptomic study that included upregulated genes after ETI treatment. As a result of Reactome pathway analysis, 297 upregulated genes were found to be associated with interferon signaling, immune system and cytokine signaling. According to miRTarBase database BTG2, MTDH and RPS19 genes were found to be experimentally validated.
Conclusion: Our preliminary findings highlight the need to further investigate the role of miR-449c-5p in the molecular response to ETI treatment. Further studies comparing miR-449c-5p expression levels in the same patients before and after ETI initiation are currently underway. ETI-associated molecular effects may extend beyond CFTR channel restoration to include miRNA-mediated regulatory mechanisms.

Keywords: Cystic fibrosis, miR-449c-5p, elexacaftor/tezacaftor/ivacaftor, microRNA, CFTR modulator

PP011
SIK2 acts as an ER-sensitive Flip-flop Switch for Robust Autophagy Induction Towards ER Homeostasis

Nazan Yılmaz[1], Ferruh Özcan[1]

[1] Gebze Technical University, Department of Molecular Biology and Genetics, Kocaeli, Turkey

Background/Aim: Hypothalamic POMC neurons control metabolism via α-MSH release and are particularly susceptible to ER stress in obesity. Transport from ER to Golgi occurs primarily via COPII vesicles, which form on ER membrane in a highly regulated assembly of coat proteins, particularly Sec31A. Sec31A acts as a "molecular rheostat" to regulate vesicle size and coating stiffness through phosphorylation and ubiquitination on ER exit sites (ERES). In addition to its role in secretion, Sec31A provides membranes for autophagosome formation via ATG9A and plays a role in Insulin Receptor signaling and cell survival. Salt Inducible Kinase 2 (SIK2), an AMPK-family metabolic kinase activated downstream of UPR pathways in response to ER stress, restricts secretion by trapping Sec31A in 'Sec Bodies' and selectively shapes cargo release from the POMC secretome. We propose that SIK2 may directly be involved in secretion via secretory vesicle formation or regulation by Sec31A. To test this hypothesis, we investigated time-dependent interaction between Sec31A and SIK2, as well as key autophagy components, under thapsigargin-induced ER stress in HEK293FT cells, as first step toward a POMC neural model.
Materials and Methods: FLAG-Sec31A expressing HEK293FT cells were treated with 1 μM thapsigargin for 30 min to 6 h. Sec31A interactions with SIK2, Beclin1, and ATG9A were analyzed via Co-IP and Western blot. Colocalization is being assessed using immunocytochemistry and fluorescence microscopy.
Results: Co-IP assays showed rapid, specific remodeling of Sec31A interactome after thapsigargin treatment. SIK2 enrichment appeared within 30 min, Beclin1 association rose between 30 min–3h, and ATG9A remained elevated. Input levels stayed stable, confirming altered interaction dynamics rather than expression changes.
Conclusion: ER stress rapidly reorganizes the Sec31A interactome, increasing its association with SIK2 and autophagy-initiation proteins. This supports a model where SIK2 diverts Sec31A from secretion toward autophagy, providing a basis for future studies in POMC neurons and obesity.

Keywords: SEC31A, COPII, SIK2, ER Stress, Autophagy

PP012
Membrane Lipid Remodelling Coordinates Mitophagy and Selective Mitochondrial-derived Vesicle Export in Doxorubicin-treated Cardiomyoblasts

Dünya Aydos[1, 2], Musa Latif Çöllüoğlu[1, 2], Neslihan Başak[1, 2], Begüm Mercan[1, 2], Ceylan Verda Bitirim[1]

[1] Stem Cell Institute, Ankara University, Ankara
[2] Graduate School of Health Sciences, Ankara University, Ankara

Background/Aim: Doxorubicin (DOX)-induced cardiotoxicity involves cumulative mitochondrial oxidative damage, but how membrane lipid organisation coordinates mitochondrial quality-control mechanisms when mitophagy is insufficient remains poorly understood. We hypothesised that DOX-induced membrane lipid remodelling promotes mitochondria-derived vesicle (MDV)-mediated export as a complementary response to impaired mitophagy. This study aimed to determine whether MDV cargo loading reflects selective sorting rather than passive membrane leakage, and whether the mitochondria-targeted antioxidant Mito-TEMPO restores this coordinated quality-control network.
Materials and Methods: H9c2 rat cardiomyoblasts were assigned to Control, DOX, and DOX+Mito-TEMPO groups. Membrane lipid order, mitochondrial function and dynamics, autophagic flux, and MDV-associated mitochondrial cargo were assessed by spectral confocal imaging, biochemical assays, and immunoblotting. Dynasore or 3-methyladenine (3-MA) was co-administered with DOX to dissect the interplay between MDV-mediated export and autophagic clearance.
Results: Under DOX, COX4I2 and VDAC — but not COX4I1 — were preferentially shifted into the MDV fraction despite unchanged intracellular abundance, supporting regulated cargo sorting rather than passive vesicle leakage; this occurred alongside reduced membrane lipid order in plasma, mitochondrial, and lysosomal membranes and mitochondrial dysfunction reflected by increased ROS, ATP depletion, membrane depolarisation, enhanced fission, and LC3-II/p62 accumulation indicative of impaired autophagic flux. This selective MDV shift was largely abolished by Mito-TEMPO, which also restored membrane order and mitochondrial function. Blocking MDV secretion with Dynasore markedly increased intracellular VDAC and LC3-II, whereas inhibiting autophagy initiation with 3-MA reduced LC3-II but markedly increased p62 — demonstrating that MDV-mediated export and mitophagy function as complementary, coordinated mitochondrial quality-control pathways.
Conclusion: Membrane lipid remodelling governs a coordinated, selective mitochondrial quality-control network directing damaged components toward either mitophagy or MDV-mediated export rather than indiscriminate release, pointing toward membrane-targeted cardioprotective strategies and EV-based biomarkers for chemotherapy-induced cardiotoxicity.

Keywords: Membrane lipid remodelling, Mitochondrial-derived vesicles, Selective cargo sorting, Mitophagy, Doxorubicin cardiotoxicity

PP013
Regulation of PI(3,5)P2 Synthesis During Mitosis in Yeast and Mammalian Cells

Maria Doukmak[1], Ahmed Alsaftawi[1], Nurhan Ersoy[1], Ayşe Koca-Çaydaşı [1]

[1] Department of Molecular Biology and Genetics, College of Sciences, Koç University, Istanbul, Türkiye

Background/Aim: Phosphatidylinositol 3,5-bisphosphate [PI(3,5)P_2] is a low-abundance phosphoinositide that is evolutionarily conserved from the unicellular yeast Saccharomyces cerevisiae to multicellular eukaryotes. Recent advances, including the development of the PI(3,5)P_2 biosensor SnxA, have enabled the visualization of this lipid in living cells. Previous studies from our laboratory demonstrated that PI(3,5)P_2 is required for timely mitotic exit in S. cerevisiae. This study aims to elucidate the regulatory mechanisms controlling mitotic PI(3,5)P_2 production in yeast and to establish the spatiotemporal dynamics of PI(3,5)P_2 during the mammalian cell cycle.
Materials and Methods: The regulation of the Fab1 complex (PIKfyve in mammals) is being investigated using molecular and cell biology approaches to characterize post-translational modifications of its scaffold protein, Vac14. In parallel, live-cell microscopy is being employed to examine the spatiotemporal dynamics of PI(3,5)P_2 during mitotic progression in HEK293T cells.
Results: Our findings indicate that Vac14 undergoes cell cycle-dependent phosphorylation by the Polo-like kinase Cdc5, which is required to maintain PI(3,5)P_2 asymmetry during anaphase in S. cerevisiae. Furthermore, preliminary data suggests that PI(3,5)P_2 is synthesized in a cell cycle-dependent manner in mammalian cells, supporting a conserved role for this phosphoinositide during mitotic progression.
Conclusion: These findings provide new insights into the mechanisms regulating mitotic PI(3,5)P_2 synthesis and establish a foundation for understanding PI(3,5)P_2 dynamics in mammalian cells. Together, this work advances our understanding of the conserved functions of phosphoinositide signaling during cell division and highlights PI(3,5)P_2 as an important regulator of mitotic progression.

Keywords: PI(3,5)P2, mitosis, Vac14, lipid signaling

PP014
Decoding the Dynamics of Translational Regulation Mediated by Nutrient-Sensing Pathways Under Nutrient Deprivation in Colorectal Cancer Cell Lines

Çağrı GÜNDÜZ[1], Elif Edanur BALÇIK[1], Özün ÖZCAN[1], Kayra AKBULUT[2], Hazal Beril Çatalak YILMAZ[1], Beyza YILMAZ[1], Sreeparna BANERJEE[1]

[1] Department of Biological Sciences, Orta Doğu Teknik Üniversitesi, Ankara
[2] Department of Molecular Biology and Genetics, Bilkent University, Ankara

Background/Aim: Tumor cells experience metabolic rewiring driven by localized changes in nutrient availability within the tumor microenvironment. To survive under these restrictive conditions, cancer cells dynamically alter their intracellular signaling networks. Global protein translation is one of the pathways that is likely to be affected significantly with low nutrient availability, since the process is highly energy-intensive. Our laboratory previously demonstrated that nutrient deprivation (ND) universally limits cell division in colorectal cancer (CRC) cell lines, yet the surviving cell populations maintain high viability. ND triggers rapid metabolic adaptations within 2 hours, characterized by the activation of canonical nutrient sensors. Intriguingly, despite proximal mTOR suppression, these cells exhibit sustained phosphorylation of Ribosomal Protein S6 (RPS6)—a critical component of the small ribosomal subunit and a downstream translational effector. We hypothesized that RPS6 may be activated in the ND cells via alternative signaling pathways such as the MAPK pathway.
Materials and Methods: HCT-116 and LoVo cells were subjected to ND and targeted pharmacological inhibitors against the mTOR and MAPK cascades. Western blotting was used to determine the activation of the MAPK pathway and translation initiation factors. Puromycylation assay was used to determine global translation, while luciferase assays were used to determine whether translation was 5'Cap dependent or independent.
Results: Cells under ND can retain 5' Cap dependent translation, although global translation decreases tremendously. The MAPK pathway activates Ribosomal protein S6 Kinase (RSK), which acts as a critical alternative driver of RPS6 phosphorylation and translation initiation.
Conclusion: Cancer cells are known to activate alternative pathways for survival, depending on the microenvironment. Cells under ND can continue survival and protein synthesis through the activation of the MAPK-RSK axis, even when mTOR is inhibited.

Keywords: Colorectal Cancer, Nutrient Restriction, Translational Regulation

PP015

Lactobacillus-derived Fatty Acids and Supernatant Modulate Sirtuin and Telomerase Expression Without Altering Chronological Lifespan in Schizosaccharomyces pombe

Alperen Kaynak[1], Gülşen Uz Ekşi[1], Görkem Yılmaz[1]

[1] Istanbul Yeni Yuzyil University, Molecular Biology and Genetics Department

Background/Aim: Probiotic metabolites, particularly short-chain fatty acids (SCFAs), remain poorly characterised, and their effects on aging are largely unexplored. The effects of fatty acids and cell-free supernatant from three probiotic Lactobacillus species (Lactobacillus acidophilus, LA; Lactobacillus plantarum, LP; Lactobacillus fermentum, LF) on aging in the fission yeast Schizosaccharomyces pombe (S. pombe) were investigated. Aging was assessed through chronological lifespan (CLS), and the expression of the sirtuin genes hst2, sir2 and the telomerase gene trt1 was examined to explore underlying mechanisms.
Materials and Methods: S. pombe was treated separately with a total fatty acid extract or cell-free supernatant of each strain, alongside solvent, medium, and oxidative-stress controls. CLS was assessed by a stationary-phase survival assay. Expression of hst2, sir2, and trt1 was measured by Real-Time PCR (RT-PCR) in three replicates as Log2 fold change relative to control, and analysed by one-way analysis of variance (ANOVA) with Dunnett's test ($p<0.05$).
Results: Neither treatment changed CLS ($p>0.05$), but expression diverged sharply by treatment type. The LF fatty acid extract strongly upregulated hst2 (Log2FC +2.79) and sir2 (+1.64; both $p<0.001$), whereas the LF supernatant abolished this effect. LP supernatant instead repressed hst2 strongly (−2.84, $p<0.001$). trt1 was repressed under both treatments, partly also in the control conditions, while LF stayed the least affected group.
Conclusion: The stimulatory effect of the LF fatty acid extract on sirtuin expression was lost in the supernatant, indicating that co-secreted components antagonize it. Despite these expression changes, CLS was unchanged, consistent with sirtuins and telomerase acting mainly on replicative rather than chronological aging, which in S. pombe is driven largely by nutrient signaling and oxidative stress. These findings show that probiotic metabolite activity depends on whether a compound acts in isolation or within the full secretome, a distinction that should guide future aging studies.

Keywords: Lactobacillus, chronological lifespan, short-chain fatty acids, Schizosaccharomyces pombe

PP016
The Centriolar Inner Scaffold Protein CCDC15 Is Required for Motile Ciliogenesis in Airway Multiciliated Epithelia

Alper İbrahim Özkan[1], İrem Sultan Dilbaz[1], Melis Dilara Arslanhan[2], Mehmet Şerif Aydın[2, 3], Elif Nur Fırat-Karalar[1, 4]

[1] Department of Molecular Biology and Genetics, Koç University, Istanbul, Türkiye
[2] Research Institute for Health Sciences and Technologies (SABITA), Istanbul Medipol University, Istanbul, Türkiye
[3] Department of Histology and Embryology, School of Medicine, Istanbul Medipol University, Istanbul, Türkiye
[4] Koç University School of Medicine, Istanbul, Türkiye

Background/Aim: Motile cilia drive mucociliary clearance in the airway, and defects in their assembly or function cause motile ciliopathies, including primary ciliary dyskinesia (PCD). Multiciliated cells generate hundreds of basal bodies that must remain structurally stable to support coordinated beating. Although the centriolar inner scaffold maintains centriole architecture, its role in airway multiciliated cells is unknown. We tested whether the inner scaffold protein CCDC15 supports basal body integrity and progression through motile ciliogenesis.
Materials and Methods: Mouse tracheal epithelial cells (MTECs) were differentiated at air–liquid interface as a staged model of motile ciliogenesis. CCDC15 localization and abundance were analyzed across differentiation by immunofluorescence and ultrastructure expansion microscopy (U-ExM) using centriole, basal body, and ciliary markers. CCDC15 function was assessed by lentiviral short hairpin RNA (shRNA)-mediated depletion, followed by stage-resolved quantification of centriole amplification, basal body organization, apical alignment, and ciliation. The related inner scaffold protein POC5 was analyzed in parallel.
Results: CCDC15 localized to the inner scaffold of centrioles and basal bodies in differentiating MTECs, with abundance changing dynamically and peaking during centriole amplification. CCDC15 depletion impaired centriole amplification, basal body docking and orientation, and cilia formation. U-ExM showed that depletion significantly reduced centriole length, supporting a role in maintaining basal body structural integrity. Cells reaching later stages displayed defective ciliation. POC5 depletion produced similar phenotypes, suggesting a broad requirement for the inner scaffold in centriole amplification, basal body maturation, and motile ciliogenesis.
Conclusion: Our findings identify CCDC15 as an inner scaffold component required for centriole amplification, basal body integrity, and efficient motile ciliogenesis in airway multiciliated cells, supporting a model in which the inner scaffold stabilizes basal bodies during multiciliogenesis. This work extends the functional relevance of the inner scaffold from primary to motile ciliogenesis and frames how centriole stability defects may contribute to airway ciliopathies, including PCD.

Keywords: centriole, inner scaffold, motile cilia, basal body, CCDC15

PP017
Mitotic Phosphorylation of Vac14 Regulates Fab1 Complex Interactions and Asymmetric Vacuolar Localization

Nurhan Ersoy[1], Ahmed Alsaftawi[1], Maria Doukmak[1], Ayşe Koca Çaydaşı[1]

[1] Koç University, İstanbul

Background/Aim: Phosphatidylinositol 3,5-bisphosphate (PI(3,5)P2), a signaling lipid of endo-lysosomal sytem, is synthesized by the Fab1-complex, consisting of Fab1, Vac14, Fig4, and Vac7. Vac14 acts as a scaffold for complex assembly. Our previous work showed that PI(3,5)P2 is synthesized in a cell cycle dependent manner in the budding yeast Saccharomyces cerevisiae. We showed that PI(3,5)P2 accumulates on the daughter vacuole while disappearing from the mother vacuole, and this asymmetry is critical for establishment of vacuolar asymmetry in yeast. Our unpublished data further showed that Vac14 is phosphorylated in a polo-like kinase dependent manner during mitosis and this is critical for PI(3,5)P2 synthesis during late mitosis. Here, I will present how polo-like kinase dependent phosphorylation of Vac14 affects Fab1-complex formation and vacuolar localization.
Materials and Methods: To arrest cells in telophase with and without polo-like kinase activity, cdc15-1 and cdc5-10 temperature sensitive mutants were used. Telophase arrest was also achieved through nondegradable Clb2 expression. Vac14 phosphorylation was assessed through protein mobility shift. Fab1-complex components were knocked out (fab1Δ, fig4Δ, and vac7Δ) to analyze their effect on Vac14 phosphorylation. Protein interactions were analyzed by co-immunoprecipitation assays. For localization studies, temperature-sensitive strains carrying mNG or GFP-tagged complex components were shifted to the restrictive temperature and imaged by fluorescence microscopy.
Results: Loss of Fab1 but not Fig4 or Vac7 caused a reduction in Vac14 phosphorylation. Less Fig4 co-immunoprecipitated with Vac14 in the presence of phosphorylation. More Vac14, Fab1 and Fig4 localized on the daughter vacuole in phosphorylated conditions, whereas this asymmetry was less evident in the unphosphorylated stage.
Conclusion: Our results suggest that polo-kinase activity promotes maintenance of the Fab1-complex in a state that favors PI(3,5)P2 synthesis. This happens likely through reduction of Fig4 association with the complex, and increased binding of the complex to the vacuolar membrane.

Keywords: Phosphatidylinositol 3,5-bisphosphate, Vac14, Polo-like Kinase, Saccharomyces cerevisiae.

PP018
"Investigating the Role of the Fab1/PIKfyve Complex in PI(3,5)P_2 Regulation and Longevity"

Asena Demircan[1], Mariam Huda[1], Ayşe Koca Çaydaşı[1]

[1] Koç University, İstanbul

Background/Aim: Phosphoinositides (PIPs) are membrane lipids that regulate cellular signaling and membrane dynamics. Among the seven human PIPs, phosphatidylinositol 3,5-bisphosphate [PI(3,5)P_2] is one of the least abundant but essential for endolysosomal homeostasis. Defects in PI(3,5)P_2 synthesis are associated with several neurodegenerative diseases. PI(3,5)P_2 is synthesized by the evolutionarily conserved Fab1/PIKfyve-complex, comprising the lipid kinase Fab1/PIKfyve, the lipid phosphatase Fig4, and the scaffold protein Vac14. Previous work from our laboratory showed that PI(3,5)P_2 synthesis is cell cycle regulated and required for daughter vacuole acidification, promoting daughter cell rejuvenation. Here, we aim to understand regulation and function of the Fab1/PIKfyve-complex particularly with a focus on its role in longevity. Furthermore, through generation of a humanized yeast model, we will study its conservation.
Materials and Methods: TurboID proximity-labeling was used to identify Fab1-complex interaction partners. Functionality of TurboID-tagged proteins was assessed by fluorescence microscopy using the vacuolar membrane marker Vph1-tdTomato and the PI(3,5)P_2 biosensor SnxA-GFP. Replicative lifespan of PI(3,5)P_2 pathway mutants was assessed by monitoring mother cell divisions using micromanipulation-based dissection microscopy. Chronological-lifespan was assessed by determining colony-forming units over a 30-day period. Human PIKfyve, Vac14, and Fig4 cDNAs are cloned into yeast expression vectors by restriction digestion cloning.
Results: Functional TurboID-tagged Fab1-complex strains were generated without significant changes in vacuole morphology, indicating that C-terminal tagging preserves protein function. Three-hour incubation with 5μM biotin produced detectable biotinylation above background in SC-complete medium, enabling streptavidin enrichment and mass spectrometry. Loss of PI(3,5)P_2 significantly shortened both chronological and replicative-lifespan. Unexpectedly, constitutively elevated and symmetric PI(3,5)P_2 production extended chronological-lifespan but shortened replicative-lifespan. Functional analysis of humanized yeast strains expressing PIKfyve-complex orthologs is in progress.
Conclusion: These findings establish a functional TurboID toolkit for identifying Fab1-complex interactors. Together with ongoing humanized yeast and lifespan studies, this work will provide insight into PI(3,5)P_2 regulation, longevity, and evolutionary conservation.

Keywords: PI(3,5)P_2, Fab1/PIKfyve-Complex, TurboID, Humanized Yeast, Yeast Aging

PP019

A Novel Mechanism for ATF4 Stabilization in Nutrient-deprived Colorectal Cancer Cells

Barın Ergüneş[1, 2], Aydan Torun[2], Sreeparna Banerjee[2]

[1] Department of Biotechnology, Uniwersytet Wrocławski, Wrocław, Poland
[2] Department of Biological Sciences, Orta Doğu Teknik Üniversitesi, Ankara, Türkiye

Background/Aim: Cancer cells frequently encounter nutrient deprivation (ND) within the tumor microenvironment, activating adaptive pathways such as the integrated stress response (ISR). Activating transcription factor 4 (ATF4), a central ISR effector, supports adaptation by regulating amino acid metabolism, redox homeostasis, protein synthesis, and survival. Phosphorylation of eIF2α at Ser51 attenuates global translation while enhancing ATF4 translation, whereas ATF4 abundance is also controlled by ubiquitin-dependent proteasomal degradation. Ribosomal S6 kinase 2 (RSK2) phosphorylates ATF4 at Ser245, a modification associated with increased ATF4 abundance and reduced ubiquitination in PIK3CA-mutant colorectal cancer (CRC) cells. Preliminary observations from our laboratory indicated increased RSK phosphorylation and ATF4 abundance following ND without a concomitant increase in eIF2α Ser51 phosphorylation. Together, published evidence and our preliminary observations led us to hypothesize that ND may promote RSK2-dependent ATF4 stabilization independent of the activation of ISR.
Materials and Methods: Canonical human ATF4 and RSK2 sequences and domain annotations were retrieved from UniProtKB. Candidate ATF4 linear motifs were analyzed using ELM, curated RSK2–ATF4 evidence was examined using OmniPath, and reported phosphorylation and ubiquitination sites were assessed using PhosphoSitePlus. Potential interaction regions were explored using AlphaFold Server-based complex modelling.
Results: Based on this literature-guided *in silico* analysis, we propose that Ser245 phosphorylation may reorganize βTrCP-bound ATF4, reducing the accessibility or productive positioning of ubiquitin-acceptor lysines toward the E2–ubiquitin complex. Alternatively, Ser245 phosphorylation may favor deubiquitinase-mediated stabilization, potentially involving USP7, or abrogate recognition by additional E3 ubiquitin ligases.
Conclusion: We suggest that cancer cells under nutrient stress promote RSK2-dependent ATF4 stabilization. Our model predicts that RSK2 signaling may decrease ATF4 ubiquitination and enhance its half-life without necessarily disrupting βTrCP binding. These predictions are currently being experimentally evaluated, providing a testable framework for sustained ATF4 signaling during ND in CRC cells.

Keywords: Stress response, ATF4, RSK2, protein stability, post-translational modification (PTM)

PP020
Purification of Fab1-Vac14-Fig4 Complex in its Native State for Structural Analysis

Mehmet Enes Şanlan[1], Ayşe Koca Çaydaşı[1]

[1] Department of Molecular Biology and Genetics, Graduate School of Sciences and Engineering, Koç University, Istanbul, Türkiye

Background/Aim: The Fab1-Vac14-Fig4 complex is a conserved multiprotein assembly responsible for both synthesis and turnover of phosphatidylinositol 3,5-bisphosphate [$PI(3,5)P_2$], a low-abundance phosphoinositide essential for endo-lysosomal homeostasis and trafficking. Dysregulation of $PI(3,5)P_2$ is linked to neurodegenerative disorders. How the complex coordinates these opposing enzymatic activities remains unknown. We hypothesize that the complex undergoes conformational shifts governing its synthesis or turnover states. This project aims to establish robust isolation strategies to determine the structural architecture of the Fab1–Vac14–Fig4 complex via single-particle cryo-electron microscopy (cryo-EM).
Materials and Methods: To isolate native complexes from Saccharomyces cerevisiae, first a strain carrying a genomic Vac14-TEV-GFP tag was constructed. For this, first, a plasmid carrying TEV-GFP was constructed, then this plasmid was used to amplify a cassette, which was eventually transformed into yeast. Anti-GFP nanobodies carrying a GST-tag were recombinantly expressed in E. coli and purified via affinity chromatography to serve as capture agents for immunoprecipitation (IP) of Vac14-TEV-GFP. In parallel, Vac14 was subcloned into pisp65 pET28a-6His-SUMO plasmid vector for recombinant expression in E. coli.
Results: Genomic integration of the Vac14-TEV-GFP cassette into S. cerevisiae was successfully achieved and verified by flourescence microscopy. High-yield, purified anti-GFP nanobodies were successfully produced in E. coli for IP. Additionally, Vac14 was successfully subcloned into the pisp65 pET28a-6His-SUMO vector, establishing a recombinant expression vector construct.
Conclusion: Combining endogenous yeast purification via TEV-cleavable GFP tags with recombinant E. coli expression provides a viable pipeline to obtain structural-grade Fab1-Vac14-Fig4 preparations. This foundation enables upcoming studies to uncover how opposing phosphoinositide metabolic activities are structurally coordinated.

Keywords: Fab1-Vac14-Fig4 complex, Recombinant expression, Affinity purification, PI(3,5)P2, Cryo-EM

PP021
Two-Step Process Regulating the Ciliary Entrance of Non-Ciliary Proteins: BBSome Functions In The First Step

Sebiha Cevik[1], Ferhan Yenisert[1], Hanife Kantarci[1], Damla Pucak[1], Onur Cakici[2], Oktay I. Kaplan[1]

[1] Rare Disease Laboratory, School of Life and Natural Sciences, Abdullah Gül University, Kayseri, Türkiye
[2] Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology – Hellas (FORTH), Heraklion, Crete, Greece

Background/Aim: Cellular compartmentalization is essential for organelle-specific functions. Cilia are microtubule-based sensory compartments that detect environmental stimuli, and their composition must be tightly regulated to maintain proper function. Protein entry into cilia is controlled by a selective barrier known as the ciliary gate, while additional mechanisms are required to prevent the accumulation of non-ciliary proteins. Ciliary proteins are selectively recognized in the cell body, loaded onto intraflagellar transport (IFT) complexes at the basal body, and transported along the axoneme by motor proteins. The BBSome complex, composed of Bardet–Biedl syndrome (BBS) proteins, plays a key role in cargo selection and trafficking. However, the mechanisms that prevent non-ciliary proteins from entering cilia remain unclear. Here, we investigate the conserved mechanisms regulating ciliary composition.
Materials and Methods: Using the nematode Caenorhabditis elegans, we investigated the effects of loss of BBSome function on ciliary protein composition. Time-lapse imaging was used to examine the loading of non-ciliary proteins onto IFT trains, while fluorescence recovery after photobleaching (FRAP) analysis was performed to assess ciliary gate integrity.
Results: Loss of BBSome function leads to abnormal accumulation of non-ciliary proteins within cilia, resembling phenotypes observed in ciliary gate mutants such as mks-5 and nphp-4. Time-lapse imaging reveals inappropriate loading of non-ciliary proteins onto IFT trains, while FRAP analysis indicates that the ciliary gate remains largely intact.
Conclusion: Together, our findings support a two-step model of ciliary composition control involving selective cargo loading and ciliary gating and highlight the role of the BBSome in maintaining ciliary protein specificity in C. elegans.

Keywords: BBSome, ciliary gate, intraflagellar transport (IFT), ciliary composition, Caenorhabditis elegans

PP022

Selective Transcriptional Downegulation of Sirt1 and Sirt3 in Antimycin A–Induced Mitochondrial Stress in C2C12 Myoblasts

İsra ŞİNİK[1], Evrim AKSU MENGEŞ[1], Burcu BALCI HAYTA[1]

[1 ١]Hacettepe University Faculty of Medicine, Department of Medical Biology, ٠٦١٠٠ Sıhhiye, Ankara, Türkiye.

Background/Aim: Mitochondrial dysfunction is a common secondary consequence of many neuromuscular disorders and contributes to skeletal muscle degeneration and disease progression. NAD^+-dependent sirtuins are key regulators of mitochondrial homeostasis, linking cellular energy metabolism with mitochondrial quality control. However, their potential roles in secondary mitochondrial dysfunction in skeletal muscle remain poorly understood. This study investigates the expression levels of SIRT1, SIRT3, SIRT4, and SIRT5 at both the mRNA and protein levels in an in vitro model of mitochondrial damage established in C2C12 myoblasts using Antimycin A (AMA), a known inhibitor of mETC complex III.
Materials and Methods: Mitochondrial damage in C2C12 cells was induced by treating the cells with AMA (25–30 µM) in serum-free medium at 37°C for 90 minutes. After mitochondrial damage was evaluated by assessing mitochondrial fragmentation using TOM20 immunofluorescence staining, ATP levels were measured using an bioluminescence assay, while mitochondrial membrane potential was assessed using MitoTracker Red CMXRos staining. Subsequently, SIRT1, SIRT3, SIRT4, and SIRT5 expression levels were determined at both the mRNA and protein levels using RT-qPCR and Western blot, respectively.
Results: AMA-treated C2C12 cells exhibited marked mitochondrial fragmentation, accompanied by reduced mitochondrial membrane potential and ATP levels, while substantial cell viability was maintained compared with controls. Both SIRT1 and SIRT3 expression levels were significantly reduced in AMA-treated cells compared with control cells at both the mRNA (Sirt1: 1.8 fold at 25 µM; 2.1 fold at 30 µM/ Sirt3: 1.7 fold at 25 µM; 1.6 fold at 30 µM) and protein levels (SIRT1: 2.4 fold and SIRT3: 1.7 fold at 30 µM), whereas SIRT4 and SIRT5 remained unchanged.
Conclusion: Mitochondrial dysfunction selectively suppresses Sirt1 and Sirt3 expression in skeletal muscle cells, highlighting their potential roles in mitochondrial damage.

This study was supported by Hacettepe University, Scientific Research Projects Coordination Unit (Project no: THD-2025-22351)

Keywords: mitochondrial damage, sirtuins, neuromuscular disorders, skeletal muscle, in-vitro model

PP023
Protective Effect of Oleic Acid Against Ferroptosis in Granulosa Cells

Hilal Bayram[1], Emine Elif Güzel Meydanlı[1]

[1] İstanbul University-Cerrahpasa, Cerrahpasa Medical School, Department of Histology and Embryology, İstanbul, Türkiye

Background/Aim: Ferroptosis is an iron-dependent form of regulated cell death driven by peroxidation of polyunsaturated fatty acid (PUFA)-containing membrane phospholipids. In contrast, monounsaturated fatty acids (MUFAs) are less susceptible to lipid peroxidation and may increase resistance to ferroptosis. Oleic acid (OA), a major MUFA, may therefore exert a protective effect by limiting peroxidizable lipids. This study investigated the protective effect of OA against ferric ammonium citrate (FAC)-induced ferroptotic damage in granulosa cells.
Materials and Methods: COV434 human granulosa cells were used. OA concentrations were selected by CCK-8 viability analysis, and 50 and 100 µM OA were chosen. Cells were pretreated with OA for 24 h and then exposed to FAC for 24 h. FAC-induced changes in cell viability were assessed using Trypan Blue. Lipid peroxidation was evaluated by malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) enzyme-linked immunosorbent assays and BODIPY 581/591 C11 fluorescence imaging. Protein concentrations were determined by bicinchoninic acid assay for normalization.
Results: FAC exposure decreased granulosa cell viability and increased lipid peroxidation compared with controls. OA alone did not markedly reduce cell viability at the selected concentrations. Pretreatment with 50 and 100 µM OA attenuated FAC-induced lipid peroxidation in biochemical and fluorescence-based analyses. These findings suggest that OA may increase resistance to iron-induced ferroptotic injury, potentially because MUFAs are less susceptible to lipid peroxidation than PUFAs.
Conclusion: OA showed a protective effect against FAC-induced ferroptotic damage in granulosa cells by limiting lipid peroxidation. Modulation of the cellular lipid environment toward less peroxidizable lipids may contribute to this effect. OA may represent a potential protective approach against cellular damage associated with iron overload and excessive lipid peroxidation. Further mechanistic and in vivo studies are required.

Keywords: ferroptosis, oleic acid, granulosa cell, iron overload, lipid peroxidation

PP024
Exploring the Impact of Environmentally Relevant Nanoplastics on Mitochondrial Morphology and ER Stress Responses

Sude Bildi[1], Hande Gündüz Koçan[2], Ebru Hacıosmanoğlu Aldoğan[3], Emir Alper Türkoğlu[4], Erkan Kıdeyş[5], Esra Çikler[6], Fulya Dal Yöntem[7], Müfide Aydoğan Ahbab[8]

[1] Koc University Graduate School of Health Sciences, Istanbul, Turkey
[2] Koc University, Chemistry Department, Rumelifeneri, Istanbul, Turkey
[3] Cerrahpasa University Faculty of Medicine, Department of Biophysics, Istanbul, Turkey
[4] University of Health Sciences Turkey, Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Turkey, İstanbul, Turkey
[5] Middle East Technical University (METU), Ankara, Turkey
[6] University of Health Sciences Turkey, Department of Histology and Embryology, Hamidiye Faculty of Medicine, İstanbul, Turkey
[7] Koc University School of Medicine, Department of Biophysics, Istanbul, Turkey
[8] University of Health Sciences Turkey, Hamidiye Institute of Health Sciences, Biotechnology Department, Istanbul, Turkey

Background/Aim: Plastic waste is a major environmental contaminant, with microplastics (MPs) and nanoplastics (NPs) increasingly detected in food and water, resulting in human exposure. While primary polystyrene nanoplastics (PSNPs) have been linked to adverse cellular responses, the effects of environmentally relevant secondary PSNPs remain poorly understood. This study investigates secondary PSNP-induced cellular stress using a model that more closely reflects real-world exposure.
Materials and Methods: Secondary PSNPs were prepared and size-fractionated to enrich approximately 100 nm particles and characterized by Fourier-transform infrared spectroscopy (FTIR), small-angle X-ray scattering (SAXS), scanning electron microscopy (SEM), and nanoparticle tracking analysis (NTA). BEAS-2B (human bronchial epithelial cells), SH-SY5Y (human neuroblastoma-derived neuronal cells), and 1.2B4 (human pancreatic beta-cell) cells were exposed to 10^4–10^8 particles/mL for 24h. Cell viability was assessed by MTT assay, and cellular uptake by Nile Red-labelled PSNPs with membrane and lysosomal staining. Mitochondrial morphology, total and mitochondrial reactive oxygen species (ROS), total antioxidant status (TAS), total oxidant status (TOS) was investigated, and ER-stress response was evaluated by Western Blotting.
Results: Characterization confirmed approximately 100 nm PS with irregular, sharp-edged morphology. Both primary and secondary PSNPs were internalized by all three cell types without significantly reducing cell viability. Mitochondrial fragmentation was qualitatively increased following PSNP exposure. No significant change was observed in total and mitochondrial ROS, whereas TOS increased and TAS decreased across all three cell lines, with more pronounced alterations observed following secondary PSNP exposure. Glucose-Regulated Protein 78 (GRP78), master regulator of the unfolded protein response was increased 3-fold in SH-SY5Y cells, with secondary PSNPs producing a stronger response than primary PSNPs.
Conclusion: Secondary PSNPs can induce cellular stress without exert cytotoxicity. Their stronger effect on GRP78 suggests that environmentally relevant secondary PSNPs may demonstrate adverse effects underestimated by conventional primary PSNP models.

Keywords: Polystyrene nanoplastics, Secondary nanoplastics, Endoplasmic reticulum stress, Mitochondria, Cellular toxicity

PP025
Molecular Effects of Rosmarinic Acid in an Il-1β-Induced Inflammatory Cartilage Injury Model

Gülsima Arık[1], Evren Önay Uçar[2]

[1] Istanbul University
[2] Istanbul University

Background/Aim: Articular cartilage has a limited regenerative capacity, and inflammatory responses following cartilage injury contribute to extracellular matrix degradation and impaired chondrocyte function. Rosmarinic acid (RosA) is a natural phenolic compound with reported anti-inflammatory and antioxidant properties. This study aimed to investigate the molecular effects of RosA in an interleukin-1 beta (IL-1β)-induced inflammatory injury model using SW-1353 cells.
Materials and Methods: An inflammatory model was established by treating SW-1353 cells with 20 ng/mL IL-1β for 8 h. Subsequently, cells were treated with 1, 10, or 25 µM RosA for 48 h. These concentrations were selected based on cell viability analysis. Cellular migration was evaluated using a scratch assay, while the expression levels of nuclear factor kappa B (NF-κB), matrix metalloproteinase-13 (MMP-13), Caspase-3, Collagen-I, HSP70, and HSP90 were assessed by Western blot analysis.
Results: IL-1β treatment increased the expression of NF-κB, MMP-13, Caspase-3, Collagen-I, HSP70, and HSP90 compared with the untreated control. RosA exerted concentration-dependent regulatory effects on these proteins. Low and moderate RosA concentrations reduced NF-κB and Caspase-3 expression, whereas 25 µM RosA markedly decreased MMP-13 and Collagen-I expression. In contrast, Caspase-3 expression increased at 25 µM RosA. In the scratch assay, RosA treatment improved the reduced scratch closure observed following IL-1β exposure, with the highest closure rate detected in the 10 µM RosA group.
Conclusion: RosA modulated inflammatory, cellular stress, matrix degradation, and apoptotic responses in a concentration-dependent manner in IL-1β-treated SW-1353 cells. These findings suggest that RosA may have protective potential against inflammatory cartilage injury, while emphasizing the importance of concentration-dependent effects.

Keywords: Rosmarinic Acid, Cartilage Injury, IL-1β, Inflammation

PP026
Molecular Characterization of MTMR8 and Development of a Strategy to Investigate Its Interactome

Nida Nur Öner[1, 2, 3], Gencay Kaan Polat[1, 2, 3], Şehime Gülsün Temel[4], Gülden Budak[5], Arzu Çelik Fuss[1, 2, 3]

[1] Department of Molecular Biology and Genetics, Bogazici University, 34342 Bebek, Istanbul, Turkiye
[2] Center for Life Sciences and Technologies, Bogazici University, 34342 Bebek, Istanbul, Turkiye
[3] Center for Targeted Therapy Technologies (CT٣), Bogazici University, Kandilli Campus, Cengelkoy, Istanbul 34685, Turkiye
[4] Department of Medical Genetics, Faculty of Medicine, Bursa Uludag University, Bursa, Turkiye
[5] Department of Medical Biology, Faculty of Medicine, Istinye University, Istanbul, Turkiye

Background/Aim: Retinitis pigmentosa (RP) comprises a genetically heterogenous group of inherited retinal disorders characterized by progressive photoreceptor degeneration. Primary driver of RP pathogenesis lies in defects in the primary cilium, essential for photoreceptor maintenance and signal transduction. Whole-exome sequencing of an RP patient identified a hemizygous variant of uncertain significance in MTMR8, encoding a phosphoinositide phosphatase implicated in membrane trafficking and phosphoinositide metabolism. Although studies of its orthologs suggest that Mtmr8 contributes to development and vascular integrity, its biological function remains unexplored. We aimed to characterize the subcellular localization of MTMR8 and establish a proximity-labeling approach to investigate its potential association with ciliogenesis and ciliary transport.
Materials and Methods: Full-length wild-type (wt) MTMR8 and a catalytically inactive variant (C338S-MTMR8) were cloned into MiniTurbo expression vectors using Gibson assembly and stably expressed in HEK293 cells. Proximity-dependent biotin labeling coupled with mass spectrometry was used to identify candidate proximal proteins under ciliated and non-ciliated conditions. For subcellular localization studies, N-terminally GFP-tagged WT-MTMR8 and C338S-MTMR8 constructs were generated using the Gateway cloning and expressed in HeLa cells. Their localization was examined by confocal microscopy using ARL13B as a primary cilium marker.
Results: Constructs encoding miniTurbo-tagged and GFP-tagged WT-MTMR8 and MTMR8-C338S were generated, and stable HEK293 cell lines were established for proximity-labeling experiments. Confocal microscopy revealed that both GFP-WT-MTMR8 and GFP-MTMR8-C338S exhibited predominantly perinuclear localization. No marked difference in localization was observed between the wild-type and inactive variant. Proximity-labeling analyses are ongoing.
Conclusion: This study establishes the molecular tools and cell models to investigate the MTMR8 proximity interactome under ciliated and non-ciliated conditions. The observed perinuclear localization of MTMR8 is aligned with intracellular membrane compartments, although its relationship to ciliary biology remains to be determined. Ongoing interactome analyses will test whether MTMR8 is associated with proteins involved in ciliogenesis, ciliary trafficking, or vesicle transport.

Keywords: Retinitis pigmentosa, MTMR8, Ciliogenesis, Primary cilia, Membrane phospholipids

PP027
Catenanes and Circular Dimers: A Topological Perspective on Cellular Evolution

Yekbun Adiguzel[1]

[1] Atilim University School of Medicine, Department of Biophysics, Ankara

Background/Aim: In 1970, Richards J. Douthart proposed a topological model explaining the formation of catenanes, circular dimers, and higher DNA oligomers. Based on Möbius-like configurations, the model demonstrated how different degrees of twisting could produce distinct circular DNA structures.
This study aims to examine the possible evolutionary significance of catenanes, particularly their potential role in early membrane-less cellular systems, and the subsequent requirement for mechanisms capable of separating interlocked DNA molecules.
Materials and Methods: A conceptual analysis was conducted using Douthart's model and its implications for DNA replication, retention, and segregation. The relationship between catenane formation and DNA-topology-modifying enzymes was considered in bacterial and eukaryotic cells. Douthart's observations concerning circular dimers in mitochondrial DNA were also reviewed.
Results: Catenanes arise during the replication of circular DNA and consist of interlocked rings. Although interlocking creates a segregation problem in modern cells, it may have been advantageous in primitive membrane-less systems by helping retain replicated genetic material and reducing its loss through diffusion. Catenanes may therefore have contributed to early cellular evolution.
With increasing cellular organization, efficient DNA separation became essential. This requirement may have favored the evolution of topological enzymes, including gyrases in bacteria and topoisomerase II in eukaryotes. Their function is essential for resolving DNA entanglements during replication and chromosome segregation. Douthart also distinguished circular dimers as class I complexes from catenanes as class II complexes and reported circular dimers in mitochondrial DNA from leukocytes of patients with leukemia, citing Clayton and Vinograd (1967).
Conclusion: Catenanes may have represented both an evolutionary advantage and a subsequent biological challenge. Their potential role in retaining genetic material in early cells suggests that DNA topology may have contributed significantly to the evolution of cellular organization and genome inheritance.

Keywords: Circular DNA, DNA topology, Topoisomerases

PP028
Development Of A Hybrid Hydrogel Mesh and Alginate-Based Microsphere Scaffold System for Cardiac Tissue Engineering

Muhammed Furkan Turan[1], Fatma Derya Öncel[3], Özgül Gök Özatay[2]

[1] Graduate School of Natural and Applied Sciences, Biomaterials Master's Program, Acıbadem Mehmet Ali Aydınlar University, Istanbul, Türkiye
[2] Department of Biomedical Engineering, Faculty of Engineering and Natural Sciences, Acıbadem Mehmet Ali Aydınlar University, Istanbul, Türkiye
[3] Materials Science and Nano Engineering, Graduate School of Engineering and Natural Sciences, Sabancı University, Istanbul, Türkiye

Background/Aim: Cardiac tissue engineering requires scaffold systems that can provide structural support while allowing the incorporation of different cellular or bioactive components. This study aimed to develop a hybrid scaffold system consisting of a three-dimensional (3D)-printed hydrogel mesh and alginate-based microspheres for potential cardiac tissue engineering applications.
Materials and Methods: Gelatin methacryloyl (GelMA), hyaluronic acid methacrylate (HAMA), and polyethylene glycol diacrylate (PEGDA) were combined to prepare a hydrogel formulation. Different formulations were initially evaluated based on viscosity, extrusion behavior, and ultraviolet (UV) curing response. The selected formulation was fabricated as a mesh scaffold using extrusion-based 3D printing. Alginate microspheres and peptide-conjugated alginate microspheres were prepared and incorporated with the printed mesh to form the hybrid scaffold system. The prepared materials were characterized using Fourier-transform infrared spectroscopy (FTIR) and rheological analysis.
Results: The selected HGP formulation demonstrated suitable extrusion behavior and UV-induced crosslinking for fabrication of the 3D-printed mesh. Alginate-based and peptide-conjugated alginate microspheres were successfully prepared, and a hybrid mesh–microsphere construct was successfully assembled. FTIR analysis provided qualitative evidence supporting the chemical structures of the starting materials, methacrylated polymers, peptide-conjugated alginate, and final formulation. Rheological analysis showed shear-dependent flow behavior of the precursor formulation, while UV crosslinking resulted in predominantly elastic hydrogel behavior.
Conclusion: The study demonstrated the feasibility of producing a hybrid scaffold composed of a 3D-printed mesh and alginate-based microspheres. The printed mesh provided structural support, while the microsphere component offered a complementary platform for further functionalization and cellular incorporation. Further physicochemical characterization and cell-based studies are required to evaluate the biological performance and long-term applicability of the hybrid scaffold for cardiac tissue engineering.

Keywords: 3D printing, hydrogel, microsphere, hybrid scaffold, cardiac tissue engineering

PP029
A Methodological Comparison of Circular RNA Isolation Approaches

Safiye Özalp[1], Gülşah Çeçener[2], Güven Özkaya[3]

[1] Department of Medical Biology, Graduate School of Health Sciences, Bursa Uludağ University, Bursa, Türkiye
[2] Department of Medical Biology, Faculty of Medicine, Bursa Uludağ University, Bursa, Türkiye
[3] Department of Biostatistics, Faculty of Medicine, Bursa Uludağ University, Bursa, Türkiye

Background/Aim: Circular RNAs (circRNAs) are promising biomolecules for transcriptomic and biomarker research, but their reliable analysis depends on robust isolation and enrichment strategies. This study aimed to systematically compare three methodological approaches for circRNA analysis from peripheral blood-derived total RNA in terms of technical performance, linear RNA depletion efficiency, and suitability for RT-qPCR.
Materials and Methods: Peripheral blood samples were collected from 20 individuals who provided written informed consent prior to sample collection. Total RNA was isolated and processed using three approaches: direct analysis without enrichment, conventional RNase R treatment, and an RPAD-like workflow involving polyadenylation, RNase R digestion, and magnetic bead-based purification. cDNA was synthesized using random primers, and circFBXW7, circERBB2, circCDR1as, circZNF609, ACTB, and GAPDH were analyzed by RT-qPCR.
Results: Conventional RNase R resulted in significantly greater circRNA recovery and higher relative expression levels than the RPAD-like method for circFBXW7, circCDR1as, and circZNF609 ($p < 0.01$). No statistically significant difference was observed for circERBB2 ($p = 0.114$). In contrast, the RPAD-like method achieved significantly more efficient depletion of residual linear RNA. Residual linear RNA levels were reduced to 13.84% for ACTB and 19.38% for GAPDH, compared with 55.46% and 73.04%, respectively, following conventional RNase R treatment ($p < 0.001$). Furthermore, the RPAD-like method exhibited markedly lower analytical variability and demonstrated good discriminatory performance in ROC analysis (ACTB: AUC = 0.837; GAPDH: AUC = 0.745).
Conclusion: Conventional RNase R appears more suitable when preservation and enrichment of target circRNAs are prioritized, whereas the RPAD-like method is advantageous when stricter linear RNA depletion is required. These findings support method selection according to analytical priorities in circRNA-focused RT-qPCR workflows.

Keywords: Circular RNA, RNase R, Polyadenylation

PP030

Effect of the Piezoelectric Field on RNA Modifications in Stem Cells Exposed to a PVDF-Based Wound Dressing Material: A Preliminary In Vitro Study

Hasan Basri Kurulay[1], Mehmetali Tibatan[1]

[1] İstanbul Sabahattin Zaim University

Background/Aim: In this study we aim to detect RNA modifications which may be caused by the piezoelectric field of PVDF wound dress material. Since the electrical field has a stimulating effect on living organisms, we would like to investigate its stress effects at the molecular level.
Materials and Methods: Besides, PVDF wound-dress material is known for its piezoelectric features caused by mechanical movements. However, this electric field may cause some abnormalities in the RNA level.
Results: We found 2-thiocytidine and 7-methylguanosine modifications in RNA from the cells exposed to piezoelectric field (beta-phase) increased PVDF material.
Conclusion: Therefore, our study results suggest that the wound dress production method and polymeric material preference should be taken into account when choosing proper production for the therapeutical applications. However, future studies are required to clarify RNA modification types and effect of these modifications on the cellular metabolism.

Keywords: RNA, Piezoelectric , HPLC, Wound Dress, PVDF

PP031
Time-dependent miRNA Responses To Nitrogen Deficiency, Drought and Combined Stress in Arabidopsis Thaliana Callus

Seda Yaşar[1, 2]

[1] Institute of Graduate Studies in Sciences, Istanbul University, Istanbul, Türkiye
[2] Current Affiliation: Joseph Gottlieb Kölreuter Institute for Plant Sciences (JKIP) Department of Molecular Biology, Karlsruhe, Germany

Background/Aim: Plants frequently encounter multiple abiotic stress conditions simultaneously, and microRNA responses may vary depending on stress type and exposure duration. This study aimed to compare the early and prolonged expression responses of miR167d, miR319c and miR396b-5p, together with callus growth responses, under nitrogen deficiency, drought and combined stress conditions.
Materials and Methods: Arabidopsis thaliana Col-0 callus were exposed to control, nitrogen-deficient, polyethylene glycol (PEG) 8000-induced drought and combined stress conditions for 7 or 14 days. Growth was evaluated using fresh-weight-based growth index analysis. MicroRNA expression was quantified by stem-loop quantitative reverse-transcription Polymerase Chain Reaction (PCR) using Actin as the reference gene and the delta-delta ct method. Three biological replicates and three technical replicates were analysed. Growth index and microRNA expression data were evaluated using two-way and one way analysis of variance, respectively followed by Tukey's multiple-comparison test, with $p<0.05$ considered significant.
Results: Combined stress reduced callus growth by 39% after 7 days, and growth remained 11% below the initial level after 14 days. miR167d was significantly up regulated under nitrogen deficiency but down regulated under combined stress at both time points. miR319c was significantly down regulated under drought and combined stress at both time points, whereas it was up regulated following 14 days of nitrogen deficiency. miR396b-5p was down regulated under all 7-day stress treatments but significantly up regulated following 14 days of combined stress.
Conclusion: The selected miRNAs displayed distinct expression patterns depending on stress condition and duration. The contrasting response of miR167d, persistent suppression of miR319c under drought-containing conditions and temporal reversal of miR396b-5p under combined stress were observed alongside sustained inhibition of callus growth. These findings highlight the complexity of microRNA responses to combined abiotic stress.

Keywords: Arabidopsis thaliana, microRNA, drought, nitrogen deficiency, combined stress

PP032

Expressions of Epithelial Ion Channels ENaC-α and SLC26A4 in Patients with Cystic Fibrosis Transmembrane Conductance Regulator Related Disorders Presenting with Pseudo-Bartter Syndrome

Alperen KORKMAZLI[1], Dilara DEMİREL[2], Burcu ÇAPRAZ-YAVUZ[2], Deniz DOĞRU[2], Nagehan EMİRALİOĞLU-ORDUKAYA[2], Uğur ÖZÇELİK[2], Ebru YALÇIN[2], Didem DAYANGAÇ-ERDEN[1]

[1] Hacettepe University, Faculty of Medicine, Department of Medical Biology, Ankara Türkiye
[2] Hacettepe University, Faculty of Medicine, Department of Pediatric Pulmonology, Ankara Türkiye

Background/Aim: Cystic fibrosis (CF) is an autosomal recessive disease caused by mutations in the CF Transmembrane Conductance Regulator (CFTR) gene. Patients exhibiting clinical features of CF but don't fulfill the current diagnostic criteria are classified as CFTR-related disorders (CFTR-RD). Pseudo-Bartter Syndrome (PBS), which is an important metabolic complication of CF, is characterized by hyponatremia, hypokalemia, hypochloremia, and metabolic alkalosis resulting from excessive electrolyte loss. In these patients ion channels in addition to CFTR play a critical role in determining the disease phenotype. The aim of this study is to analyze the expression levels of ENaC-α and SLC26A4 gene and their role in pathophysiology of PBS.
Materials and Methods: Total RNA was isolated from nasal swabs collected from 9 pediatric patients with at least one episode of PBS, grouped as CFTR-RD with PBS (n=5) and CF with PBS (n=4). The expression levels of ENaC-α and SLC26A4 genes were determined using quantitative-real time PCR (qPCR). Actin-B (ACTB) was used as the reference gene.
Results: The expression level of SLC26A4 was decreased 1.56 fold in CFTR-RD with PBS group compared to CF with PBS group. Also, ENaC-α expression was down regulated 1.20 fold in CFTR-RD with PBS group compared to CF with PBS group.
Conclusion: Further research with an increased sample size and additional ion channel candidates will provide valuable insights into the molecular mechanisms and pathophysiology of CFTR-RD and PBS, thereby contributing to the early diagnosis and effective management of PBS.
This study is funded by Hacettepe University Scientific Research Projects Coordination Unit(THD-2025-21598)

Keywords: CFTR-RD, CF, PBS, SLC26A4, ENaCA

PP033
Investigating the Role of Ribosome-associated Quality Control in Prostate Cancer

Ayşe Nilay SEYHAN[1], Duygu KUZUOĞLU ÖZTÜRK[1, 2, 3]

[1] Department of Molecular Biology, Genetics and Bioengineering, Faculty of Engineering and Natural Sciences, Sabanci University, Istanbul, Turkey
[2] Department of Urology, University of California, San Francisco, San Francisco, CA, USA
[3] Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, San Francisco, CA, USA

Background/Aim: Prostate cancer is one of the leading causes of cancer-related mortality in men and is primarily driven by androgen receptor (AR) signaling. Although androgen deprivation therapy is initially effective, many patients eventually develop castration-resistant prostate cancer (CRPC), in which AR signaling remains active. While the transcriptional regulation of AR has been extensively studied, the mechanisms controlling its translation remain poorly understood. Ribosome-associated quality control (RQC) maintains translational homeostasis by recognizing stalled and collided ribosomes and promoting their resolution. This study aimed to investigate whether AR mRNA translation is regulated through its coding sequence during translation elongation and to determine the contribution of RQC to its regulation in CRPC.
Materials and Methods: Polysome profiling was performed in 22Rv1 cells to evaluate global translational activity, and the distribution of polysome-associated AR mRNA in polysome fractions was evaluated by RT-qPCR. The effect of canonical RQC pathway factors on AR expression and its translation was investigated by depleting and overexpressing RQC factors in 22Rv1 cells. Following transcriptional inhibition, the effect of the RQC pathway on AR mRNA stability was assessed.
Results: Our study in 22Rv1 cells showed that modulation of canonical RQC factors altered AR expression and affected AR mRNA stability. These findings demonstrate that distinct RQC factors differentially regulate AR expression and suggest that AR expression is regulated through the RQC pathway. Although the precise ribosome stalling sites and the underlying molecular mechanisms remain to be identified, this study reveals a previously unrecognized layer of post-transcriptional regulation of AR expression.
Conclusion: Overall, our findings identify the interaction between AR expression and RQC in CRPC and highlight translation elongation and the RQC pathway as promising therapeutic targets for future CRPC treatment.

Keywords: translation, androgen receptor, ribosome-associated quality control, prostate cancer

PP034

A New Role for an Old Cancer Driver: Transcriptome-Wide RNA Binding by a Nuclear Receptor and Its Splice Variant

Cansu YILMAZ[1], Duygu KUZUOĞLU-ÖZTÜRK[1, 2, 3]

[1] Molecular Biology, Genetics and Bioengineering, Faculty of Engineering and Natural Sciences, Sabancı University, İstanbul, Türkiye
[2] Department of Urology, University of California, San Francisco, San Francisco, CA, USA
[3] Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, San Francisco, CA, USA

Background/Aim: The main drivers of prostate cancer are the androgen receptor (AR), which is a ligand-dependent nuclear receptor, and its constitutively active splice variant, androgen receptor variant 7 (AR-V7). Both proteins are well-characterized transcription factors, and their DNA-binding activity is considered essential for their role in prostate cancer progression. Androgen deprivation therapy, the primary clinical treatment for the patients, aims to inhibit the DNA-binding capacity of these oncogenes. However, emerging evidence highlights an additional layer of complexity: Recent studies reported that transcription factors, including members of the hormone receptor family encompassing AR, also interact with RNA. Importantly, their RNA-binding capacity actively contributes to their oncogenic function. In this study, we first identify the novel RNA-binding capacity of AR and its splice variant and then investigate how AR directly interacts with cellular RNAs using a prediction method combined with experimental data.
Materials and Methods: To identify how cellular mRNAs interact with AR and its splice variant AR-V7, we integrated experimental cross-linking immunoprecipitation sequencing (CLIP-seq) with in silico structural modeling. Using RNA sequences derived from our CLIP-seq data, protein-RNA complexes were co-folded utilizing Boltz-2, with a specific focus on interactions mapping to the 3'UTR. The frequency with which each residue appeared in the interface sphere (5 Å radius) was recorded, and the critical residues were grouped based on frequency.
Results: Despite the well-known and characterised DNA signalling roles of these proteins in prostate cancer progression, our preliminary data indicate a non-canonical role of AR and ARV7 proteins, which is significantly associated with interaction with coding RNAs. The residue frequency indicated high-occupancy regions in both receptors. Molecular dynamics simulations of the alanine-mutated variants identified the critical residue groups in both receptors.
Conclusion: Overall, in silico analysis indicated that the AR and AR-V7 have non-canonical RNA-Binding regions for mature cytoplasmic mRNAs through their shared domains.

Keywords: adnrogen receptor, prostate cancer, CLIP-seq, molecular dynamics simulations

PP035
Functional Interrogation of MYC Transcription Factor Binding Sites in Triple-Negative Breast Cancer Using CRISPR/Cas9-Based Screening

Ezgi Nur Çatı[1, 3], Hande Özkan Coşkun[3], Begüm Durdar[2, 3], Ferzan Betül Berber[2, 3], Deniz Gülfem Öztürk[3, 5], Gözde Korkmaz[3, 4]

[1] Graduate School of Health Sciences, Koc University, Sariyer, İstanbul, Türkiye
[2] Graduate School of Sciences and Engineering, Koc University, Sariyer, İstanbul, Türkiye
[3] Koc University Research Center of Translational Medicine (KUTTAM), Sariyer, Istanbul, Turkey
[4] School of Medicine, Koc University, Sariyer, İstanbul, Türkiye
[5] Albert Einstein College of Medicine, Bronx, New York, USA

Background/Aim: Triple-negative breast cancer (TNBC) is an aggressive subtype of breast cancer with limited targeted treatment options. MYC is a key oncogenic driver in TNBC, regulating transcriptional programs that promote cell proliferation, metabolism, and survival. However, MYC remains difficult to target therapeutically. Therefore, we aimed to identify and characterize MYC-bound regulatory regions that may serve as novel therapeutic targets.
Materials and Methods: To identify functional MYC-bound regulatory regions, a pooled CRISPR/Cas9 dropout screen was conducted in the TNBC cell lines MDA-MB-231 and BT-549 using the LIB_MYC library. Following the low-MOI (Multiplicity of Infection) transduction, cells were collected at three different time points. Genomic DNA was isolated, and sgRNA abundance was assessed by amplifying guide sequences through a two-step PCR protocol followed by next-generation sequencing. Bioinformatic analysis of the sequencing data was carried out using an optimized CRISPR screening pipeline, including MAGeCK-based sgRNA counting, normalization, and robust rank aggregation (RRA) analysis for hit identification.
Results: The CRISPR/Cas9 dropout screen identified multiple candidate MYC-bound regulatory regions whose disruption led to progressive sgRNA depletion in both MDA-MB-231 and BT-549 cell lines, suggesting a functional contribution to TNBC cell survival and proliferation. Bioinformatic analyses demonstrated the robustness and reproducibility of the screening approach. The top candidate hit was further validated in both MDA-MB-231 and BT-549 cell lines, confirming its functional role in TNBC.
Conclusion: Our targeted CRISPR/Cas9 dropout screen identified candidate functional MYC-bound regulatory regions that are essential for TNBC cell fitness. Functional characterization of the top candidate confirmed its biological significance in both MDA-MB-231 and BT-549 cell lines. Collectively, these findings highlight the potential of MYC-associated regulatory elements as novel therapeutic targets in TNBC.

Keywords: Triple-negative breast cancer , MYC, CRISPR/Cas9 screening, Regulatory elements

PP036
ETC-159 Suppresses WNT-Driven Fibrotic and Inflammatory Signaling in Human Fibroblast and Fibrosarcoma Cells

Yunus Güngörmez[1], Dilara Nur Kılıç[1], Ayse Koçak Sezgin[1]

[1] Kütahya Health Sciences University

Background/Aim: Aberrant activation of WNT signaling plays a central role in fibrosis by promoting fibroblast activation, extracellular matrix deposition, and chronic inflammation. Porcupine inhibitors, which prevent WNT ligand secretion, have emerged as promising therapeutic agents for WNT-dependent disorders. ETC-159 is a potent and selective Porcupine inhibitor; however, its molecular effects on fibrosis-associated signaling pathways remain incompletely understood.
This study investigated the effects of ETC-159 on cell viability, apoptosis, inflammatory cytokine production, TGF-β secretion, and WNT ligand expression in human fibroblast and fibrosarcoma cell models.
Materials and Methods: IC_{50} values of ETC-159 were determined in WEHI, CCD-1135, and HT1080 cells using a cell viability assay. Based on these values, cells were treated with the selected inhibitory concentration. mRNA expression of BCL2, BAX, TP53, WNT1, WNT3, WNT5A, WNT7A, WNT7B, WNT10A, and WNT10B was quantified by quantitative real-time PCR. Secreted IL-6, IL-1β, and TGF-β levels were measured by ELISA. Statistical analyses compared treated and untreated groups.
Results: ETC-159 significantly attenuated inflammatory responses in human fibroblast and fibrosarcoma cells, reducing IL-6 and IL-1β production compared with controls. Decreased TGF-β levels indicated suppression of profibrotic signaling. Quantitative real-time PCR revealed broad downregulation of WNT1, WNT3, WNT5A, WNT7A, WNT7B, WNT10A, and WNT10B, confirming inhibition of WNT signaling. Expression of apoptosis-related genes (BCL2, BAX, and TP53) showed a tendency toward modulation, but changes were not statistically significant. Overall, ETC-159 strongly inhibited WNT-mediated inflammatory and fibrotic pathways while exerting only modest effects on apoptosis.
Conclusion: ETC-159 suppressed WNT-dependent inflammatory and profibrotic signaling through downregulation of WNT ligands and reduced TGF-β production in fibroblast and fibrosarcoma cell models. These findings suggest that Porcupine inhibition may represent a promising therapeutic strategy for WNT-driven fibrotic diseases and warrants further preclinical investigation.

Keywords: ETC-159, Fibrosarcoma, WNT signaling

PP037
Macrophage–Hepatic Stellate Cell Crosstalk Under Metabolic Stress Modulates Epithelial–Mesenchymal Plasticity in Colorectal Cancer Cells

Gülhas Solmaz Kavak[1, 2], Duygu Gündüz[2, 3], Peyda Korhan[2, 4], Safiye Neşe Atabey[2, 4, 5]

[1] Medical Laboratory Techniques Program, Vocational School of Health Services, Izmir Tinaztepe University, Buca, Izmir, Türkiye
[2] Galen Research Center, Izmir Tinaztepe University, Buca, Izmir, Türkiye
[3] Department of Nutrition and Dietetics, Faculty of Health Sciences, Izmir Tinaztepe University, Buca, Izmir, Türkiye
[4] Department of Medical Biology, Faculty of Medicine, Izmir Tinaztepe University, Buca, Izmir, Türkiye
[5] Izmir Biomedicine and Genome Center (IBG), Balçova, Izmir, Türkiye

Background/Aim: Metabolic dysfunction-associated steatohepatitis (MASH) creates a pro-inflammatory and remodeled hepatic microenvironment that functions as a pre-metastatic niche, facilitating colorectal cancer (CRC) progression and liver colonization. While macrophages and hepatic stellate cells (HSCs) are key components of this niche, the impact of their reciprocal crosstalk on CRC epithelial–mesenchymal plasticity (EMP) remains poorly understood. This study aimed to investigate how paracrine signals derived from a three-dimensional (3D) MASH-like microenvironment influence CRC cell plasticity.
Materials and Methods: A collagen type I-based 3D culture model was established using LX-2 HSCs and PMA-differentiated THP-1 macrophages under monoculture and co-culture conditions. MASH-associated metabolic stress was induced using a metabolic medium containing high glucose, fructose, insulin, and a lipid mixture of palmitic and oleic acids. The conditioned media from 3D cultures were collected and applied to SW480 and HCT-116 CRC cells. Cellular responses were evaluated by XTT viability assay, cell-cycle analysis, wound healing (migration) assay, colony formation assay, and RT-qPCR analysis of key EMP-related genes.
Results: Conditioned media from macrophage–HSC co-cultures elicited distinct CRC phenotypic responses compared with monoculture-derived media, with the strongest effects observed under MASH-associated metabolic stress. Molecular profiling revealed a hybrid epithelial–mesenchymal phenotype. Although metabolically challenged co-culture-derived conditioned media reduced metabolic activity and proliferation, it promoted dispersed colony organization and altered colony morphology in CRC cells, suggesting that the MASH microenvironment favors phenotypic plasticity over rapid proliferation.
Conclusion: Our findings suggest that MASH-associated metabolic stress reshapes macrophage–HSC crosstalk, resulting in altered stromal-derived paracrine signals associated with a hybrid EMP phenotype and altered clonogenic properties in CRC cells. This study highlights the role of immune–stromal crosstalk in the MASH-associated microenvironment and its potential as a target for limiting CRC metastatic adaptation and liver colonization.
Funding: This study was supported by the TÜBİTAK 3501 Career Development Program (Project No: 323S506).

Keywords: Metabolic dysfunction-associated steatohepatitis (MASH), colorectal cancer, hepatic stellate cells, macrophages, epithelial–mesenchymal plasticity (EMP)

PP038

Supercritical CO_2 Extract of Salvia heldreichiana Suppresses Cancer Stem Cell-Associated Markers and Enhances Oxaliplatin Efficacy in Colorectal Cancer

Nagehan Betül Çelik[1], Amine Erdoğanlı[1], Süleyman Doğu[2], Suray Pehlivanoğlu[1]

[1] Necmettin Erbakan University, Faculty of Science, Department of Molecular Biology and Genetics,Konya, Türkiye
[2] Necmettin Erbakan University, Medical and Cosmetic Plants Application and Research Center, Konya, Türkiye

Background/Aim: According to the World Health Organization, colorectal cancer ranks as the third most commonly diagnosed malignancy and the second leading cause of cancer-related mortality worldwide. The resistance to chemotherapy represent the major obstacles to treatment. This phenomenon is generally attributed to the presence of cancer stem cells. In this context, the present study aimed to determine the therapeutic effects of the supercritical CO_2 extract of Salvia heldreichiana on cancer stem cell biomarkers and oxaliplatin resistance.
Materials and Methods: The colorectal cancer-associated target genes PROM1 (CD133), ALDH1, ALDH2, and CXCR4 were evaluated in silico with transcriptomic profiles (TCGA) and functional interaction networks (STRING). The cytotoxic effects of the extract administered alone or in combination with oxaliplatin, were evaluated in HT-29 and Caco-2 cells using the MTT reagent, gene expression levels were determined by qRT-PCR, and clonogenic properties were assessed by the colony formation assay in vitro.
Results: According to TCGA database, the expression profiles of the target genes were determined in colorectal cancer tissue samples. The elevated levels of PROM1 (CD133) and ALDH1 were found to be associated with reduced patient survival. The extract exhibited cytotoxic effects against HT-29 and Caco-2 colorectal cancer cells and enhanced the cytotoxic and antiproliferative effects of oxaliplatin treatment. Furthermore, the extract suppressed the mRNA expression levels of PROM1 (CD133), ALDH1, ALDH2, and CXCR4 genes. STRING analysis revealed a strong functional interaction of PROM1 (CD133) with ALDH1 and CXCR4.
Conclusion: This study demonstrated that the supercritical CO_2 extract of S. heldreichiana exhibited anticancer activity in colorectal cancer cells, enhanced the therapeutic efficacy of oxaliplatin, and suppressed the expression of cancer stem cell (CSC)-associated genes. The findings suggest that the extract holds promise as a potential adjunctive therapeutic approach for the treatment of advanced colorectal cancer.

Keywords: Cancer Stem Cells, Colorectal Cancer, Oxaliplatin, Salvia heldreichiana

PP039
Investigation of the Relationship Between Cancer and the Homologous Recombination Repair (HRR) Mechanism

Adar İntaş[1], Berke Muslubaş[1], Berin Yılmazer Aktar[1], Arzu Aysan[2], Soner Aksu[1]

[1] Istanbul Health and Technology University, Department of Molecular Biology and Genetics, Istanbul, Turkey
[2] Gebze Technical University, Department of Molecular Biology and Genetics, Kocaeli, Turkey

Background/Aim: A strong correlation exists between Double-strand breaks (DSBs), genomic instability, and cancer development. Homologous Recombination Repair (HRR) is the primary repair mechanism for highly accurate DSB repair. This study aimed to investigate the relationship between cancer and selected 12 genes involved in the HRR mechanism.
Materials and Methods: Using the RT-qPCR analysis differences in the expression levels of the Ataxia Telangiectasia Mutated (ATM), BRCA1 Interacting Protein C-Terminal Helicase 1 (BRIP1), Fanconi Anemia Complementation Group D2 (FANCD2), Tumor Protein p53 Binding Protein 1 (TP53BP1), DNA Polymerase Theta (POLQ), RAD54 Like (RAD54L), AT-Rich Interaction Domain 1A (ARID1A), RAD52 DNA Repair Protein (RAD52), Bloom Syndrome Protein (BLM), SWI/SNF-Related Matrix-Associated Actin-Dependent Regulator of Chromatin Subfamily A Containing DEAD/H Box 1 (SMARCAD1), Werner Syndrome ATPDependent, Helicase (WRN) and RecQ Like Helicase 4 (RECQL4) genes between cancer and normal cell lines were investigated. Frequently used 10 cancer and 2 normal cell lines were used in this study.
Results: It was determined that RAD52 was significantly up-regulated only in colon cancer cell lines. ARID1A was significantly down-regulated, particularly in breast and lung cancer. BLM, WRN, TP53BP1, ATM and RAD54L were down-regulated in all cancer types. RECQL4 was down-regulated in breast and lung cancer, but up-regulated in liver and brain cancer. BRIP1 was down-regulated in breast, brain, and lung cancer, but up-regulated in liver and colon cancer cell lines. FANCD2 was up-regulated only in lung cancer, and significantly down-regulated in all other cancer types. POLQ was significantly down-regulated especially in lung cancer.
Conclusion: This study has provided preliminary results suggesting that the expression differences observed in the interested genes could be used as a molecular indicator for the carcinogenesis. Meaningful results have also been obtained indicating that diagnostic panels based on gene expression levels can be developed for monitoring cancer progression.

Keywords: Double-strand breaks,Homologous Recombination Repair,Cancer Cell Lines,Gene Expression,RT-qPCR

PP040

A Brain-related Kinase Regulates Pediatric Glioma Proliferation, Infiltration, and Autophagy

Nehir Dalgic[1], Suheda Duran[1], Gamze Deveci[1], Ibrahim Bayramoglu[3], Yunus Akkoc[1, 2], Joern Dengjel[3], Devrim Gozuacik[1, 2, 4]

[1] Research Center for Translational Medicine (KUTTAM), Koç University, Istanbul, Türkiye
[2] Department of Medical Biology, School of Medicine, Koç University, Istanbul, Türkiye
[3] Department of Biology, Faculty of Science and Medicine, Fribourg University, Fribourg, Switzerland
[4] SUNUM Nanotechnology Research and Application Center, Istanbul, Türkiye

Background/Aim: Gliomas are highly aggressive malignancies with poor survival rates driven by pronounced tissue infiltration capacity and limited therapeutic options. Although adult and pediatric gliomas are pathologically and molecularly distinct, clinical treatment strategies remain largely identical, but prognosis remains very poor in pediatric cases.
Materials and Methods: We investigated the role of a novel stress-related brain kinase in pediatric glioma cells. We established CRISPR-Cas9 knockout, kinase, and inactive kinase overexpression models in pediatric glioma cell lines. Western blotting and quantitative PCR were performed to analyze key molecular markers. Phenotypic changes were evaluated using viability, migration, and autophagy assays. Downstream targets and altered signaling pathways were identified through comparative proteomics of wild-type and knockout cells.
Results: Modulating kinase expression significantly impacted autophagy and migratory capacity of pediatric glioma cells. Proteomic profiling further uncovered distinct pathway alterations and candidate molecular targets regulated by the kinase.
Conclusion: We introduced a kinase expressed in the brain as a novel stress-related kinase controlling pediatric glioma cell migration and discovered its relevant phosphorylation targets. The kinase expression profile and its key targets might be used as molecular sub-classification markers, and open novel avenues for therapy development in pediatric neuro-oncology.

Keywords: pediatric glioma, serine-threonine kinase, infiltration, autophagy, phospho-proteomics

PP041
CTF1 is a Tumor-derived Cytokine and a Critical Mediator of Stroma-assisted Breast Cancer Cell Migration, Invasion and Metastasis

Omrum Naz Nalkiran[1], Yunus Akkoc[1, 6], Dyana Sari-Turk[1], Ece Dilege[3], Joern Dengjel[4], A. Işın Dogan-Ekinci[5], Devrim Gozuacik[1, 6, 2]

[1] Koç University School of Medicine, Research Center for Translational Medicine (KUTTAM), ٣٤٠١٠ Istanbul, Turkey
[2] Sabanci University Nanotechnology Research and Application Center (SUNUM), Department of Biotechnology, 34956 Istanbul, Turkey.
[3] Koç University Hospital, Department of General Surgery, Koç University Medical School, ٣٤٠١٠ Istanbul, Turkey.
[4] University of Fribourg, Department of Biology, Chemin Du Musée 10, CH-1700 Fribourg, Switzerland
[5] Acibadem Mehmet Ali Aydınlar University School of Medicine, Department of Pathology, 34752 Istanbul, Turkey
[6] Koç University School of Medicine, Medical Biology ٣٤٤٥٠ Istanbul, Turkey.

Background/Aim: Cardiotrophin-1 is a 21.5-kilodalton cytokine belonging to the IL-6 cytokine family. It's role in hypertension, cardiac hypertrophy, and heart failure has been previously studied. The role of the cytokine in cancer was not clear. In this study, we showed that cancer-derived CTF1 plays a key role in tumor-microenvironment interactions.
Materials and Methods: The breast cancer cells MCF7, mouse embryonic fibroblasts (MEFs), and patient-derived human cancer-associated fibroblasts (CAFs) were used to investigate CTF1-driven cancer invasion and migration. Transwell migration and invasion experiments were performed using cancer cell-fibroblast co-cultures. Additionally, breast adenocarcinoma and paired non-tumor breast tissue frozen and histopathology samples from patients were grouped according to clinical stage (T1/T2 and N0/N1/N2 based on AJCC classification) and analyzed.
Results: Co-culture experiments revealed that while cancer invasion and migration increased when MCF7 cells were co-cultured with MEF and CAF cells, this effect was attenuated upon the treatment with a CTF1-neutralizing antibody. Furthermore, comparison of tumor and non-tumor sections in patient samples revealed that tumor tissues that showed higher expression of CTF1 were enriched in CAF cells and higher CTF1 levels positively correlated with T2 tumors (larger size) and lymph node metastasis.
Conclusion: We showed for the first time that CTF1 is an important mediator of tumor-stroma interactions and cancer metastasis. Our results strongly indicated that CTF1 may be used as a novel cancer diagnosis marker and a potential drug target, disrupting tumor-stroma interactions.

Keywords: Cardiotrophin-1, Carcinoma-associated fibroblasts (CAFs), Tumor microenvironment (TME), Diagnostics, Drug target

PP042
The Determination of Tyrosine Kinase Inhibitors that Overcome FGFR1 V561M Gatekeeper Mutation Using a Computational Method

Elif Başer[1]

[1] Nesibe Aydın Yıldızlar Science High School, Ankara, Türkiye

Background/Aim: Fibroblast growth factor receptor 1 (FGFR1) is a tyrosine kinase (TK) receptor, and its mutations can lead to tumor progression. Among FGFR1 mutations, the V561M mutation plays a significant role in drug resistance to TK inhibitors (TKIs). This study aimed to evaluate the binding affinities of different TKIs to wild-type FGFR1 and the V561M gatekeeper mutant using computational approaches, and to elucidate the mechanistic basis of gatekeeper mutation evasion.
Materials and Methods: The RCSB Protein Data Bank was used to obtain the crystal structures of FGFR1. For comparative analysis, wild-type FGFR1 complexed with Lucitanib and the V561M gatekeeper mutant complexed with AZD4547 were selected. To perform molecular docking simulations, the AutoDock Vina system implemented via the dockstring Python package was used. RDKit chemical computing software was used for ligand processing.
Results: The docking protocol successfully replicated the steric hindrance property of the FGFR1 V561M gatekeeper mutation. Lucitanib exhibited a binding affinity of -10.30 kcal/mol, AZD4547 -9.0 kcal/mol, Imatinib -9.50 kcal/mol, and Ponatinib -9.80 kcal/mol against the wild-type kinase domain. With the addition of the V561M mutation, these affinities decreased to -7.70, -7.00, -7.30, and -7.50 kcal/mol, respectively (resistance gap ranging from 2.00 to 2.60 kcal/mol). Similar destabilization was observed with other TKIs, including Pemigatinib, Infigratinib, Futibatinib, and Selpercatinib. These inhibitors exhibited a resistance gap ranging from 2.10 to 2.30 kcal/mol. Both Irpagratinib and Pralsetinib achieved high mutant binding affinities (-7.20 kcal/mol, for both). Compared to other inhibitors, Irpagratinib exhibited a relatively small resistance gap, while this gap was higher for Pralsetinib (0.90 kcal/mol and 2.50 kcal/mol, respectively).
Conclusion: This study found that Irpagratinib has a strong mutant binding affinity for the V561M gatekeeper mutant and exhibits a significantly lower resistance gap compared to other TKIs. This suggests that Irpagratinib could be a potent inhibitor of mutant FGFR1.

Keywords: Fibroblast growth factor receptor, mutation, tyrosine kinase inhibitor, computational method

PP043
Repurposing Teriflunomide for Bladder Cancer: Proteome-Wide Analysis Reveals Antitumor Effects and Potential Therapeutic Targets

Zeynep Sude KONGUR[1], Mehmet SARIHAN[1], Banu ISKENDER [1]

[1] Kocaeli University Faculty of Medicine Department of Medical Biology Protein Research and Proteomics Laboratory, Umuttepe, 41001, Izmit, Kocaeli-Turkiye

Background/Aim: Teriflunomide inhibits mitochondrial dihydroorotate dehydrogenase (DHODH), which limits pyrimidine synthesis and thereby impairs DNA/RNA production and proliferation of rapidly dividing cells, including cancer cells. DHODH inhibition affects apoptosis, cell-cycle progression, PI3K/Akt signalling, autophagy, and mitochondrial stress in cancers such as triple-negative breast and non-small cell lung cancer, supporting potential activity in bladder cancer. This study evaluated bladder cancer-specific dose responses to teriflunomide and proteomic changes underlying its mechanism of action.
Materials and Methods: We used a CCK-8 assay to evaluate the effects of teriflunomide on cell viability and proliferation of human bladder cancer cell line T24 in comparison to the normal urothelial cell line SV-HUC-1. Colony-forming and scratch assays evaluated clonogenicity and migration. Label-free LC-MS/MS profiled proteomic changes, with raw data analysed using Proteome Discoverer 2.5, UniProt, GO datasets, Venny 2.1, and iDEP for protein identification, differential expression, enrichment, clustering, and visualisation.
Results: Our results suggest that teriflunomide inhibits bladder cancer cell viability, clonogenicity and migration in a dose- and time-dependent manner. Teriflunomide reshaped the proteome of bladder cancer cells, introducing stage-specific variations. The proteins responsive to teriflunomide treatment were further analysed for pathway enrichment and kinase effects. Proteomic analysis focused on epithelial-mesenchymal transition, apoptotic process, mitochondrion, cell membrane, kinase activity and cellular response to xenobiotic stimulus. Teriflunomide treatment strongly regulated the desmosomal proteins and cell division proteins.
Conclusion: In conclusion, teriflunomide is a promising repurposed candidate for bladder cancer, acting mainly through DHODH inhibition and broader proteomic remodelling. The enrichment of desmosomal proteins may reflect restoration of epithelial cell-cell adhesion and partial reversal of an invasive EMT-like phenotype. However, because desmosomal adhesion may have context-dependent roles in urothelial tumour attachment, membrane localisation and functional validation are required. Further studies in additional models, 3D cultures, and in vivo systems are needed to confirm therapeutic efficacy and resistance mechanisms.

Keywords: Teriflunomide, , bladder cancer, , proteomics, , drug repurposing.

PP044
Combined Delivery of sap53 and siMDM2 Modulates the p53–MDM2 Axis and Suppresses Malignant Cellular Phenotypes in A549 Lung Adenocarcinoma Cells

İrem Nur MENEVŞE[1], Muhammed DÜNDAR[1]

[1] İnönü Üniversitesi, Malatya

Background/Aim: The p53–MDM2 signaling pathway plays a central role in regulating cell proliferation, apoptosis, and tumor development. An increase in MDM2's negative regulatory effect on p53 can contribute to the weakening of p53's tumor-suppressing functions and to tumor progression. In this study, in A549 lung adenocarcinoma cells expressing wild-type p53, the co-administration of a small interfering RNA (sap53)—which enhances p53 transcription—and a small interfering RNA (siMDM2) that suppresses MDM2 expression—using the same cationic lipid-based delivery system.
Materials and Methods: In this study, the A549 human lung adenocarcinoma cell line, which carries wild-type p53, was used as a model. The cells were transfected using the same cationic lipid-based transfection system with small activating RNA (sap53), which increases p53 transcription, and small interfering RNA (siMDM2), which suppresses MDM2 expression. Seventy-two hours after transfection, gene and protein expression levels were assessed using qPCR and Western blot methods, respectively. Cellular antitumor responses were examined through analyses of cell viability, apoptosis, cell cycle, colony formation, migration, and invasion. The obtained data were statistically analyzed using Student's t-test.
Results: The combined application of sap53 and siMDM2 increased the expression of the p53, p21, and PUMA genes and proteins, while decreasing MDM2 expression. The combination treatment reduced cell viability and colony formation, increased cell cycle arrest in the G0/G1 phase and apoptosis, and also suppressed migration and invasion capacity. The combination group exhibited the most pronounced biological response in the majority of molecular and functional analyses, and statistically significant superiority over single-agent treatments was observed for certain parameters.
Conclusion: The findings demonstrate the potential of simultaneously targeting the enhancement of p53 transcription and MDM2-mediated negative regulation using the same cationic lipid-based delivery system to reactivate the p53 signaling pathway and promote antitumor cellular responses in lung adenocarcinoma cells expressing wild-type p53.

Keywords: p53, MDM2, saRNA, siRNA

PP045
Effects Of Fasting Stress and Resveratrol on Cell Viability and Migration in U87 Glioblastoma Cells

Şehide BAŞTÜRK[1], MehmetAli TİBATAN[1], Serap ANDAÇ[1]

[1] Istanbul Sabahattin Zaim Üniversitesi

Background/Aim: Glioblastoma is the most aggressive primary brain tumor and is characterized by rapid proliferation, extensive invasion, and resistance to conventional therapies. Fasting stress has emerged as a promising metabolic intervention to improve the efficacy of anticancer agents. Resveratrol, a natural polyphenolic compound, has demonstrated anticancer activity in various tumor models. This study aimed to investigate the effects of fasting stress and resveratrol on cell viability, migration, and stress-related protein expression in U87 glioblastoma cells.
Materials and Methods: Human U87 glioblastoma cells were cultured under normal (LGS+) and fasting stress (LGS−) conditions and treated with resveratrol (10–1000 µM). Cell viability was assessed after 24 h using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, and half-maximal inhibitory concentration (IC_{50}) values were determined by four-parameter nonlinear regression analysis. Cell migration was evaluated using scratch wound-healing assays at IC_{30} and IC_{50} concentrations. Nuclear morphology was examined by 4′,6-diamidino-2-phenylindole (DAPI) staining. Midnolin and ubiquitin protein expression levels were evaluated by Western blot analysis.
Results: Resveratrol reduced cell viability in a dose-dependent manner under both culture conditions. Fasting stress significantly enhanced resveratrol-induced cytotoxicity, resulting in a lower IC_{50} value (77.6 µM) compared with normal conditions ($p < 0.0001$). Cell migration was significantly reduced following resveratrol treatment under both conditions ($p < 0.001$), with a greater inhibitory effect under fasting stress. DAPI staining demonstrated reduced cell density and nuclear morphological alterations. Western blot analysis revealed changes in Midnolin and ubiquitin protein expression following fasting stress and resveratrol treatment.
Conclusion: Fasting stress enhanced the anticancer effects of resveratrol by reducing cell viability and migration in U87 glioblastoma cells. These findings suggest that metabolic stress may improve the therapeutic potential of resveratrol, while alterations in Midnolin and ubiquitin expression warrant further investigation.

Keywords: glioblastoma, fasting stress, resveratrol, cell migration, midnolin

PP046

Effects of Young and Senescent Fibroblast-Derived Extracellular Vesicles on MDA-MB-231 and MCF-7 Breast Cancer Cell Lines

FIRAT AVCI[1], Sena YAYLACIOĞLU[1], Dilek KAAN[2]

[1] Betul Ziya Eren Genome and Stem Cell Center, Erciyes University, Kayseri 38039, Turkey
[2] Halil Bayraktar Vocational Health College, Erciyes University, Kayseri 38039, Turkey

Background/Aim: Metastatic progression and therapeutic resistance remain major challenges in breast cancer management. Senescent stromal fibroblasts can influence breast cancer cell behavior through extracellular vesicles (EVs) carrying distinct molecular cargo. This study aimed to compare the effects of EVs derived from young and senescent fibroblasts on migration, apoptosis, and mitochondrial membrane potential in MCF-7 and MDA-MB-231 breast cancer cells.
Materials and Methods: Young and senescent fibroblasts were cultured in serum-free medium for 24 h before EV isolation. EVs were isolated by ultracentrifugation (110,000 × g, two rounds, 70 min each) and characterized by nanoparticle tracking analysis, transmission electron microscopy, and flow cytometry. EV protein concentrations were quantified by Bradford assay for treatment normalization. Cellular senescence was confirmed by β-galactosidase staining. MCF-7 and MDA-MB-231 cells were treated with young or senescent fibroblast-derived EVs. Migration, apoptosis, and MMP were evaluated using scratch assays and flow cytometry.
Results: Scratch assays revealed that both EV populations increased wound closure in MCF-7 cells compared with controls, with a stronger effect observed after Young-EV treatment. In MDA-MB-231 cells, Young-EVs reduced wound closure, whereas Senescent-EVs promoted the highest migration. Total apoptotic rates remained below 6% in all groups, indicating that these changes were not associated with significant apoptosis. MMP analysis showed significantly higher active mitochondrial populations in MDA-MB-231 cells treated with both Young-EVs and Senescent-EVs compared with controls, while no significant changes were detected in MCF-7 cells.
Conclusion: Fibroblast-derived EVs induced subtype-dependent responses in breast cancer cells. Although both EV types enhanced mitochondrial activity in MDA-MB-231 cells, only Senescent-EVs increased migratory capacity, whereas Young-EVs showed an opposite effect. These findings suggest that stromal fibroblast senescence status may regulate breast cancer cell behavior through EV-mediated communication.

Keywords: Breast cancer, Extracellular vesicles, Cellular senescence, Fibroblasts

PP047
Development of an Oligonucleotide-Based PROTAC Strategy Targeting IRF4 In Melanoma

Özennur Ünal[1], Tolga Emre[1]

[1] Boğaziçi University, Istanbul

Background/Aim: Interferon Regulatory Factor 4 (IRF4) is a transcription factor that functions as a critical dependency in melanoma by regulating gene expression programs required for tumor cell survival. Despite its therapeutic potential, direct targeting of IRF4 remains challenging because transcription factors generally lack suitable ligand-binding pockets. Oligonucleotide-based proteolysis targeting chimeras PROTACs (oPROTACs) have emerged as a promising strategy for the selective degradation of transcription factors by using sequence-specific DNA oligonucleotides to bring transcription factors into proximity with E3 ubiquitin ligases, thereby promoting ubiquitination and proteasomal degradation. Therefore, our aim is to develop an oPROTAC for the targeting and degradation of IRF4 in melanoma.
Materials and Methods: Wild-type and mutant IRF4-binding oligonucleotides were designed based on structural and biochemical studies of IRF4–DNA interactions and synthesized with 5′ biotin and phosphorothioate modifications to enhance nuclease resistance. Their binding to endogenous IRF4 was evaluated using biotin pull-down assays with nuclear extracts from SK-MEL-28, G361, and MALME-3M melanoma cells, followed by immunoblotting. Binding specificity was further assessed by competition assays using unlabeled oligonucleotides.
Results: Wild-type oligonucleotides efficiently captured endogenous IRF4 from melanoma nuclear extracts, whereas mutant oligonucleotides showed reduced binding. Competition assays confirmed the sequence-specific binding of IRF4 to the designed oligonucleotide.
Conclusion: These findings validate the designed oligonucleotide as a sequence-specific IRF4-binding ligand and provide a foundation for the future development of IRF4-targeting oPROTACs as a potential therapeutic strategy for melanoma.

Keywords: IRF4, melanoma, PROTAC, cancer theraphy

PP048
Pooled CRISPR/Cas9 Library Screen Identifies Novel Synthetic Lethal Partners of IRF4 In Melanoma Cells

Nusaibah Tawfik Alsanabani[1], Batuhan Balkaya[1], Ulduz Sobhiafshar[2], N. C. Tolga Emre[1]

[1] Boğaziçi University, Istanbul
[2] The Roslin Institute, University of Edinburgh, Scotland,

Background/Aim: IRF4 is a transcription factor that our lab has shown to play critical roles in melanoma cell dependency and survival. Identifying genes that become essential upon IRF4 loss — synthetic lethal partners — represents a promising therapeutic strategy. This study aims to identify novel genetic dependencies in IRF4-knockout SK-MEL-28 melanoma cells using pooled CRISPR/Cas9 dropout screens.
Materials and Methods: SK-MEL-28 cells stably expressing doxycycline-inducible Cas9 were transduced with either an IRF4-targeting single-guide RNA (KO) vector or an empty vector (EV) control, then with two sub-genomic single-guide RNA (sgRNA) libraries: ACOC (targeting 3005 apoptosis/cancer-related genes) and GEEX (targeting 2335 gene expression regulator genes). Cells were harvested and DNA-sequenced to assess genomic sgRNA abundance investigation at days 0 and day 21 post-infection across two biological replicates. Sequence reads were processed using MAGeCK robust rank aggregation (RRA) and maximum likelihood estimation (MLE) algorithms. Pathway enrichment was performed using GO, KEGG, Reactome, and MSigDB Hallmarks Genesets/Ontologies with library-restricted background.
Results: Screen quality was validated by significant depletion of core essential genes (CEGv2). Using RRA and MLE analysis, hits were identified at an FDR < 0.05 cutoff. In addition to the core essential genes, several other genes showed up as essential for melanoma fitness, some of which showed only in IRF4 knockout (KO) samples. Enrichment analysis consistently identified ribonucleoprotein complex biogenesis and RNA metabolism across both libraries and methods. Cell cycle regulation, DNA replication, chromosome organization, and proteasomal degradation pathways were enriched in ACOC-KO hits; spliceosome components and rRNA processing were prominent in GEEX-KO hits.
Conclusion: This screen identifies novel dependencies in IRF4-KO melanoma cells, providing a prioritized candidate list for validation and potential co-targeting strategies.

Keywords: CRISPR/Cas9 screen, synthetic lethality, IRF4, melanoma, gene essentiality

PP049
An IRF4 Enhancer-Driven Reporter Platform for High-Throughput Screening of Small-Molecule Inhibitors Targeting IRF4 Expression in Melanoma

Doğan Duran[1], N. C. Tolga Emre[1]

[1] Department of Molecular Biology and Genetics, Boğaziçi Üniversitesi, Bebek, Istanbul, Türkiye

Background/Aim: Interferon Regulatory Factor 4 (IRF4) is a transcription factor involved in tumor cell proliferation and survival in several malignancies, including melanoma, highlighting IRF4 as a potential therapeutic target. Initially identified through genetic studies linking the rs12203592 single nucleotide polymorphism in IRF4 to pigmentation traits, including skin color, hair color, and freckling, this intronic regulatory element was subsequently shown to control IRF4 expression through a melanocytic enhancer mechanism . Building on this knowledge, we used this validated regulatory element to develop an IRF4 enhancer-driven reporter platform for high-throughput screening of small-molecule inhibitors of IRF4 expression in melanoma.
Materials and Methods: pGL3-based reporter constructs containing the IRF4 intron 4 enhancer region with either the wild-type (C) or rs12203592 variant (T) allele, previously described by Praetorius et al. 2013, were transiently introduced into SK-MEL-28 melanoma cells, A-375 melanoma cells, and HEK-293 cells via liposomal delivery. Enhancer activity was quantified by dual-luciferase reporter assay. In parallel, the validated IRF4 intron 4 enhancer sequences were cloned into a lentiviral dual-reporter plasmid containing luciferase and GFP reporters to establish a stable reporter system for screening.
Results: The wild-type IRF4 intron 4 enhancer demonstrated approximately fourfold higher transcriptional activity than the rs12203592-T variant in SK-MEL-28 cells. In contrast, no allele-dependent difference was observed in A-375 or HEK-293 cells, indicating enhancer activity depends on the appropriate melanocytic and IRF4-expressing cellular context. These findings validate the IRF4 intronic regulatory element as a reporter of IRF4 transcription. The enhancer-based system is being optimized through lentiviral reporter generation to enable stable expression and fluorescence validation for future screening.
Conclusion: This study establishes a melanoma-relevant IRF4 enhancer-driven dual-reporter platform for monitoring IRF4 transcriptional regulation. By integrating quantitative luciferase measurement with fluorescence validation, this platform enables future high-throughput screening to identify small molecules that suppress IRF4 expression in melanoma.

Keywords: IRF4, melanoma, enhancer, reporter platform, high-throughput screening

PP050
Leakage-controlled Multi-cohort Machine Learning Identifies a Ferroptosis-linked Prognostic Programme in Lung Adenocarcinoma

Rana Salihoğlu[1]

[1] Department of Computer Engineering, Recep Tayyip Erdogan University, Rize, Türkiye
[2] Chair of Bioinformatics, Julius-Maximilians-Universität Würzburg, Würzburg, Germany

Background/Aim: Lung adenocarcinoma (LUAD) outcomes vary substantially within clinicopathological stage groups. Existing ferroptosis-related signatures often rely on single-cohort feature selection and limited external validation. We aim to derive a reproducible ferroptosis-linked prognostic programme and characterise its immune context.
Materials and Methods: Candidate genes were constrained using FerrDb V3 and weighted gene co-expression network analysis in the independent GSE81089 cohort. TCGA-LUAD, GSE31210, GSE72094 and GSE136961 (n=1,149; 344 overall-survival events) were used for the model development. Preprocessing, gene screening, feature selection and hyperparameter tuning were performed within leave-one-cohort-out (LOCO) folds. Bagged cross-cohort Cox screening and stability locking generated a 25-gene panel. The development-selected model was evaluated unchanged in GSE50081, GSE68465 and GSE30219 (n=912; 507 events). Pathway, protein-interaction and tumour-microenvironment analyses characterised the prioritised programme.
Results: The selected co-expression module is correlated strongly with the ferroptosis trait (r=0.595; false-discovery rate=3.7×10^{-19}). Extra Survival Trees achieved a mean LOCO Uno's C-index of 0.723. External Harrell's C-indices ranged from 0.605 to 0.682, while 5-year Uno's C-indices ranged from 0.605 to 0.696. The continuous score showed a pooled external hazard ratio of 1.42 (95% confidence interval 1.15–1.75; p=0.001). In TCGA-LUAD, the stored out-of-fold score remained prognostic after TNM-stage adjustment (hazard ratio per standard deviation 2.32; 95% confidence interval 1.39–3.87; p=0.0013). TNM-plus-score models were closely calibrated but provided modest discrimination gains and decision-curve benefit only across restricted thresholds. Low-risk tumours showed an interferon-enriched, immune-inflamed phenotype with greater lymphocyte signals and lower tumour purity.
Conclusion: A reproducible ferroptosis-linked prognostic signature was identified in LUAD. Although the model showed moderate performance across independent cohorts, its added clinical value beyond TNM stage was limited. Further prospective and functional validation is required before clinical implementation.

Keywords: Ferroptosis, lung adenocarcinoma, machine learning, survival analysis, tumour microenvironment

PP051
Dose and Time-dependent Effects of Warfarin on Metabolic Activity, Mitochondrial Alterations and Apoptosis in MDA-MB-231 Breast Cancer Cells

Enes BAL[1], Zehra Nur YILDIRIM[1], Ada BILGIN[1], Buse OZDEN[1], Pelin OZFILIZ KILBAS[1], Burcu AYHAN SAHIN[1]

[1] Department of Molecular Biology and Genetics, Faculty of Science and Letters, Istanbul Kültür University, Istanbul, Türkiye

Background/Aim: Warfarin is a vitamin K antagonist widely used to prevent and treat thromboembolic disorders. Beyond its anticoagulant activity, it has attracted attention as a potential drug-repurposing candidate due to its potential effects on cancer cell survival and metabolism. However, its direct effects on triple-negative breast cancer cells remain insufficiently characterized. This study investigated the dose- and time-dependent effects of warfarin on metabolic activity, mitochondrial status, membrane integrity and apoptosis in MDA-MB-231 cells.
Materials and Methods: MDA-MB-231 cells were treated with vehicle (DMSO) or 0.5, 1, 1.5, and 2 mM warfarin for 24, 48, and 72 hours (h). Metabolic activity was assessed by MTT assay. At 48 h, MitoTracker staining and propidium iodide (PI) fluorescence were evaluated by fluorescence microscopy and flow cytometry. Apoptotic profiles were analyzed using Annexin V/PI staining. Experiments were performed in three independent replicates and analyzed using one-way or two-way ANOVA, as appropriate.
Results: Warfarin reduced MTT-derived metabolic activity in a concentration- and time-dependent manner. At 2 mM, relative metabolic activity decreased to approximately 44%, 26%, and 10% after 24, 48, and 72 h, respectively. Flow cytometry at 48 h showed concentration-dependent changes in MitoTracker fluorescence by warfarin treatment, indicating altered mitochondrial status in the direction of reducing mitochondrial activity. Propidium iodide fluorescence also increased with increasing warfarin concentrations, suggesting impaired membrane integrity. Annexin V/PI analysis revealed a modest but progressive increase in apoptosis with increasing concentration and exposure time. At 72 h, total apoptosis reached approximately 6% at 1.5 mM and 9% at 2 mM.
Conclusion: Warfarin reduced metabolic activity, altered mitochondrial status, and promoted dose- and time-dependent apoptotic changes in MDA-MB-231 cells, with the strongest effects at 1.5–2 mM and prolonged exposure. Further studies are needed to distinguish cytostatic effects from alternative cell-death mechanisms, including ferroptosis.

Keywords: Warfarin,, Breast cancer,, MDA-MB-231,, apoptosis

PP052
Investigation of The Effects of The P2X7 Gene on Tumor Development-Related Proliferation and Invasion in Liver Cancer Cells

Tuğba Gezmiş[1], Adem Kara[1]

[1] Erzurum Teknik University, Faculty of Science, Department of Molecular Biology and Genetics, Erzurum

Background/Aim: There are limited treatment options for hepatocellular carcinoma (HCC), the most common subtype of liver cancer and the second leading cause of cancer-related death worldwide. Late-stage diagnosis, together with the uncontrolled proliferation and increased invasion capacity of HCC tumor cells, poses a major obstacle in clinical management. The purinergic receptor P2X7, a ligand-gated cation channel, exerts indirect regulatory effects on tumor cell proliferation, migration, and metastasis through immune cell activation and inflammatory responses; however, its role in HCC progression and cell survival has not yet been fully elucidated.
Materials and Methods: In this study, the HepG2 cell line was used as an in vitro HCC model. The cellular and molecular effects of siRNA-mediated silencing of the P2X7 gene and paclitaxel (PTX) treatment were investigated using cell viability, apoptosis, cell cycle, wound healing, and transwell migration/invasion assays. Changes in the expression of proliferation- and signaling-related genes were determined by real-time polymerase chain reaction (qRT-PCR), and alterations in protein levels were assessed by Western blot (WB) analysis.
Results: Silencing the P2X7 gene reduced HepG2 cell viability, increased apoptosis, and induced cell cycle arrest, indicating an antiproliferative effect. P2X7 knockdown also suppressed wound healing, migration, and invasion capacity, pointing to a regulatory role in tumor cell motility. Combining P2X7 silencing with PTX further enhanced the chemotherapeutic response compared with either intervention alone.
Conclusion: These findings indicate that P2X7 contributes to proliferation, migration, and invasion in HCC and that its inhibition sensitizes tumor cells to chemotherapy. P2X7 may therefore represent a potential molecular target for HCC treatment, particularly in combination with conventional chemotherapeutics.

Keywords: Hepatocellular Carcinoma, P2X7, Paclitaxel, Proliferation, Invasion

PP053
Mpg is A Functional Effector of Nek2 Mediated Chemoresistance Through Base Excision Repair

Beste Kanevetçi[1], Beyza Tütüncüler[1], Enes Çiçek[1], Selahattin Can Özcan[2], Batuhan Mert Kalkan[3], Ayşe Nur Aycı[4], Ceyda Açılan[4]

[1] Graduate School of Health Sciences, Koç University, Istanbul, Türkiye
[2] School of Medicine, Columbia University, New York, United States
[3] Faculty of Medicine, University of Geneva, Geneva, Switzerland
[4] School of Medicine, Koç University, Istanbul, Türkiye

Background/Aim: NEK2 (NIMA-related kinase 2) is a serine/threonine kinase best characterized for its role in centrosome separation and mitotic regulation, but its involvement in the DNA damage response (DDR) remains poorly defined. This study investigated whether NEK2 physically associates with DDR components in a cell cycle dependent manner, and whether this association contributes functionally to base excision repair (BER) and chemoresistance.
Materials and Methods: Doxycycline-inducible TurboID-tagged wild-type (WT) or kinase-dead (KD) NEK2 was expressed in cells synchronized by double thymidine block into G1/S, late S, and G2/M phases. Biotinylated proximal proteins were enriched with streptavidin beads and identified by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Candidate interactions were validated by co-immunoprecipitation in multiple cell lines.
Results: Pathway enrichment analysis showed that the NEK2-WT interactome was enriched for BER components, including N-methylpurine DNA glycosylase (MPG), APEX1, NTHL1, and FEN1. Co-immunoprecipitation confirmed the NEK2-MPG interaction, and MPG knockout abolished NEK2-mediated 5-FU resistance.
Conclusion: TurboID-based interactome profiling suggests that NEK2 may associate with base excision repair components in a cell cycle dependent manner, with MPG emerging as a potential downstream effector linked to chemoresistance. These findings point toward a possible, previously unrecognized role for NEK2 in the DNA damage response, warranting further investigation into the NEK2-MPG axis as a potential contributor to 5-FU resistance.

Keywords: Nek2, MPG, DNA damage response, chemoresistance

PP054
From Biological Insight to Therapeutic Targeting: A Translational Journey of S100A9

Aysegul Kaymak Ozdemir[1], Hüseyin Saygın Portakal[2], Cihangir Yandim[3], Osman Doluca[4]

[1] Department of Biochemistry, Faculty of Pharmacy, Ege University, Izmir, Türkiye
[2] Graduate School of Natural and Applied Science, Department of Biotechnology, Izmir, Türkiye
[3] Department of Genetics and Bioengineering, Faculty of Engineering, Izmir University of Economics, Izmir, Türkiye
[4] Department of Biomedical Engineering, Faculty of Engineering, Izmir University of Economics, Izmir, Türkiye

Background/Aim: S100A9 is an alarmin involved in chronic inflammation, immune dysregulation, cancer progression, and hyperinflammatory disorders. While investigating Zuotin-related factor 1 (ZRF1), an epigenetic transcriptional co-regulator implicated in breast cancer progression, transcriptomic profiling identified S100A9 as a markedly dysregulated downstream gene. Functional studies highlighted the ZRF1–S100A9 axis as a potential therapeutic target regulating the tumor microenvironment in breast cancer. However, the limited efficacy of current S100A9 inhibitors, together with the challenges of directly targeting transcriptional co-regulators such as ZRF1, prompted a structure-based drug discovery campaign targeting S100A9.
Materials and Methods: Stable ZRF1 knockdown and overexpression models representing multiple breast cancer subtypes were used to investigate S100A9 regulation. S100A9 expression, secretion, and stability were evaluated by qPCR, Western blotting, ELISA, and pharmacological modulation of protein degradation. Structure-based drug discovery integrated virtual screening, molecular docking, molecular dynamics simulations, and in silico absorption, distribution, metabolism, excretion, and toxicity (ADMET) profiling. Selected candidates were validated by Drug Affinity Responsive Target Stability (DARTS), Cellular Thermal Shift Assay (CETSA), cytotoxicity, and cell-based anti-inflammatory assays.
Results: ZRF1 regulated S100A9 expression, secretion, and proteostasis in a nutrient-dependent manner. Under metabolic stress, ZRF1 depletion altered proteasome-dependent S100A9 turnover and reduced extracellular S100A9 secretion, whereas Bortezomib partially restored S100A9 accumulation. The current inhibitor Tasquinimod showed limited efficacy. Guided by these findings, structure-based virtual screening identified novel S100A9 binders. Molecular dynamics simulations prioritized lead compounds, while DARTS and CETSA validated target engagement. Oleanolic Acid and Imatinib emerged as the most promising lead compounds, exhibiting low cytotoxicity and anti-inflammatory activity.
Conclusion: This study illustrates how a biological discovery can be translated into a therapeutic opportunity. From the identification of S100A9 as a downstream effector of ZRF1 to structure-based drug discovery, we identified promising S100A9-targeting lead compounds, establishing a translational framework for therapeutic development in breast cancer.

Keywords: ZRF1, S100A9, breast cancer, tumor microenvironment, structure-based drug discovery

PP055
Cloning of Endophilin A2 Gene from Hepatocellular Cancer Cells

Ilayda Sezer[1], Feray KOCKAR[1]

[1] Balikesir University, Faculty of Science and Literature, Department of Molecular Biology and Genetics, Cagis Campus, Balikesir, Türkiye

Background/Aim: Delayed early diagnosis complicates cancer treatment, a leading cause of global mortality. The Endophilin family is an important group of proteins whose expression has been demonstrated in leukemia, colorectal cancer, glioblastoma, osteosarcoma, and breast cancer in the literature. Endophilin A2 (EA2 /SH3GL1) plays a role in the formation of plasma membrane invaginations, clathrin-mediated endocytosis, and signal transduction. This study aims to clone EA2 gene from hepatocellular cancer cells.
Materials and Methods: The gene sequence was retrieved from NCBI, expression and ORF (Open Reading Frame) cloning primers were designed by optimizing Tm temperatures and GC contents. For expression study, Liver cancer (Hep3B, Huh-7, SNU-398), colorectal cancer (HT-29, SW480), osteosarcoma (Saos-2), breast cancer (MCF-7), prostate cancer (PC-3), and glioblastoma (A-172), were cultured in medium supplemented with 10% FCS under incubator conditions of 5% CO_2. Total RNA isolation was performed from the cells using the InnuPREP RNA Mini Kit. cDNA synthesis was carried out using the Thermo cDNA Synthesis Kit with 1 µg of template RNA. Amplicons were visualized by 2% agarose gel electrophoresis. The region, EA2 Open Reading Frame was cloned into the pGEM-T Easy vector by PCR-based strategy. Recombinant colonies were screened via blue-white screening using IPTG and X-Gal.
Results: As a result of our study, EA2 expression was detected in the Hep3B cell line. The EA2 gene was cloned into the pGEM-T Easy vector by PCR-based stategy. Sequencing analysis confirmed the integrity of the sequence of the gene.
Conclusion: This study elucidates the endogenous EA2 expression in different cancer models. The EA2 gene will be subcloned into the prokaryote expression vector for the recombinant protein production.

Keywords: Cancer, Endophilin A2, mRNA Expression, Cloning

PP056
SACM1L Overexpression Differentially Regulates PI3K Signalling and Autophagic Flux in Breast and Lung Cancer Cells

Eda Efe Sözeyataroğlu[1], Mehmet Gümüş[1], Selin Kurtulmuş[1], Eylül Cevrem[1], Özden Yalçın-Özuysal[1]

[1] İzmir Institution of Technology, İzmir, Türkiye

Background/Aim: SACM1L (SAC1) is a phosphoinositide phosphatase localized to the Golgi apparatus and endoplasmic reticulum, where it regulates phosphoinositide homeostasis. This study aimed to investigate the role of SACM1L overexpression in phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) signalling, macro-autophagy, and cancer cell behaviour in breast and lung cancer models.
Materials and Methods: Stable SACM1L overexpression was established using lentiviral vectors in breast (MCF7) and lung (A549) cancer cell lines. PI3K/AKT signalling was evaluated by Western blot analysis, and FOXO3 subcellular localization was examined by using reporter system under fluorescence microscopy. Cell proliferation was assessed using the MTT assay, migration by wound-healing assay. Autophagic activity was investigated by Western blot analysis of key autophagy markers and tandem fluorescent LC3 reporter imaging under nutrient starvation and pharmacological modulation (LY294002, and SF1670) to monitor autophagic flux.
Results: SACM1L overexpression exerted cell-type-dependent effects on PI3K signalling and autophagy. In A549 cells, SACM1L enhanced PI3K activation and autophagic activity, whereas in MCF7 cells it reduced PI3K signalling and significantly suppressed migration. Functional analyses demonstrated that the observed alterations in migration and autophagic flux were dependent on PI3K signalling. SACM1L overexpression did not significantly affect proliferation but produced distinct migration dynamics among the cell lines. Tandem fluorescent LC3 reporter analysis demonstrated efficient autophagic cargo degradation following autophagy induction, indicating preserved autophagic flux without evidence of lysosomal blockade. Pharmacological studies further suggested that SACM1L modulates cellular adaptation to PI3K pathway activation through protein-level feedback mechanisms.
Conclusion: These findings demonstrate that SACM1L functions as a context-dependent regulator of PI3K signalling and autophagy. By maintaining phosphoinositide homeostasis, SACM1L contributes to the regulation of autophagic flux and cellular responses to signalling stimulation, supporting its potential role in cancer-associated signalling networks.

Keywords: SACM1L, PI3K/AKT/mTOR, Autophagy, Cancer

PP057
Investigation of the Effect Of Nradd Expression on Apoptosis Under Neuroinflammatory Conditions

Sevcan HACOĞLU[1, 2], Güneş ÖZHAN[1, 2]

[1] Izmir Institute of Technology
[2] Izmir Biomedicine and Genome Center

Background/Aim: The Wnt/β-catenin signaling pathway plays a critical role in tumor growth and apoptosis resistance in neuroblastoma. The zebrafish neurotrophin receptor-associated death domain (nradd) gene, which encodes a structural homolog of the human p75 neurotrophin receptor (p75NTR), functions as a negative feedback regulator of Wnt signaling. Furthermore, studies have shown that expression of this gene in human cells induces apoptosis. However, the molecular mechanisms underlying this process remain unclear. This study aims to investigate the mechanistic effect of zebrafish Nradd expression on apoptosis and cellular migration in human neuroblastoma cells specifically under neuroinflammatory conditions.
Materials and Methods: The study utilizes an in vitro co-culture simulation model. Murine N9 microglia cells are activated with lipopolysaccharide (LPS) to produce pro-inflammatory conditioned medium. This conditioned medium is then applied to human SH-SY5Y neuroblastoma cells to compare those expressing the zebrafish nradd gene with their respective controls. Apoptotic responses under these inflammatory conditions are evaluated by measuring key apoptotic marker proteins using Western blot analysis. Additionally, cellular migration capacity is quantitatively assessed over a designated time course through a wound healing assay.
Results: The exposure of nradd-expressing neuroblastoma cells to pro-inflammatory conditioned medium altered their apoptotic and migratory behaviors compared to the control group. Specifically, zebrafish Nradd expression is assumed to sensitize these cancer cells to microglia-derived inflammatory factors, thereby enhancing immune-mediated cell death. Additionally, migration analyses demonstrate the impact of inflammatory stress conditions on the overall cellular motility of the neuroblastoma cells.
Conclusion: Understanding how the zebrafish nradd gene influences cell death during an immune response offers critical insights into tumor biology. By acting as a vital link between immune-mediated cell death and the Wnt signaling pathway, Nradd represents a promising new therapeutic target. In conclusion, these findings could inform the development of innovative combination therapies to overcome apoptosis resistance in cancer.

Keywords: Wnt/β-catenin signaling pathway, Nradd, p75NTR, zebrafish, immune response

PP058
Discovery of a Novel Indole-derived Dual-acting FLT3-ITD/WT Inhibitor as a Potential Lead Scaffold

Zeynep Nur KILIM[1], Ismail CELIK[2], Onur BENDER[1], Beyza OZTEKIN[2], Merve YILDIRIM[2, 3], Ulviye ACAR CEVIK[4], Arzu ATALAY[1]

[1] Biotechnology Institute, Ankara University, Ankara, Turkey
[2] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Erciyes University, Kayseri, Turkey
[3] The Graduate School of Health Sciences, Ankara University, Ankara, Turkey
[4] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Anadolu University, Eskişehir, Turkey

Background/Aim: FLT3 is a key therapeutic target in (AML) acute myeloid leukemia where aberrant signaling driven by mutations or persistent WT (wild-type) activation contributes to disease progression and poor prognosis. In this study, we screened 14 newly synthesized indole derivatives against FLT3. Using in vitro and in silico approaches, we aimed to identify the most active compound and evaluate its biological activity and predicted binding characteristics.
Materials and Methods: Initial screening of the 14 compounds was performed on FLT3-ITD mutant MV4-11 and FLT3-WT THP-1 cells at 10 µM and 50 µM for 48 and 72 hours using CCK-8 assay. The lead compound was further evaluated in time and dose-dependent experiments. To elucidate ligand-target interactions and structural dynamics, molecular docking and molecular dynamics simulations were conducted against the FLT3 kinase domain via Schrödinger Small-Molecule Drug Discovery Suite.
Results: ICA3 was identified as the lead compound, exhibiting strong growth-inhibitory activity against both FLT3-ITD and FLT3-WT leukemia cells. ICA3 showed the strongest predicted interaction with FLT3, achieving the lowest docking score (-11.730 kcal/mol). Docking analysis using the DFG-out conformation of FLT3 (PDB ID:4XUF) revealed that ICA3 occupied the ATP-binding pocket and established key interactions with the hinge-region residue Cys694, the αC-helix residue Glu661, and the DFG-motif residue Asp829. Furthermore, molecular dynamics simulations supported structural stability throughout the dynamic trajectory.
Conclusion: ICA3 represents a promising dual-active FLT3 inhibitor with comparable activity against both FLT3-ITD and FLT3-WT AML cells. Although currently available FLT3 inhibitors have significantly improved outcomes in FLT3-mutated AML, therapeutic resistance and disease relapse remain major clinical challenges, and persistent FLT3-WT signaling may contribute to treatment failure. By simultaneously targeting both FLT3 mutant and wild-type receptors, ICA3 may represent a strategy to overcome these limitations. Therefore, we propose ICA3 as a promising lead scaffold for the development of next-generation FLT3-ITD/WT co-targeting therapeutics.

Keywords: Acute Myeloid Leukemia, FLT3, Indole, Molecular docking, Molecular Dynamics

PP059
Comparison Of the Combined Effect of the Amantadine Molecule on Temozolomide Sensitive and Resistant Glioblastoma Multiforme Cells

Ömer Faruk KOR[1], Dicle AĞGİL[2], Öykü Sıla ÖZÇELİK[3], Öykü GÖNÜL GEYİK[4]

[1] Istinye University, Faculty of Engineering and Natural Sciences, Department of Molecular Biology and Genetics, Istanbul, Türkiye
[2] Istinye University, Faculty of Medicine, Istanbul, Türkiye
[3] Istinye University, Faculty of Pharmacy, Istanbul, Türkiye
[4] Istinye University, Faculty of Medicine, Department of Medical Biology, Istanbul, Türkiye

Background/Aim: Glioblastoma (GBM) is a highly agressive and lethal type of brain tumor, and its resistance to first line chemothepeutic agent temozolomide (TMZ) remains to be a major barrier to its treatment. Amantadine is an N-Methyl-D-Aspartate (NMDA) receptor antagonist currently used in the treatment of Parkinson's. This study is aimed to research the cell viability and drug resistance outcome of combining Amantadine with TMZ in TMZ-sensitive U87 and TMZ-resistant T98G glioblastoma cell lines.
Materials and Methods: To determine the cell viability, Sulforhodamine B (SRB) assay was used at 24, 48 and 72 hours in both of the cell lines treated with TMZ, Amantadine, and their combination.The protein expression levels of LC3A/B, ABCC2 and ABCG2 were determined by using Western Blotting.
Results: TMZ IC50 value was 400 µM and Amantadine IC50 value was 1000 µM in U87 cells at 72 hours. In T98G cells, Amantadine IC50 value was 1000µM at 72-hour-timepoint. Similar to Amantadine, TMZ also reduced cell viability in T98G cells in a concentration and time-dependent manner.
Conclusion: Our study showed that Amantadine and Temozolomide combination modulated the expressions of LC3A/B, ABCC2 and ABCG2 proteins.

Keywords: Amantadine, Temozolomide, Glioblastoma, Drug Resistance

PP060
Targeting RNA Regulatory Networks, Metabolic Reprogramming, and Nanotherapy to Overcome 5-Fluorouracil Resistance in Colorectal Cancer

Farnaz Ghanbari Ilkhechi[1], Mohammad Kafili[2]

[1] Department of Biology, Faculty of Basic Sciences, Islamic Azad University, Tabriz Branch, Tabriz, Iran
[2] Faculty of Pharmacy, Istanbul University, Istanbul, Turkey

Background/Aim: 5-Fluorouracil (5-FU) remains a cornerstone of chemotherapy for colorectal cancer (CRC); however, acquired resistance and treatment-related toxicity limit its clinical effectiveness. Emerging evidence indicates that, beyond thymidylate synthase inhibition and DNA damage, RNA damage responses, non-coding RNA dysregulation, metabolic reprogramming, autophagy, and cellular stress pathways influence the response to 5-FU. This narrative review summarizes recent mechanisms underlying 5-FU resistance and evaluates therapeutic strategies based on RNA modulation, metabolic targeting, and nanomedicine.
Materials and Methods: A structured PubMed search was conducted to identify English-language studies published between 2018 and 2026. Relevant studies addressing 5-FU response or resistance in CRC were reviewed, with particular emphasis on RNA-related mechanisms, non-coding RNAs, epitranscriptomic regulation, metabolic adaptation, autophagy, and nanoparticle-based drug delivery.
Results: The reviewed evidence demonstrates that 5-FU can induce an RNA damage response during ribosome biogenesis, resulting in the degradation of damaged ribosomal RNA and ribosomal proteins. Dysregulated RNA networks also contribute to resistance. Reduced miR-375-3p expression decreases sensitivity to 5-FU through thymidylate synthase upregulation, whereas lncRNA H19 promotes resistance via the miR-194-5p/SIRT1/autophagy axis. METTL1-modified tsRNA-GlyGCC enhances resistance through SPIB/JAK1/STAT6 signaling, while YTHDC2/LIMK1/eIF2α signaling promotes stress granule formation. In addition, HIF-1α-driven glycolysis and activation of the pentose phosphate pathway support resistant phenotypes, whereas altered HMBOX1/HACE1/ATG5 signaling enhances autophagy-mediated resistance. Preclinical nanoparticle systems, including lipid-coated calcium carbonate, poly(β-amino ester), and silk fibroin nanocarriers, have improved -ᵒFU delivery, enhanced chemosensitivity, inhibited tumor growth, and reduced gastrointestinal toxicity.
Conclusion: Resistance to 5-FU in CRC is driven by interconnected RNA-, metabolism-, autophagy-, and stress adaptation-related mechanisms. Combining RNA modulation with metabolic or autophagy-targeting approaches, together with nanodelivery systems, may improve therapeutic efficacy while reducing toxicity. However, further clinical validation is required before these strategies can be translated into routine clinical practice.

Keywords: Colorectal Cancer, 5-Fluorouracil, RNA Regulation, Metabolic Reprogramming, Nanomedicine

PP061
Effects Of Foxo1 Inhibition with AS1842856 and TMZ in TMZ-Sensitive and TMZ-Resistant Cells

Enes Bera GÜMÜŞER[1], Melike GÜNEŞ[1], Dilara KIZILKAYA[1, 2], Caner GEYİK[3], Öykü GÖNÜL GEYİK[4]

[1] Istinye University, Faculty of Engineering and Natural Sciences, Department of Molecular Biology and Genetics, Istanbul, Türkiye
[2] Istinye University, Faculty of Engineering and Natural Sciences, Department of Chemistry, Istanbul, Türkiye
[3] Istinye University, Faculty of Medicine, Department of Medical Biochemistry, Istanbul, Türkiye
[4] Istinye University, Faculty of Medicine, Department of Medical Biology, Istanbul, Türkiye

Background/Aim: Glioblastoma (GBM) is the most aggressive primary brain tumor, and resistance to the first-line chemotherapeutic agent temozolomide (TMZ) remains a major challenge in its treatment. FOXO1 is a transcription factor involved in the regulation of cancer stem cell maintenance and DNA damage repair induced by alkylating agents. This study aimed to investigate the antitumor effects of combining FOXO1 inhibition with TMZ in TMZ-sensitive U87 and TMZ-resistant T98G glioblastoma cell lines.
Materials and Methods: Both cell lines were treated with TMZ, the FOXO1 inhibitor AS1842856, and their combination. IC50 values were determined using the Sulforhodamine B (SRB) assay. The mRNA expression levels of GADD45A, RFC2, CDK2, CDKN1B, CDKN1A, BMP6, ERK1, ERK2, MAPK14, nNOS, iNOS, and FOXO1 were analyzed by qRT-PCR.
Results: TMZ IC50 values in U87 cells were >1000, 600–800, and 400–600 μM at 24, 48, and 72 hours, respectively. IC50 values of AS1842856 in U87 were ~100, ~50, and ~40 μM across the same time points. Both TMZ and the FOXO1 inhibitor reduced cell viability in T98G cells in a concentration- and time-dependent manner. The combination treatment altered cell viability compared with TMZ alone for both cell lines. All treatment groups modulated the expression of the genes analyzed by qPCR in U87 and T98G cells.
Conclusion: Combinatory treatments using FOXO1 inhibitors along with TMZ shows promise for future GBM therapy strategies.

Keywords: Glioblastoma Multiforme, FOXO1, Temozolomide, Combination therapy

PP062

Pathway-Level Characterization of Transcriptomic Reprogramming Induced by ATF4 Silencing and ERK Inhibition Under Nutrient Deprivation in Colorectal Cancer

Kerem Yazıcı[1], Leman Nur Nehri[2], Özün Özcan[3], Sreeparna Banerjee[3]

[1] Tekirdağ Namık Kemal High School, Tekirdağ, Türkiye; Department of Biological Sciences, Middle East Technical University, Ankara, Türkiye
[2] Data Science in Life Sciences Group, Institute for Chemistry and Bioanalytics, School of Life Sciences, FHNW University of Applied Sciences and Arts Northwestern Switzerland, Muttenz, Switzerland
[3] Department of Biological Sciences, Middle East Technical University, Ankara, Türkiye

Background/Aim: Cancer cells can survive nutrient depletion (ND) by metabolic adaptation through the rewiring of stress-response pathways. Activating Transcription Factor 4 (ATF4) and extracellular signal-regulated kinase (ERK1/2) signaling are major regulators of cellular adaptation to metabolic stress; however, their coordinated effects on transcriptomic remodeling remain incompletely understood. We aimed to investigate the molecular changes induced by ND, ATF4 silencing, and ERK1/2 inhibition using a pathway-level transcriptomic approach, building upon an in silico pipeline previously developed in our laboratory.
Materials and Methods: RNA-seq data obtained from colorectal cancer (CRC) cells incubated in complete medium (nutrient rich, NR), 1% of the nutrients found in NR (ND), stable silencing of ATF4, and ERK1/2 inhibition (with U0126) were analyzed in the R environment. Differential gene expression analysis was performed using DESeq2 based on a 6 × 6 experimental comparison matrix comprising six conditions: ND, ND+U0126, ND+ATF4, ND+ATF4+U0126, NR, and NR+U0126. The differentially expressed genes (DEG) identified across the 30 comparisons were then subjected to STRING based protein-protein interaction (PPI) network analysis, Gene Ontology (GO) enrichment analysis, and hub gene identification. Finally, biological process specific gene clusters were integrated into an R-based pathway scoring algorithm that combined DEG, PPI, and the functional classification of pathway regulators supported by the literature to illustrate condition-specific pathway rewiring.
Results: ATF4 suppression and ERK inhibition in ND cells revealed extensive changes in DEG and the reorganization of functional pathways. GO-based pathway activity scoring revealed a significant increase in angiogenesis, migration and immune response in the ND cells with ATF4 and ERK1/2 perturbations, suggesting a coordinated transcriptomic reprogramming that can help with cell survival.
Conclusion: Our systems-biology based approach offers a scalable strategy for prioritizing biologically significant pathways and selecting high confidence candidate genes for subsequent experimental validation, thus facilitating future mechanistic studies and the identification of potential therapeutic targets.

Keywords: ATF4, ERK, Nutrient Deprivation, Systems Biology, Colon Cancer

PP063

Cellular Adaptations and Modulation of Chemoresponse to Nutrient Deprivation in Colorectal Cancer: Implication of P-Glycoprotein and Lysosomes in 5-Fluorouracil Resistance

Elif Deniz[1], Sema Zabcı[1], Aliye Ezgi Güleç Taşkıran[1]

[1] Başkent University, Ankara

Background/Aim: Nutrient deprivation (ND), a hallmark of the tumor microenvironment, can impose metabolic and organelle-level adaptations that promote therapeutic resistance, frequently through P-glycoprotein (P-gp)-mediated mechanisms. This study investigates impact of ND on organelle abundance and drug response in T84 and CaCo-2 cells and evaluates the potential of the P-gp inhibitor verapamil to restore 5-fluorouracil (5-FU) efficacy under ND.
Materials and Methods: Organelle marker expression and clinical relevance were evaluated in silico utilizing UCSC Xena and Kaplan-Meier analyses. T84 and CaCo-2 cells were cultured in nutrient rich and limited media. Western Blotting determined the effects on lysosome abundance and P-gp expression, while Nile Red staining determined lipid droplet (LD) accumulation. Molecular docking characterized P-gp interactions with 5-FU and verapamil. Chemosensitivity and 5-FU/Verapamil combination efficacy were evaluated using MTT, synergy scoring and colony formation assays.
Results: LAMP1, TOMM20, PLIN2 and ABCB1 expression was elevated in primary tumor cells when compared to normal tissue, with high LAMP1, PLIN2 and ABCB1 correlating with decreased overall and relapse-free survival. ND increased lysosome abundance and P-gp expression, without affecting LD abundance and enhanced the 5-FU IC50 values in T84 cells. Molecular docking revealed that verapamil binds with stronger affinity to P-gp than 5-FU (−7.52 vs. −6.36 kcal/mol), despite exhibiting weaker inhibitory effect (Ki: 2.14 mM vs. 21.66 μM). These indicates that while 5-FU is transported by P-gp without forming a stable complex, verapamil forms a stable interaction, validating its inhibitory role. Combinatorial functional assays confirmed that verapamil eradicates this adaptive resistance, effectively restoring 5-FU cytotoxicity and implicating P-gp as the driver of the phenotype.
Conclusion: ND drives adaptive resistance in colorectal cancer cells through increased lysosome abundance and P-gp activity. Verapamil restores chemosensitivity under ND. These findings highlight the microenvironment's role in treatment failure, supporting targeted therapies against organelles and efflux pumps to overcome chemoresistance.

Keywords: Colorectal Cancer, 5-fluorouracil, Verapamil, Nutrient Deprivation

PP064
Selective Antiproliferative and Proapoptotic Effects of an Acylhydrazone-Containing Diphenylurea Derivative in HepG2 Cells

Furkan Yaşaryıldız[1], Cemil Caner Arıkan[2], Necla Kulabaş[2], İlkay Küçükgüzel[3], Dilek Telci[1]

[1] Department of Genetics and Bioengineering, Yeditepe University, Istanbul, Turkiye
[2] Department of Pharmaceutical Chemistry, Marmara University, Istanbul, Turkiye
[3] Department of Pharmaceutical Chemistry, Fenerbahçe University, Istanbul, Turkiye

Background/Aim: Primary liver malignancies including hepatocellular carcinoma and hepatoblastoma remain challenging to treat and the multikinase inhibitor sorafenib is constrained by toxicity towards non-malignant cells. Hydrazone-containing diphenylurea derivatives preserve sorafenib's urea pharmacophore while allowing wide structural variation. We aimed to identify derivatives active against hepatic tumour cells that spare normal liver cells.
Materials and Methods: 46 derivatives were screened in HepG2 hepatoblastoma/hepatocellular carcinoma cells (5,000/well; 25 µM; 72 h) using MTT assay, where the cell counts below the seeding density defined cytotoxicity. The candidate derivative CA-38 and sorafenib were reassessed in human hepatic fibroblasts (HHF) and hepatic stellate cells (HHSC). Half-maximal inhibitory concentrations (IC50; 0–25 µM) were derived by four-parameter logistic curve fitting. Cell cycle (propidium iodide; 36/48 h at respective IC50) and apoptosis (Annexin V/propidium iodide; 25 µM; 72 h) were analysed by flow cytometry.
Results: Only CA-38, a 5-nitrofuran-derived acylhydrazone, reduced HepG2 counts below the seeding density while sorafenib reduced counts to undetectable levels. In HHF cells, CA-38 preserved cell viability at 100% of the control, whereas sorafenib reduced it to 0.3%. CA-38 inhibited HepG2 growth with an IC50 of 15 µM (95% CI: 13–17 µM), compared with 11 µM for sorafenib (95% CI: 8–15 µM). Relative to the vehicle control, CA-38 raised G0/G1 by 14% at 36 h ($p<0.0001$) and reduced G2/M by 18% at 48 h ($p<0.0001$). CA-38 raised total apoptosis to 60% versus 20% in vehicle control ($p<0.0001$). Necrosis stayed below 0.3% in all groups.
Conclusion: CA-38 showed potency comparable to sorafenib against HepG2 cells and left hepatic fibroblasts unaffected, whereas sorafenib was equally lethal to malignant and non-tumorigenic cells. This substantially wider selectivity window identifies CA-38 as a promising lead.

Keywords: HepG2, Sorafenib, Acylhydrazone, Diphenylurea derivatives, Selective cytotoxicity

PP065
Comparative Effects of Three TREN-based Nanomaterials on the Metabolic Viability of U87 Glioblastoma Cells

Seher Berna KIRDÖK TANŞU[1], Güzide ŞATIR BAŞARAN[2]

[1] Ankara Üniversitesi, Ankara
[2] Erciyes Üniversitesi, Kayseri

Background/Aim: Glioblastoma is an aggressive primary brain tumor with limited therapeutic options, highlighting the need to investigate novel nanoscale materials with potential biological activity. This study aimed to compare the concentration-dependent effects of three TREN-based nanomaterials—TREN Dicisal, TREN 3Br Cisal, and TREN Dinitsal—on the metabolic viability of U87 glioblastoma cells and to determine their half-maximal inhibitory concentrations (IC_{50}).
Materials and Methods: U87 cells were seeded in 96-well plates at a density of 1×10^4 cells/well and treated with each nanomaterial at concentrations of 7.81, 15.63, 31.25, 62.5, 125, 250, and 500 µg/mL for 48 h. Nanomaterial suspensions were freshly prepared in sterile ultrapure water, sonicated, and diluted in complete culture medium. Following exposure, cells were incubated with 5 mg/mL MTT for 4 h. Formazan crystals were dissolved in 100 µL DMSO, and absorbance was measured at 560 nm. Each concentration was tested in quadruplicate. Cell viability was normalized to untreated controls, and IC_{50} values were calculated by nonlinear regression using GraphPad Prism.
Results: All three nanomaterials reduced U87 metabolic viability in a concentration-dependent manner. The IC_{50} values were 235.9 µg/mL for TREN Dicisal (95% CI: 221.0–252.3; $R^2 = 0.9728$), 254.1 µg/mL for TREN 3Br Cisal (95% CI: 234.7–276.0; $R^2 = 0.9595$), and 283.0 µg/mL for TREN Dinitsal (95% CI: 258.0–310.8; $R^2 = 0.9485$). TREN Dicisal showed the comparatively greatest inhibitory effect.
Conclusion: The three TREN-based nanomaterials displayed measurable but relatively modest activity against U87 cells, with TREN Dicisal emerging as the most active candidate. These preliminary findings support further structural optimization and validation through normal-cell selectivity and mechanistic cell-death assays.

Keywords: glioblastoma, cell viability, MTT assay, nanomaterials, N-GQDs

PP066
Epigenetic Modulation of Ferroptosis in Triple-Negative Breast Cancer

Ezgi Saygun[1, 2], Gizem Dogan Yilmaz[1, 2], Yiğit Emre Altın[1, 2], Tugba Bagci-Onder[1, 2]

[1] Koç University School of Medicine, Istanbul, Turkey
[2] Koç University Research Center for Translational Medicine, Istanbul, Turkey

Background/Aim: Triple-negative breast cancer (TNBC) is an aggressive subtype lacking estrogen/progesterone receptors and HER2 expression, limiting targeted therapy options and making treatment largely chemotherapy-dependent. Its aggressiveness and therapy resistance highlight the need for alternative therapeutic strategies. Ferroptosis, an iron-dependent regulated cell death driven by lipid peroxidation and redox imbalance, potentially target therapy-resistant cancer cells. This study aimed to identify epigenetic drugs that cooperate with ferroptosis inducers in TNBC and determine how key ferroptosis regulators influence combination responses.
Materials and Methods: MTT assays were used to select working concentrations of the ferroptosis inducers (FINs) Erastin, RSL3, and ML162. Colony formation assays were performed with or without the ferroptosis inhibitor Ferrostatin-1 to confirm mode of cell death. CRISPR/Cas9-mediated knockout of major ferroptosis pathway modulators, GPX4, ACSL4, and SLC7A11, was validated by Western blot. Control and knockout cells were treated with FINs ± Ferrostatin-1. FIN-induced lipid peroxidation was assessed by flow cytometry. A library of 124 epigenetic probes was screened, and selected HDAC inhibitors were followed up in combination with Erastin.
Results: FIN treatment reduced colony formation in a concentration-dependent manner, while Ferrostatin-1 rescued this effect, supporting ferroptosis-dependent cell death. GPX4- and SLC7A11-knockout cells showed increased FIN sensitivity, whereas ACSL4-knockout cells gained resistance. Ferrostatin-1 rescued the enhanced cell death observed in GPX4- and SLC7A11-knockout cells. RSL3 and ML162 increased lipid peroxidation, which was reduced by Ferrostatin-1. The screen identified HDAC inhibitors as a major drug class cooperating with Erastin. Among these, MS-275 alone had limited effects alone but enhanced Erastin-induced viability loss, indicating synergy.
Conclusion: Pharmacological ferroptosis induction was complemented by genetic modulation of key pathway regulators, establishing a chemical-genetic framework to dissect ferroptosis sensitivity. Subsequent epigenetic compound screening identified candidate regulators, highlighting promising therapeutic opportunities. Combining epigenetic modulation with ferroptosis induction may therefore represent a promising strategy to overcome treatment resistance in TNBC.

Keywords: Triple-negative breast cancer, ferroptosis, CRISPR/Cas9, epidrug

PP067
Integrated Transcriptomic Analyses Determines IGDCC3 as a Marker of a Distinct Tumor Cell State in High-Grade Serous Ovarian Cancer

Aylin Naz Mumcuoğlu[1], Zeynep Tokcaer Keskin[1, 2]

[1] Department of Molecular Biology and Genetics, Graduate School of Natural and Applied Sciences, Acıbadem Mehmet Ali Aydınlar University, İstanbul, Türkiye
[2] Department of Molecular Biology and Genetics, Faculty of Engineering and Natural Sciences, Acıbadem Mehmet Ali Aydınlar University, İstanbul, Türkiye

Background/Aim: Ovarian cancer (OC) is the deadliest gynecological malignancy and fifth leading cause of death among women. High-grade Serous Ovarian Carcinoma (HGSOC) is a type of epithelial ovarian cancer (EOC), and constitutes approximately 70% of EOC cases. Cellular plasticity and cancer stemness contribute to high recurrence rate and probability of developing chemoresistance. That's why finding novel genes that may have therapeutic properties in the treatment of OC is important. IGDCC3 is a membrane-bound protein playing role in neuromuscular and cell-adhesion activities. Although IGDCC3 is commonly appeared in cancer-related bioinformatics studies as hub gene, its functional characterization in context of HGSOC remains unexplored. The aim of this study was to characterize transcriptional role of IGDCC3 in HGSOC.
Materials and Methods: Integrative bioinformatics analysis was performed using publicly available bulk-RNA-seq and single-cell RNA-seq (scRNA-seq) data from HGSOC patient cohorts. To identify the transcriptional function of IGDCC3 in HGSOC, correlation analyses with cancer-related programs, pathway enrichment, transcription factor enrichment, gene signature score, trajectory interference and pseudo time bifurcation analyses were applied across independent bulk-RNA-seq and scRNA-seq patient datasets.
Results: Transcription factor enrichment analysis revealed activation of pathways related with stemness and epithelial-to-mesenchymal transition (EMT) in IGDCC3-high samples and Bulk-RNA-seq correlation analysis indicated a positive correlation of stemness, plasticity, cancer stemness, EMT plasticity scores with IGDCC3 expression. ScRNA-seq results confirmed findings in bulk-RNA-seq data showing elevated stemness and cellular plasticity characteristics in IGDCC3-positive cells and IGDCC3-enriched tumor clusters. Cell states (epithelial, mesenchymal, hybrid) and transcriptional dynamics analyses highlighted that IGDCC3 is a marker of a distinct hybrid EMT tumor cells state.
Conclusion: Our findings indicate that IGDCC3-enriched tumor cell populations might represent a distinct tumor cell state showing characteristics of stemness-related programs and cellular plasticity. These results provide insight into transcriptional role of IGDCC3 in HGSOC and pave the way for functional mechanistic investigations.

Keywords: IGDCC3, HGSOC, Cancer Stemness, Cellular Plasticity

PP068

The Multifaceted Impact of the COVID-19 Pandemic on Colorectal Cancer Care: A Systematic Review of Management Disruptions, Stage Migration, and Survival Outcomes

Khaled Bonna[1], Abdulrahman Alshawaf[2], Faisal Abusharkh[2], Afaf Bazerbashi[5], Jamil Aljehany[4]

[1] Mansoura University, Mansoura, Egypt
[2] Istinye Universitesi, Istanbul, Türkiye
[3] Tabuk Health Cluster, Tabuk, Saudi Arabia
[4] Taibah University College of Medicine, Madinah, Saudi Arabia
[5] TC Uskudar Universitesi, Uskudar, Türkiye

Background/Aim: The COVID-19 pandemic disrupted colorectal cancer (CRC) screening, diagnosis, and treatment worldwide, raising concern that care delays could increase advanced-stage presentation and worsen outcomes. This systematic review evaluated its impact on CRC management, treatment patterns, and survival.
Materials and Methods: The review followed Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidance and was registered in PROSPERO (CRD42025638100). PubMed and Scopus were searched for English-language peer-reviewed studies published from 2020 onward involving adults with CRC and reporting pandemic-related changes in care or outcomes. Titles and abstracts were screened, followed by full-text assessment. Data were extracted using a standardized form, observational studies were assessed with the Newcastle–Ottawa Scale, and findings were synthesized narratively.
Results: Of 1,754 records identified, 155 studies were included. Most were retrospective cohorts, supplemented by population-based, modelling, prospective, cross-sectional, and qualitative studies. Fecal immunochemical testing declined by 9%–67%, colonoscopy volumes by up to 92%, and new CRC diagnoses by 13%–36%. Several cohorts reported more advanced disease, including an increase in stage IV presentation from 22% to 37%, with more T4 tumors, nodal involvement, emergency presentations, and stoma formation. Elective surgical activity decreased by 5.4% to more than 45%, while neoadjuvant strategies increased, including a 200% rise in short-course radiotherapy in one setting. Some studies reported preserved short-term surgical safety, whereas survival findings were heterogeneous; one large registry found a 25% higher mortality risk during the pandemic. Disruptions disproportionately affected socioeconomically disadvantaged and racial or ethnic minority populations.
Conclusion: The pandemic substantially disrupted the CRC care continuum and associated with reduced screening and diagnosis, treatment modification. Predominantly retrospective evidence, heterogeneous methods, and limited long-term follow-up restrict causal inference. Resilient screening systems, risk-stratified pathways, and protected cancer services are needed during future healthcare crises.

Keywords: Fecal immunochemical testing, diagnostic delay, stage migration, surgical oncology

PP069
Establishment of a Multi-Site Patient-Derived Ovarian Cancer Organoid Biobank for Molecular Characterisation and Functional Drug Screening

Berranur Sert Bal[1], Sümeyye Akçelik Deveci[1], Eray Çetin[1], Sümeyye Koç[1], Emin Erhan Dönmez[2], Selma Atiye Kolcu[2], Sevim Baykal Koca[3], Murat Api[4], Esra Keleş Peker[4], Fatih Şanlıkan[4], Ümmügülsüm Kuyucu[4], Damlanur Yücel[4], Uğur Kemal Öztürk[4], Öncel İpekçi[5], Sırma Çetin[5], Gökçe Hande Benli[5], Damla Karaman[5], Nedim Turan[6], Elif Kubat Öktem[7], Emine Kazanç[1, 8, 9]

[1] Department of Molecular Biology and Genetics, Faculty of Arts and Sciences, Boğaziçi University, İstanbul, Türkiye
[2] Department of Obstetrics and Gynaecology, Samatya İstanbul Training and Research Hospital, İstanbul, Türkiye
[3] Department of Medical Pathology, Samatya İstanbul Training and Research Hospital, İstanbul, Türkiye
[4] Department of Gynaecologic Oncology, Kartal Dr. Lütfi Kırdar City Hospital, İstanbul, Türkiye
[5] Department of Medical Pathology, Kartal Dr. Lütfi Kırdar City Hospital, İstanbul, Türkiye
[6] Department of Medical Oncology, Kartal Dr. Lütfi Kırdar City Hospital, İstanbul, Türkiye
[7] İstanbul Medeniyet University, İstanbul, Türkiye
[8] Division of Cell and Molecular Biology, The Institute of Cancer Research, London, UK
[9] Department of Surgery and Cancer, Faculty of Medicine, Imperial College London, London, UK

Background/Aim: Ovarian cancer remains one of the most lethal gynaecological malignancies because of late-stage diagnosis, recurrence, treatment resistance, and substantial inter- and intra-patient heterogeneity. Tumours from different anatomical sites, including the ovary, fallopian tube, and metastatic deposits, may contain distinct cell populations and display different therapeutic vulnerabilities. Therefore, a single tumour sample may not fully represent an individual patient's biological and drug-response profile. Patient-derived organoids (PDOs) preserve tumour-specific features and provide physiologically relevant ex vivo models for functional testing. This study aimed to establish a multi-site ovarian cancer PDO biobank and evaluate its utility for molecular characterisation and therapeutic response profiling.
Materials and Methods: Fresh primary ovarian tumours, fallopian tube lesions, and omental metastases were mechanically and enzymatically dissociated, and isolated cells were embedded in Matrigel for three-dimensional culture. Organoid establishment, morphology, and expansion capacity were assessed across tissue types. For drug screening, PDOs were seeded in 384-well plates and treated with olaparib, capivasertib, or A-443654. Cell viability was measured after 72 hours using a luminescence-based three-dimensional assay. Dose-response curves, IC50 values, and area-under-the-curve metrics were calculated. PDOs and matched parental tissues were characterised by immunohistochemistry, immunofluorescence, and RT-qPCR using subtype-specific markers.
Results: Specimens were collected from 40 patients, and PDOs were established with an overall efficiency of approximately 60%. The models showed sustained three-dimensional growth, site-specific morphology, and concordance with matched parental tissues. Drug screening revealed heterogeneous responses to all three agents. In one patient, an ovarian tumour-derived PDO was more sensitive than the matched fallopian tube-derived PDO, whereas in another patient, bilateral tumour-derived PDOs were resistant while the matched non-malignant model remained sensitive.
Conclusion: Multi-site ovarian cancer PDOs capture clinically relevant inter- and intra-patient heterogeneity. Integrating functional drug profiling with molecular characterisation provides a translational platform for identifying site-specific therapeutic vulnerabilities and supporting precision oncology strategies.

Keywords: Ovarian Cancer, Patient-Derived Organoids, Tumour Heterogeneity, Drug Screening, Precision Medicine, AKT Inhibition

PP070
Timing Of Germline Genetic Testing and Initial Treatment Decision-Making In Newly Diagnosed Breast Cancer

Mustafa Ramadan[1], Abdullah Jamal[5]

[1] Mustafa Ramadan
[2] Maein Mare Mohammed Alshaykhi
[3] Faisal Abusharkh
[4] ABDULRAHMAN ALSHAWAF
[5] Abdullah Jamal

Background/Aim: Germline testing is useful in helping in the early management of breast cancer only if there are test results prior to making such decisions. We analyzed the impact of testing and time to results on treatment decision-making.
Materials and Methods: We searched for articles published between 2016 and 2026 on adults diagnosed with primary breast cancer. The eligible articles should have addressed the timing of germline testing in relation to diagnosis or initiation of treatment and should have had data on treatment, timing, testing, or decision making.
Results: Seventy-five reports were included, mainly observational. Availability of pathogenic breast cancer susceptibility genes 1 and 2 (BRCA1/2) results before surgery was associated with more bilateral or contralateral risk-reducing mastectomy: 76.4% versus 14.7%, 45.0% versus 2.0%, and an odds ratio of 8.23 (95% confidence interval, 2.55–26.59). In ductal carcinoma in situ, corresponding rates were 46.9% before versus 10.8% after surgery. A randomized trial found that rapid genetic counseling and testing increased presurgical counseling (57.8% versus 38.7%) but did not significantly increase presurgical result receipt (30.6% versus 27.4%) or bilateral mastectomy (26.6% versus 21.8%). One cohort found no difference in time to surgery (33 versus 34 days; p=0.45). Testing at diagnosis identified poly(ADP-ribose) polymerase inhibitor eligibility in 1.8% overall and approximately 10% of triple-negative cases. Radiotherapy evidence was limited. Variation between studies prevented quantitative pooling and causal conclusions.
Conclusion: Testing is helpful when the result is back before treatment is done; otherwise, testing will be helpful only once it is ordered. The rapid testing process can help to get tests faster without any delay in surgeries, but counseling alone might not provide results.

Keywords: breast cancer , Germline genetic testing , BRCA1/2 , Treatment timing

PP071

Aurora B Inhibition Enhances the Antitumor Effects of Cisplatin, Oxaliplatin, and 5-Fluorouracil in Colorectal Cancer Cells

Yaren EVLER[1], Elif Rümeysa ÖZSOY[1], Sibel SARI TÜRK[1]

[1] Abdullah Gül University, Department of Molecular Biology and Genetics, Kayseri, TÜRKİYE

Background/Aim: Aurora kinase B (AURKB) is a serine/threonine kinase that plays a central role in chromosome segregation and cytokinesis. Its overexpression in colorectal cancer (CRC) has been associated with chromosomal instability, poor prognosis, and resistance to chemotherapy. This study evaluated the antitumor effects of the selective AURKB inhibitor BI 831266, alone and in combination with cisplatin, oxaliplatin, or 5-fluorouracil (5-FU), in DLD-1 and SW480 colorectal cancer cells.
Materials and Methods: The effects of BI 831266 and chemotherapeutic agents on cell viability, clonogenic survival, and cell migration were assessed using the MTT assay, 2D clonogenic survival assay, and wound-healing assay, respectively. Drug interactions were evaluated by calculating combination index (CI) values using CompuSyn software.
Results: BI 831266 reduced cell viability and clonogenic survival in a dose-dependent manner in both cell lines. Its combination with cisplatin, oxaliplatin, or 5-FU produced synergistic antitumor effects at the tested concentrations. The strongest synergy was observed with oxaliplatin, with minimum CI values of 0.033 in DLD-1 cells and 0.241 in SW480 cells. Synergistic interactions were also observed with 5-FU and cisplatin. BI 831266 additionally impaired cell migration as a single agent. Combination treatment further increased the remaining wound area for 5-FU and oxaliplatin in both cell lines and for cisplatin in SW480 cells, indicating enhanced antimigratory activity under these conditions.
Conclusion: Selective AURKB inhibition potentiated the antitumor effects of standard CRC chemotherapeutic agents, particularly oxaliplatin. These findings support further mechanistic and in vivo investigation of BI 831266-based combination strategies. This study was supported by TÜBİTAK-1002A (Project No. 125Z822).

Keywords: Aurora Kinases, BI 831266, Combination theraphy, Colorectal cancer

PP072
De Novo Design and In Silico Safety Profiling of CLDN4 Binders for Solid Tumor CAR-T Therapy

Ozan Göçmen[1, 2], Yasin Kaymaz[1]

[1] Bioengineering Department, Ege University, Izmir, Türkiye
[2] Graduate School of Natural and Applied Sciences, Biotechnology, Ege University, Izmir, Türkiye

Background/Aim: Generative artificial intelligence (GenAI)-based diffusion models enable rapid de novo design of thousands of binders for chimeric antigen receptor T-cell (CAR-T) therapies, yet reliable in silico methods to screen these candidates remain lacking. We developed a universal, high-throughput computational filtering pipeline and validated with de novo single-chain variable fragment (scFv) antibodies targeting the Claudin-4 (CLDN4) tumor antigen.
Materials and Methods: CLDN4 expression was confirmed by analyzing pseudobulk data from a lung cancer single-cell RNA sequencing (scRNA-seq) atlas and validated at the protein level using tissues from 45 non-small cell lung cancer (NSCLC) patients. Using the RFantibody tool, 1,000 CLDN4-specific scFv candidates were generated. A combinatorial off-target library comprising homologous Claudin family members was constructed, and antibody–off-target interactions were modeled with AlphaFold3. Structural confidence metrics (iPTM, PTM, PAE, clashes, and disorder scores) at protein–protein interfaces served as features for a scoring model quantifying binding interface quality. Final candidates were selected via negative selection maximizing the therapeutic index. Binding stability of the lead candidate was assessed through 500 ns molecular dynamics (MD) simulations using GROMACS, with the complex embedded in a lipid bilayer prepared via CHARMM-GUI. The scoring model was further benchmarked against clinically tested anti-claudin monoclonal antibodies from the Thera-SAbDab database.
Results: The leading candidate, scFv_73_0, demonstrated high-confidence binding to CLDN4 (score: 0.846) with minimal cross-reactivity to other claudins (mean score: 0.374), representing a 54.9% reduction in off-target binding tendency. MD simulations of the membrane-embedded complex confirmed structural stability, yielding a mean root-mean-square deviation (RMSD) of 2.90 Å.
Conclusion: This study delivers a highly specific lead candidate for in vitro validation and provides a scalable, target-agnostic computational framework for predicting and mitigating off-target binding of any engineered binder, thereby accelerating the translation of safer, next-generation CAR-T therapies into clinical application.
Acknowledgements: TÜBİTAK (321S233, 1649B022406380), TÜSEB (16428), UHEM(4024472025).

Keywords: CAR-T cell Therapy, De novo protein design, Claudin-4, Immunoinformatics

PP073
CASPEX-Mediated Proximity Labeling For Investigating Epigenetic Regulation Of PD-L1

Beyza Bursali[1], İpek Bulut[2], Ceyda Acilan Ayhan[3]

[1] Koc University Graduate School of Health Sciences, Istanbul, Turkey
[2] Koc University Research Center for Translational Medicine, Istanbul, Turkey
[3] Koc University School of Medicine, Istanbul, Turkey

Background/Aim: Immune checkpoint inhibitors enhance anti-tumor T-cell activity by disrupting signaling through the PD-1/PD-L1 axis. Although this pathway plays a critical role in maintaining immune tolerance, tumor cells frequently upregulate PD-L1 to evade immune surveillance and develop resistance to therapy. Identifying tumor-specific epigenetic regulators of PD-L1 expression may therefore reveal novel therapeutic targets. CASPEX is an advanced CRISPR-based proximity-labeling system that integrates dCas9-guided genomic targeting with APEX2-mediated biotinylation, enabling the locus-specific characterization of promoter-associated protein complexes. In this study, we established CASPEX models in triple-negative breast cancer and head and neck squamous cell carcinoma cells to investigate epigenetic regulators associated with PD-L1 expression.
Materials and Methods: Stable CASPEX-expressing MDA-MB-231 and HN30 cell lines were generated through co-transfection with CASPEX and transposase plasmids. Doxycycline-inducible CASPEX expression was induced using 2 µg/mL doxycycline for 48 hours and evaluated by flow cytometry and Western blotting. Stable guide RNA-expressing cell lines were subsequently established by lentiviral transduction and hygromycin selection (400 µg/mL for MDA- 200µg/mL for HN30). The subcellular distribution of CASPEX was examined using subcellular fractionation, Western blotting, and immunofluorescence. Recruitment of CASPEX to the targeted region of the PD-L1 promoter was assessed by chromatin immunoprecipitation followed by quantitative PCR (ChIP-qPCR).
Results: Following doxycycline induction, CASPEX expression was detected in 97.35% of MDA-MB-231 cells and 98.65% of HN30 cells. Subcellular fractionation confirmed the presence of CASPEX in both nuclear and cytoplasmic compartments. ChIP-qPCR demonstrated significant CASPEX enrichment at the targeted PD-L1 promoter region, confirming locus-specific recruitment. Successful protein biotinylation was further validated by Western blotting.
Conclusion: Highly efficient doxycycline-inducible CASPEX models were successfully established in TNBC and HNSCC cell lines. The observed enrichment at the PD-L1 promoter confirms locus-specific CASPEX recruitment and provides a robust experimental platform for the subsequent streptavidin-based enrichment and mass spectrometric identification of PD-L1 promoter-associated protein complexes.

Keywords: PD-L1, CASPEX, epigenetic regulation, proximity labeling

PP074
Investigation of the Relationships of SALL4 Gene with Resistance Genes in Head and Neck Cancer Cells

Arzugül Tanas Yılmaz[1], Kübra Solak[2, 3], Adem Kara[1, 4]

[1] Department of Molecular Biology and Genetic, Faculty of Science, Erzurum Teknik University, Erzurum, Türkiye.
[2] Department of Molecular Biology and Genetic, Faculty of Science, Atatürk University, Erzurum, Türkiye.
[3] East Anatolia High Technology Application and Research Center (DAYTAM), Atatürk University, ٢٥٢٤٠ Erzurum, Türkiye.
[4] High Technology Application and Research Center (YUTAM), Erzurum Teknik University, Erzurum, Türkiye.

Background/Aim: Sal-like protein 4 (SALL4) is a transcription factor expressed in embryonic stem cells that has been shown to be overexpressed in several cancers, including head and neck squamous cell carcinoma (HNSCC), where it contributes to tumor development, progression, and metastasis. This study aimed to investigate the role of SALL4 in the development of drug resistance and its relationship with resistance-related genes in HNSCC cells.
Materials and Methods: FaDU cells, in both parental and paclitaxel (PTX)-resistant forms, were divided into six experimental groups: positive control, SALL4 inhibition, PTX treatment, combined inhibition and PTX treatment, and negative control. SALL4 knockdown was confirmed by real-time polymerase chain reaction (qRT-PCR) and Western blot (WB) in both cell lines. The relationship between SALL4 and resistance-associated genes was assessed using molecular genetic analyses, and cell cycle distribution, migration ability, and apoptosis were further evaluated across all groups.
Results: SALL4 was successfully silenced in both parental and resistant FaDU cells. SALL4 inhibition enhanced the pro-apoptotic effect of PTX, exerted an antiproliferative effect through cell cycle arrest, and produced a synergistic effect when combined with PTX, particularly in resistant cells.
Conclusion: These findings suggest that SALL4 contributes to drug resistance in HNSCC and that its inhibition may enhance chemosensitivity. Targeting SALL4 could represent a promising therapeutic strategy to improve treatment response in HNSCC patients.
The Scientific and Technological Research Council of Türkiye (TÜBİTAK) provided financial support for this work (Project No. 124Z890).

Keywords: SALL4, Head and Neck Squamous Cell Carcinoma, Drug Resistance, Paclitaxel, Apoptosis

PP075
Preliminary Evaluation of the Protective Effects of β-Carotene Extract Against Methylglyoxal- and Glyoxal-Induced Cytotoxicity in Caco-2 Cells

Büşra Yusufoğlu[1]

[1] Istanbul Technical University

Background/Aim: Reactive α-dicarbonyl compounds, particularly methylglyoxal (MGO) and glyoxal (GO), are important precursors of advanced glycation end products (AGEs) and contribute to intestinal oxidative stress and epithelial dysfunction. β-Carotene has been widely recognized for its antioxidant activity; however, its potential to attenuate dicarbonyl-induced cellular injury remains largely unexplored. This study investigated the anti-dicarbonyl potential of β-carotene in an intestinal epithelial cell model.
Materials and Methods: Caco-2 cells were exposed to increasing concentrations of MGO and GO to determine their cytotoxic profiles using the MTT assay. The calculated IC_{50} values were 1.26 mM for MGO and 1.44 mM for GO**.** β-Carotene was extracted under light-protected conditions and tested at serial dilutions (1:10–1:50), with vehicle (DMSO) controls included to eliminate solvent-associated effects. Based on viability screening, non-cytotoxic β-carotene concentrations were selected for subsequent co-incubation and pre-incubation experiments, using the IC_{50} values for MGO and GO.
Results: LC–MS/MS analysis further demonstrated that the β-carotene preparation contained chlorogenic acid (7778.00 ng/mL), quinic acid (2999.90 ng/mL), fumaric acid (1998.80 ng/mL), caffeic acid (769.82 ng/mL), 4-hydroxybenzoic acid (694.82 ng/mL), ferulic acid (212.48 ng/mL) and rosmarinic acid (168.66 ng/mL). β-Carotene alone exhibited minimal cytotoxicity at the selected dilutions. However, co-incubation with MGO or GO did not significantly improve cell viability compared with cells exposed to MGO or GO alone. Pre-incubation experiments suggested treatment-sequence-dependent differences, with modest improvements observed in selected conditions; however, these effects were inconsistent and did not demonstrate a robust cytoprotective response. Similarly, β-carotene pretreatment for 24 h before MGO or GO exposure did not restore cell viability to control levels.
Conclusion: Under the experimental conditions employed, β-carotene did not exert a marked protective effect against MGO- or GO-induced cytotoxicity in Caco-2 cells. Nevertheless, the absence of a significant improvement in cell viability does not exclude a possible anti-dicarbonyl mechanism acting independently of acute cytoprotection.

Keywords: beta caroten, advanced glycation end products, di-carbonyl

PP076
In Silico Discovery and Repurposing Of Dual Monoamine Oxidase Inhibitors for Glioblastoma Treatment Through Machine Learning and ADMET Profiling

Ayse Mina ARSLANBAS[1, 2], Halil Ibrahim GULER[1]

[1] Karadeniz Technical University, Faculty of Science, Department of Molecular Biology and Genetics, ٦١٠٨٠, Trabzon, Turkiye
[2] Karadeniz Technical University, Graduate Institute of Natural and Applied Sciences, Department of Molecular Biology and Genetics, ٦١٠٨٠, Trabzon, Turkiye

Background/Aim: Glioblastoma is an aggressive central nervous system (CNS) malignancy necessitating innovative treatments. Monoamine oxidase A (MAO-A) and B (MAO-B) enzymes are highly promising therapeutic targets. This study aims to identify effective, CNS-penetrable dual MAO inhibitors by executing two distinct computational strategies: drug repurposing and novel lead discovery via machine learning (ML).
Materials and Methods: Two parallel in silico workflows were systematically executed. Data preprocessing and Extended Connectivity Fingerprint 4 (ECFP4) extractions utilized KNIME and RDKit. The ML pipeline employed Random Forest and CatBoost algorithms, augmented by SHAP (SHapley Additive exPlanations) analysis for model explainability. The drug repurposing approach screened 5000 FDA-approved drugs from the ChEMBL database, while the novel lead discovery screened extensive chemical libraries. Promising hits underwent comprehensive Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) profiling via ADMETlab 3.0, SwissADME, and ProTox-II. Tanimoto similarity against Safinamide was calculated to ensure the structural novelty of the candidates.
Results: The dual-strategy pipeline yielded three elite candidates for future validation. The drug repurposing workflow identified the FDA-approved drug Chlorzoxazone, possessing a highly safe Class 4 toxicity profile (LD50: 1230 mg/kg) suitable for neuro-oncology. The novel lead workflow identified two distinct compounds: CHEMBL2106281 and CHEMBL2105544. Tanimoto scores (ranging from 0.09 to 0.14) confirmed these novel leads do not structurally resemble existing MAO inhibitors. ADMET profiling revealed that all three candidates possess high blood-brain barrier (BBB) penetration probabilities (>0.900) alongside exceptionally low major organ toxicity profiles.
Conclusion: Executing parallel computational workflows integrating KNIME, CatBoost, and SHAP analysis successfully identified three equally valuable, CNS-penetrable dual MAO-A/B inhibitor candidates: one repurposed drug (Chlorzoxazone) and two structurally novel chemical leads. These compounds establish a robust foundation for Glioblastoma therapy and are prioritized for upcoming structural validation via molecular docking and molecular dynamics (MD) simulations.

Keywords: Glioblastoma, Drug Repurposing, Novel Lead Discovery, Machine Learning, ADMET

PP077
ZEB2 Directly Regulates ROR1 Expression in Glioblastoma Cells

Samet Koçarslan[1], Arzu Aysan[1], Tamer Yağcı[1]

[1] Department of Molecular Biology and Genetics, Faculty of Sciences, Gebze Technical University, Kocaeli, Türkiye

Background/Aim: Glioblastoma is an aggressive primary brain tumor characterized by extensive proliferation, invasion, and resistance to treatment. ZEB2 is a transcription factor involved in tumor progression, whereas the receptor tyrosine kinase-like orphan receptor 1 (ROR1) has been associated with cancer cell survival and migration. This study aimed to determine whether ZEB2 directly binds to the ROR1 locus and regulates ROR1 expression in glioblastoma cells.
Materials and Methods: U87-MG glioblastoma cells were used throughout the study. Chromatin immunoprecipitation followed by quantitative PCR (ChIP-qPCR) was performed using a ZEB2-specific antibody and primer pairs targeting three regions located 1, 30, and 50 kb upstream of the ROR1 transcription start site. Enrichment was calculated relative to the IgG2a isotype control. ZEB2 expression was subsequently silenced using two independent lentiviral shRNA constructs, shZEB2-83 and shZEB2-87. Knockdown efficiency and the associated changes in ROR1 expression were evaluated by quantitative PCR and Western blotting.
Results: ChIP-qPCR analysis revealed 2.21-, 1.53-, and 0.98-fold enrichment at the −1, −30, and −50 kb regions of the ROR1 locus, respectively. The strongest enrichment was detected at the proximal −1 kb promoter region, whereas no enrichment was observed at the −50 kb region, supporting the regional specificity of ZEB2 recruitment. Moreover, ZEB2 knockdown using two independent shRNA constructs resulted in reduced ROR1 expression compared with parental U87-MG cells.
Conclusion: These findings demonstrate that ZEB2 occupies specific regulatory regions of the ROR1 locus and positively regulates ROR1 expression in glioblastoma cells. To our knowledge, this study provides the first evidence that ZEB2 directly occupies regulatory regions of the ROR1 locus and positively regulates ROR1 expression in glioblastoma cells. Collectively, the results identify ROR1 as a downstream target of ZEB2 and suggest that the ZEB2–ROR1 regulatory axis may contribute to the malignant phenotype of glioblastoma.

Keywords: Glioblastoma, ZEB2, ROR1, chromatin immunoprecipitation, transcriptional regulation

PP078
Investigating the Role of SFPQ in Multi-Lineage Cell Profiles to Identify Vulnerabilities in IDH-Mutant Glioma

Aybala Samancioglu[1, 2], Ebru Yilmaz[1, 2], Sedef Sarac-Yalcinkaya[1, 2], Tugba Bagci-Onder[1, 2]

[1] Koç University School of Medicine, Istanbul, Turkey
[2] Koç University Research Center for Translational Medicine, Istanbul, Turkey

Background/Aim: Gliomas, the most common and aggressive form of brain tumors, are responsible for the majority of primary brain tumor-related deaths. Isocitrate dehydrogenase (IDH) mutant gliomas are characterized by discrete molecular mechanisms and epigenetic landscape. Recently, mutant IDH inhibitors Vorasidenib and Ivosidenib have been shown to block D-2-HG production; however identifying epigenetic vulnerabilities and reversing epigenetic hypermethylation of IDH-mutant gliomas is crucial for developing therapeutic strategies.SFPQ is identified as one of the top hit genes from a CRISPR-Cas9 knockout screen on an IDH mutant model. SFPQ is a multifunctional DNA- and RNA-binding protein but its role in glioma biology is undocumented. This study aims to identify the critical role of SFPQ in tumor survival by phenotyping the consequences of SFPQ loss across different cell lines, to determine whether SFPQ dependency directly relies on mutant IDH activity and to elucidate the biological basis of this vulnerability.
Materials and Methods: CRISPR/Cas9-mediated SFPQ knockout was validated via Western blot and RT-qPCR. Proliferation and viability were assessed using MTT, CTG, and colony formation assays across endogenous IDH1-mutant glioma lines (MGG119, MGG152), fibrosarcoma line as a well-established de novo mutant model (HT1080), and isogenic IDH-wt and IDH-mutant U87MG pairs. Additionally, HEK293T, HBVP, INHA were used as non-malignant cell models.
Results: Our studies revealed that SFPQ loss does not confer a significant selective disadvantage in INHA and HBVP. However, IDH-mutant cell lines including MGG119, MGG152 and HT1080 highlighting a potential tumor-specific vulnerability especially in IDH mutant perspective.
Conclusion: Targeting SFPQ represents a promising therapeutic vulnerability for tumors without disadvantage on healthy brain tissue, providing strong basis to advance novel therapeutic approaches for overcoming treatment limitations. Future work will define the structural domains and RNA regulatory networks through which SFPQ supports cell survival in IDH-mutant gliomas.

Keywords: IDH-mutant glioma, SFPQ, CRISPR-Cas9, Functional characterization

PP079
ABCB1 Deficiency Delays Recovery from Paclitaxel-Induced Drug Tolerance Through Cell Cycle Reprogramming

Deniz Yıldız[1], İpek Bulut[2], Arda Işıklar[1], Ceyda Açılan[3]

[1] Graduate School of Health Sciences, Koç University, Sarıyer, İstanbul, Türkiye
[2] Koç University Research Center for Translational Medicine (KUTTAM), Sarıyer, İstanbul, Türkiye
[3] School of Medicine, Koç University, Sarıyer, İstanbul, Türkiye

Background/Aim: ABCB1 is a well-established mediator of paclitaxel resistance through drug efflux; however, the mechanisms enabling cancer cell survival in its absence remain poorly understood. This study investigated how ABCB1 deficiency influences the transition from drug tolerance to long-term adaptation, with particular emphasis on cell-cycle regulation and recovery dynamics.
Materials and Methods: CRISPR/Cas9-generated ABCB1 knockout (KO) and guide non-targeting (gNT) H460 cells were exposed to paclitaxel. Drug-tolerant persister (DTP) cells were generated following 9 days of treatment, whereas drug-tolerant expanded persisters (DTEPs) were obtained after drug withdrawal and recovery. Long-term adaptation was assessed using cells subjected to gradual paclitaxel escalation for over one year. Cell proliferation and recovery kinetics were monitored throughout chronic treatment. Cell-cycle distribution was analyzed by flow cytometry. Transcriptomic profiling was performed by RNA sequencing on untreated, 9 days DTP, and DTEP populations.
Results: Although ABCB1-deficient cells survived prolonged paclitaxel exposure by entering a drug-tolerant persister state, they exhibited delayed recovery and failed to acquire stable resistance compared with gNT cells. Cell-cycle analysis demonstrated persistent proliferative defects in ABCB1 KO cells during recovery. RNA sequencing revealed marked transcriptional reprogramming characterized by altered cell-cycle, mitotic progression, and stress-response pathways across adaptation stages, indicating that loss of ABCB1 reshapes the trajectory of cellular adaptation rather than preventing survival.
Conclusion: Our findings suggest that ABCB1 deficiency does not eliminate drug tolerance but limits the transition toward stable adaptation by disrupting proliferative recovery. Integrating functional assays with transcriptomic profiling provides a temporal framework for understanding how ABCB1 influences non-genetic adaptation to paclitaxel beyond its canonical drug-efflux function.

Keywords: Cancer, Drug Efflux, Drug Tolerant Persisters

PP080
Rational Peptide Design Targeting The p53 R273H – BAG5 Interaction

Lamia Berra Aksit[1], Nazli Keskin[1, 2]

[1] Department of Molecular Biology and Genetics, Graduate School of Natural and Applied Sciences, Acibadem Mehmet Ali Aydinlar University, Istanbul, Turkiye
[2] Department of Molecular Biology and Genetics, Faculty of Engineering and Natural Sciences, Acibadem Mehmet Ali Aydinlar University, Istanbul, Turkiye

Background/Aim: p53, also known as “Guardian of The Genome”, is found to be mutant in approximately half of all the cancer incidences. Hotspot mutations in the TP53 gene can cause the protein to either lose its tumor-suppressor functions or gain new cancer-promoting functions. Moreover, mutant p53 can maintain a stable conformation by being able to escape from the negative regulators such as MDM2 and CHIP. The BAG5 co-chaperon is one of the contributors of this escape mechanism through directly binding to mutant p53. Therefore, we aim to design an inhibitory peptide that targets the interaction of BAG5 and the most frequent hotspot mutant p53, R273H.
Materials and Methods: Protein-protein interactions were modeled through global and local docking using ClusPro and HADDOCK, respectively. A peptide library was constructed based on the p53 R273H – BAG5 complex through Rosetta Peptiderive, PSSM and PEPFOLD4. After modeling the peptide - p53 R273H interactions, ΔGbinding and Kd values were predicted by using PRODIGY and the complexes were subjected to MD simulations via GROMACS.
Results: The interaction models of p53 R273H and BAG5 domains 4/5 exhibited a common binding site on the DNA binding domain of p53 R273H, named as the “binding-groove”. Inhibitory candidate peptide library was constructed by considering the interface, cell penetration, length and flexibility. The best candidate peptide was able to successfully target the “binding groove” in the global and local docking models. In addition, it was also able to maintain this interaction with moderate conformational changes after a 500ns MD simulation.
Conclusion: The proposed candidate peptide is designed as an inhibitor of p53 R273H – BAG5 interaction and after further improvement it should be tested through in vitro and in vivo studies.
Acknowledgement: The numerical calculations reported in this paper were fully/partially performed at TUBITAK ULAKBIM, High Performance and Grid Computing Center (TRUBA resources).

Keywords: Mutant p53, BAG5, Peptide Design, Targeting Protein Interactions

PP081
Investigating The Role of FIBIN Overexpression in U-87MG Human Glioblastoma Cell Line Model

Gizem Su Ayar[1], Nazli Keskin[1, 2]

[1] Department of Molecular Biology and Genetics, Graduate School of Natural and Applied Sciences, Acibadem Mehmet Ali Aydinlar University, Istanbul, Turkiye
[2] Department of Molecular Biology and Genetics, Faculty of Engineering and Natural Sciences, Acibadem Mehmet Ali Aydinlar University, Istanbul, Turkiye

Background/Aim: Glioblastoma (GBM) is the most common type of brain tumors and considering its complexity, heterogeneity and lethality, identifying the fundamental molecular mechanisms which drive the aggressiveness of glioblastoma is an urgent priority. Although the roles and alterations of some genes such as IDH, EGFR and MGMT are well-studied, there are many genes whose functions are still unknown in glioblastoma. FIBIN is first identified in zebrafish as a player in developmental biology and we observed that its mRNA and protein expression were significantly elevated in glioblastoma patients. However, its specific functions in this cancer type remain unknown. Therefore, in this study, we aimed to elucidate the functional roles of FIBIN overexpression in U-87MG human glioblastoma cell line model.
Materials and Methods: Total RNA was isolated from U-87MG cells and converted to cDNA. FIBIN cDNA was PCR amplified and cloned into mammalian expression plasmids. U-87MG cells were transfected with either FIBIN cDNA containing or empty (mock) plasmids. Stable transfected cells were positively selected with G418 (Geneticin). After selection, FIBIN protein levels were assessed by western blotting. The functional outcomes of FIBIN overexpression were evaluated through CCK-8 cell proliferation, wound healing and matrigel coated transwell assays.
Results: Our preliminary data show that compared to mock transfected control cells, FIBIN overexpressing U-87MG cells had lower doubling time in CCK8 cell proliferation assay, closed the wound area more efficiently at early time points in migration assay and had more invasion capability in transwell assay.
Conclusion: Although, in the literature, FIBIN is suggested as a potential tumor suppressor in lung cancer and Wilms tumor, considering our findings in U-87MG glioblastoma cells, FIBIN may have a context dependent dual role in cancer. Further studies are necessary to reveal its specific functions in different glioblastoma cell line models.

Keywords: Glioblastoma, GBM, FIBIN, U-87MG Cell Line

PP082
Bidirectional Crosstalk Between Breast Cancer Cells and Fibroblasts Under EGF Stimulation

Kemal Utku Aktaş[1], Arzu Aysan[1], Tamer Yağcı[1]

[1] Gebze Technical University / Kocaeli

Background/Aim: Breast cancer progression is influenced by reciprocal interactions between tumor cells and stromal components within the tumor microenvironment. Epidermal growth factor (EGF) signaling may affect both cancer cell behavior and fibroblast activation; however, the consequences of sustained EGF exposure for ROR1 expression and tumor–stromal communication remain unclear. This study investigated the effects of sustained EGF stimulation on EGFR, ROR1, and Caspase-8 expression in breast cancer cells and evaluated bidirectional communication between breast cancer cells and fibroblasts using conditioned-media transfer.
Materials and Methods: ROR1-high MDA-MB-231 and ROR1-low MCF-7 cells were exposed to increasing EGF concentrations (0–120 ng/mL). EGFR, ROR1, and Caspase-8 protein levels were assessed by Western blotting. Conditioned media from EGF-treated cancer cells were transferred to BJ fibroblasts, and fibroblast activation-associated changes were evaluated by measuring FAP and PDGFR-β expression. Conditioned media subsequently collected from BJ fibroblasts were applied to breast cancer cells. EMT-associated markers were analyzed by Western blotting and quantitative real-time PCR, while migratory behavior was evaluated using a wound-healing assay.
Results: Sustained EGF exposure reduced EGFR and ROR1 protein levels and increased Caspase-8 protein level in both cell lines. Cancer cell-derived conditioned media produced EGF concentration- and cell line-dependent alterations in FAP and PDGFR-β expression in BJ fibroblasts. Fibroblast-derived conditioned media subsequently modulated EMT-associated markers in a subtype-dependent manner, including reduced Vimentin expression in MDA-MB-231 cells and increased E-cadherin expression in MCF-7 cells. Furthermore, fibroblast-derived conditioned media significantly reduced wound closure in MDA-MB-231 cells under serum-free conditions ($p < 0.05$).
Conclusion: Sustained EGF stimulation altered EGFR, ROR1, and Caspase-8 expression in breast cancer cells and modified their communication with fibroblasts. Conditioned-media transfer revealed context-dependent changes in fibroblast activation- and EMT-associated markers, together with reduced migratory behavior in MDA-MB-231 cells. These findings demonstrate that prolonged EGF exposure modulates reciprocal tumor–stromal signaling in a cell line- and concentration-dependent manner.

Keywords: Breast cancer, EGF, ROR1, Cancer-associated fibroblast, Tumor microenviroment

PP083
Computational Design of Peptide Inhibitors Targeting the NONO–TRIM25 Interaction

Engin Mert Yenen[1], Nazli Keskin[1, 2]

[1] Department of Molecular Biology and Genetics, Graduate School of Natural and Applied Sciences, Acibadem Mehmet Ali Aydinlar University, Istanbul, Turkiye
[2] Department of Molecular Biology and Genetics, Faculty of Engineering and Natural Sciences, Acibadem Mehmet Ali Aydinlar University, Istanbul, Turkiye

Background/Aim: Glioblastoma (GBM) is the most aggressive form of the central nervous system tumors. However, the life expectancy of the patients has only been increased to 12-15 months, even after conventional treatments. For this reason, it is very important to suggest novel treatment options targeting the molecular mechanisms causing the aggressiveness of GBM. TRIM25, an E3 ubiquitin ligase was shown to bind and mediate K63-linked ubiquitination of NONO, a DBHS family member. This interaction eventually causes the activation of c-Myc pathway and leads to the progression of GBM tumorigenesis. Therefore, the aim of this study is to design an inhibitory peptide targeting the TRIM25-NONO interaction.
Materials and Methods: TRIM25-NONO interaction was modeled by performing global and local docking with ClusPro and HADDOCK, respectively. After analyzing the protein-protein interaction interface using PDBsum, candidate peptides were generated by using Peptiderive and PSSM, modeled with PEPFOLD4 and docked both globally and locally to the target proteins. All the modeled complexes were further evaluated through MD simulations with GROMACS.
Results: Considering different possible and energetically favorable dock models of TRIM25-NONO interaction, a peptide library composed of peptides with variable lengths was designed. Among all the tested peptide candidates, a 25 aa-long peptide, Peptide3M1, successfully bind to NONO and maintained the greatest consistency and stability across different MD runs. Moreover, the binding energy of the peptide-protein interaction was more negative than that of protein-protein interaction indicating more favorable binding.
Conclusion: The proposed candidate inhibitory peptide in this study, is predicted to target and inhibit the NONO-TRIM25 interaction, which may be a promising therapeutic strategy for GBM in case of further in vitro and in vivo experimental validation studies.
Acknowledgment:
The numerical calculations reported in this paper were fully performed at TUBITAK ULAKBIM, High Performance and Grid Computing Center (TRUBA resources).

Keywords: Glioblastoma, NONO, TRIM25, Targeting protein-protein interactions, Peptide design

PP084
Lentiviral Vector and GMP-Compliant Process Development for Clinically Applicable CD19-Targeted Academic CAR-T Cell Manufacturing in Türkiye

Elif Çelik[1], Betül Çakıcı[2], Mertkaya Aras[3], Beyza Nur Uysak[2], Esra Erdal[4], Batu Erman[2, 5], Tolga Sütlü[1, 2, 5]

[1] Acıbadem Mehmet Ali Aydınlar University, Graduate School of Health Sciences, Department of Translational Medicine, Istanbul, Türkiye
[2] Acıbadem Mehmet Ali Aydınlar University, Graduate School of Natural and Applied Sciences, Department of Molecular Biology and Genetics, Istanbul, Türkiye
[3] Sabancı University, Faculty of Engineering and Natural Sciences, Department of Molecular Biology, Genetics and Bioengineering, Istanbul, Türkiye
[4] Izmir Biomedicine and Genome Center, Izmir, Türkiye
[5] Acıbadem Mehmet Ali Aydınlar University, Faculty of Engineering and Natural Sciences, Department of Molecular Biology and Genetics, Istanbul, Türkiye

Background/Aim: Chimeric antigen receptor (CAR) T-cell therapy has transformed cancer immunotherapy by redirecting autologous T cells against antigens such as CD19 on B-cell malignancies. However, vector design, transduction efficiency, expansion protocols and cost-effective large-scale manufacturing continue to limit its clinical accessibility. This study aims to develop a lentiviral vector and a scalable, GMP-compliant manufacturing process for clinically applicable CD19-targeted CAR-T cells in Türkiye.
Materials and Methods: A clinically applicable lentiviral vector, pANTER23, encoding an FMC63-derived CD19 CAR with CD28 and CD3ζ costimulatory domains was designed. Third-generation lentiviral particles were produced at large scale and purified by fast protein liquid chromatography (FPLC) to increase titer, purity and transduction efficiency. CAR function was validated in Jurkat activation assays. Primary T-cell culture and transduction conditions were optimized using TexMACS medium, low-dose IL-2, T cell Transact beads and protamine sulfate. The validated protocol was then applied to MACS-enriched T cells from three patients and transferred to a GMP-compliant G-Rex culture system.
Results: Patient T cells (84.5–99.1% purity) transduced with pANTER23_FMC63 (MOI 5) and reached day-5 transduction efficiencies of 25.8–93.5% (mean: 53.2%).CAR-T cells expanded 64- to 216-fold by day 14 in the G-Rex system, and CAR expression persisted through day 21. Co-culture with CD19+ Raji cells and autologous B cells elicited increased CD107a degranulation and TNF-α/IFN-γ production compared with antigen-negative K562 and no-target conditions. CD4/CD8 ratios and exhaustion markers (LAG3, PD1, TIM3) remained trackable across all patients.
Conclusion: These results show that FPLC-purified, pANTER23-based lentiviral vectors combined with a GMP-compliant G-Rex process yields functional, CD19-specific CAR-T cells directly from patient samples with high transduction efficiency and robust expansion, supporting clinical translation of this academic CAR-T platform in Türkiye.
This project was supported by TÜBİTAK 1004 (Project No: 20AG006).

Keywords: Chimeric Antigen Receptor, T cells, Lentiviral Vector

PP085
Identification of a Candidate Regulator of Chemotherapy Resistance in Luminal Breast Cancer

Tuğçem Müge Gür[1, 2], Arda Burak Karagöz[1, 2], Gozde Korkmaz[2, 3]

[1] Graduate School of Health Sciences, Koc University, Sarıyer, İstanbul, Türkiye
[2] Koc University Research Center of Translational Medicine (KUTTAM), Sarıyer, Istanbul, Turkey
[3] School of Medicine, Koc University, Sarıyer, İstanbul, Türkiye

Background/Aim: Acquired drug resistance is a major cause of treatment failure and relapse in cancer. Tumour microenvironmental acidification, driven partly by altered cancer metabolism, can activate proton-sensing receptors that support tumour cell survival and therapy resistance. However, their role in breast cancer remains unclear. This study aimed to identify and functionally characterize proton-sensing receptors associated with chemotherapy resistance in breast cancer.
Materials and Methods: RNA-sequencing data from parental, doxorubicin-resistant and paclitaxel-resistant MCF7 cells were obtained from GEO dataset GSE288840 and analyzed using fastp, STAR, RSEM and DESeq2. Receptor X (RX) expression was examined by qPCR in breast cancer and non-tumorigenic breast cell lines. RX was targeted in luminal breast cancer cells using CRISPR–Cas9, followed by qPCR validation, colony-formation assays and three-dimensional spheroid culture.
Results: RNA-sequencing analysis revealed that RX expression was increased in both doxorubicin- and paclitaxel-resistant MCF7 cells compared with drug-sensitive parental cells. Quantitative real-time PCR analysis further demonstrated that RX was expressed at higher levels in luminal breast cancer cell lines than in basal-like breast cancer cells. CRISPR–Cas9-mediated depletion of RX was confirmed by quantitative real-time PCR. In colony-formation assays, RX-deficient cells exhibited decreased clonogenic growth compared with control cells. The colony-forming capacity of RX-deficient cells under paclitaxel or doxorubicin treatment was reduced relative to control cells. In three-dimensional culture, RX depletion resulted in reduced spheroid growth.
Conclusion: These findings identify RX as a proton-sensing receptor associated with the drug-resistant phenotype of luminal breast cancer cells. Ongoing functional analyses will determine whether RX contributes directly to breast cancer cell growth and chemotherapy response and may clarify its potential as a therapeutic target for overcoming drug resistance.

Keywords: Luminal Breast Cancer, Drug Resistance, Proton-Sensing Receptors

PP086
Integrated Bioinformatic and Functional Characterization of the miR-1825/ TSC2/mTOR Axis in Prostate Cancer Aggressiveness

Ozel Capik[1]

[1] Erzurum Technical University, Erzurum, Turkey

Background/Aim: Prostate cancer (PCa) is the second most commonly diagnosed cancer and the fifth leading cause of cancer-related mortality among men worldwide. Understanding the underlying molecular mechanisms of PCa is essential for developing more effective therapeutic strategies and identifying novel molecular targets for therapeutic intervention. This study investigated the role of miRNA-1825 in promoting the malignant characteristics of PCa cells, particularly its involvement in the TSC2/mTOR axis.
Materials and Methods: To elucidate the molecular mechanisms underlying the oncogenic effects of miR-1825, bioinformatic analyses were initially performed to identify potential target genes and ıts roles. The involvement of the miR-1825/TSC2/mTOR signaling axis was subsequently investigated in DU145 and PC3 PCa cells using complementary in vitro approaches, including qRT-PCR, Western blotting, luciferase reporter assays, and functional assays of cell viability and migration.
Results: Bioinformatic prediction analyses identified TSC2 as a candidate target of miR-1825, as it is a known negative regulator of the mTOR signaling pathway, and revealed a putative miR-1825 binding site within the 3′-UTR of TSC2 mRNA. Moreover, miR-1825 overexpression significantly reduced TSC2 expression at both the mRNA and protein levels, accompanied by a concomitant increase in mTOR expression in PCa cells. Conversely, inhibition of miR-1825 increased TSC2 expression and concomitantly decreased mTOR levels. These findings suggest that miR-1825 may regulate the TSC2/mTOR axis in PCa cells and support TSC2 as a potential target of miR-1825. Furthermore, dysregulation of miR-1825 significantly enhanced cell viability and motility in PCa cells. Collectively, in silico analysis and in vitro functional assay results demonstrate that miR-1825 promotes tumorigenic phenotypes in PCa cells by disrupting the TSC2/mTOR signaling axis.
Conclusion: our findings demonstrate that miR-1825 acts as an oncogenic regulator in PCa by targeting TSC2 and modulating the mTOR pathway. The miR-1825/TSC2/mTOR axis may represent a promising therapeutic target for the treatment of PCa.

Keywords: Prostate cancer, miR-1825, TSC2, mTOR, Cancer progression

PP087
EPIKOL Screening Uncovers RBBP7 as a Chromatin-Regulatory Dependency in IDH-Mutant Glioma

Sedef Sarac-Yalcinkaya[1, 2], Elisa Simone[2], Ebru Yilmaz[1, 2], Mina Kucuktas[1, 2], Fidan Seker-Polat[1, 2], Alisan Kayabolen[1, 2], Ali Cenk Aksu[1, 2], Tugba Bagcı-Onder[2, 3]

[1] Koç University Graduate School of Health Sciences, Istanbul
[2] Koç University Research Center for Translational Medicine, Istanbul
[3] Koç University School of Medicine, Istanbul

Background/Aim: Diffuse gliomas are the most common malignant primary tumors of the adult central nervous system (CNS) and remain difficult to treat, as they infiltrate the brain, recur, and resist surgical cure. The 2021 World Health Organization (WHO) classification defines adult-type diffuse gliomas by molecular features, distinguishing isocitrate dehydrogenase (IDH)-mutant tumors from IDH-wildtype glioblastoma. Mutant IDH acquires neomorphic activity, converting alpha-ketoglutarate to the oncometabolite D-2-hydroxyglutarate (2-HG), which inhibits alpha-ketoglutarate-dependent dioxygenases, disrupting DNA and histone demethylation. We aimed to identify chromatin-regulatory dependencies specific to the IDH-mutant context as potential therapeutic targets.
Materials and Methods: We established isogenic IDH-wildtype and IDH-mutant lines by overexpressing the respective enzymes in A172 cells and characterized them by phenotype, growth rate, 2-HG accumulation, and protein expression. Using the Epigenetic Knock-out Library (EPIKOL), we screened for selective dependencies, then integrated the results with patient data to prioritize candidates. The top hit was validated in multiple models, including patient-derived IDH-mutant primary cells, by colony and spheroid formation assays. RNA sequencing of the A172 pair and a primary IDH-mutant glioma line (TS603) characterized transcriptomic changes following knockout (KO).
Results: Mutant cells showed ~100-fold higher 2-HG, ~10-fold slower proliferation, and reduced colony & sphere formation. The screen revealed a stronger dependence on epigenetic modifiers in mutant cells, particularly in chromatin regulation, transcription, and RNA processing. Integrating screen and patient data, we identified Retinoblastoma Binding Protein 7 (RBBP7) as a selective vulnerability. RBBP7 KO reduced growth, colony, and sphere formation. RNA sequencing showed suppression of proliferative programs (E2F targets, G2M checkpoint, MYC targets, DNA repair) alongside increased stress-response, inflammatory, hypoxia, and interferon/TNF-NFκB signaling.
Conclusion: RBBP7 is a selective vulnerability in IDH-mutant gliomas, validated across multiple models. ATAC sequencing after KO is ongoing to assess chromatin reorganization, and rescue experiments with domain-specific RBBP7 mutants will define the functional region driving this dependency.

Keywords: IDH-mutant glioma, RBBP7, epigenetic vulnerability, CRISPR screen

PP088
AT-101 Induces Ferroptosis-Associated Cellular Alterations and Suppresses Proliferation in Glioblastoma Cells

Sertan Güçtür[1], Kubilay Göçücü[2], Ferdi Oğuz[3], Demet İnci[4], Ahmet Cingöz[4], Duygu Aydemir[5], Fatma Dağıstanlı[6]

[1] İstanbul University-Cerrahpaşa, Institute of Graduate Studies, Istanbul, Türkiye
[2] İstanbul Kent University, Departmen of Pathology Laboratory Techniques, Vocational School of Health Sciences, Istanbul, Türkiye
[3] Koç University, Graduate School of Health Sciences, Istanbul, Türkiye
[4] Beykoz Institute of Life Sciences and Biotechnology (BILSAB), Bezmialem Vakıf University, Istanbul, Türkiye
[5] İstanbul University Institute of Child Health, Department of Pediatric Basic Sciences, Division of Medical Genetics, İstanbul, Türkiye
[6] İstanbul University-Cerrahpaşa, Cerrahpaşa Faculty of Medicine, Department of Medical Biology, İstanbul, Türkiye

Background/Aim: Glioblastoma (GBM) is the most aggressive primary brain tumor and is associated with poor prognosis because of its invasive nature and resistance to conventional therapies. Targeting ferroptosis-related cellular processes has emerged as a potential therapeutic strategy for GBM. This study investigated the effects of the pan-BCL-2 inhibitor AT-101 on cell proliferation and ferroptosis-related metabolic alterations in GBM cells.
Materials and Methods: Human glioblastoma cell lines LN229 and T98G were treated with AT-101 at their respective IC_{50} concentrations (LN229: 10.17 μM and T98: 4.272 μM) for 48 hours. Cell viability was assessed using the MTT assay. Cellular proliferation was evaluated by Ki-67 immunocytochemistry. Ferroptosis-related cellular alterations were assessed by Prussian Blue staining for intracellular iron accumulation and Oil Red O staining for neutral lipid accumulation.
Results: AT-101 treatment reduced cell viability in both LN229 and T98G cells and was associated with fewer Ki-67-positive proliferating cells compared with untreated controls. Prussian Blue staining revealed increased intracellular iron accumulation following AT-101 treatment, while Oil Red O staining demonstrated reduced neutral lipid accumulation in AT-101-treated cells compared with untreated controls. Collectively, these findings indicate that AT-101 suppresses glioblastoma cell growth and is associated with increased intracellular iron accumulation and altered cellular lipid metabolism.
Conclusion: AT-101 exhibited anti-glioblastoma activity by reducing cell viability and proliferation and was associated with ferroptosis-related cellular alterations. These findings support the potential involvement of ferroptosis-related mechanisms in the cellular response to BCL-2 family inhibition and provide a rationale for further mechanistic and preclinical investigation of AT-101 in glioblastoma.

Keywords: Glioblastoma, AT-101, bcl2 family inhibition, ferroptosis, Cell proliferation

PP089
Green synthesized carbon quantum dots as potential anti-cancer agents

Selcen Umay Barutçugil[1], Elif Demir[2], Beste Dipçin[3], Bükem Tanören[4]

[1] Department of Molecular Biology and Genetics, Faculty of Engineering and Natural Sciences, Acibadem Mehmet Ali Aydinlar University, Istanbul, 34638, Turkey
[2] Department of Biomedical Engineering, Faculty of Engineering and Natural Sciences, Acibadem Mehmet Ali Aydinlar University, Istanbul, 34638, Turkey.
[3] Department of Natural Sciences, Faculty of Engineering and Natural Sciences, Acibadem Mehmet Ali Aydinlar University, Istanbul, 34638, Turkey.
[4] Department of Natural Sciences, Faculty of Engineering and Natural Sciences, Acibadem Mehmet Ali Aydinlar University, Istanbul, 34638, Turkey.

Background/Aim: Carbon quantum dots (CQDs) are organic-based semiconductor nanocrystals with easy functionalization, good chemical and photostability, fluorescence, and biocompatibility. CQDs are used in bioimaging, drug delivery fields, and as biosensors, anti-cancer or anti-microbial agents. The study aimed to investigate the coffee waste's roasting level effects on synthesized CQDs' anti-cancer properties.
Materials and Methods: Microwave-assisted pyrolysis technique was used to synthesize CQDs from Turkish Coffee ground waste with differing roasting levels (0, 15, 30, 45 minutes) at 250°C. CQD's synthesis was optimized for pyrolysis time (30 and 60 minutes), ethanol concentration (5% and 10%), and pyrolysis temperature (220°C and 240°C) at 600 rpm. The synthesized CQDs were characterized by FTIR, DLS, SEM, EDS, UV-VIS, and ZetaSizer instruments after neutralization and purification. The effect of the CQDs' roasting level on the cell viability was tested on HepG2, MCF-7 and HEK293 cell lines utilizing CCK-8 Kit in 24 hours with different concentrations (highest: 2 mg/mL).
Results: The most optimized CQD synthesis conditions were determined as ٪١٠ Ethanol, °٢٢٠C pyrolysis temperature, and ٦٠ minutes of pyrolysis time depending on the characterization results. The CQDs exhibited surface charge between ٣٦,٦٠- and ٢٧,٦٠- mV, spherical morphology, high carbon content, absorbance under ٢٣٠ nm indicating π-π* stacking, and hydrodynamic diameter between ١٦٠-١٢٠ nm supporting CQD presence. After ٢٤h of incubation, CQDs exhibited more cytotoxic effects on cancerous cells and less cytotoxic effects to HEK٢٩٣ cells suggesting CQDs' potential as anticancer agents.
Conclusion: In conclusion, CQDs were able to be synthesized from coffee waste successfully and exhibit promising preliminary results as anticancer agents. The treatment concentration will be increased to 3 mg/mL to establish precise IC50 values and select the most effective roasting level. Additionally, chitosan coating will be performed on the selected roasting level to enhance cellular uptake, and increase the anticancer potential of the CQDs.

Keywords: Green Synthesis, Carbon Quantum Dots, Cell Viability, Bioimaging

PP090
Deciphering the Genome-Wide Functions of CXXC5 in Estrogen Signaling in Breast Cancer

Gizem Güpür[1], Hazal Ayten[1, 2], Hamit İzgi[1], Büşra Bınarcı[1], Gizem Turan Duman[1], Bora Ergin[3], Efe Dallı[3], Burçak Otlu Sarıtaş[4], Mesut Muyan[1]

[1] Department of Biological Sciences, Middle East Technical University, ٠٦٨٠٠ Çankaya, Ankara, Türkiye
[2] Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, MD ٢١٢٠٥, USA
[3] Intergen Genetic and Rare Diseases Diagnosis Research and Application Center, Çankaya, Ankara, Türkiye
[4] Department of Health Informatics, Graduate School of Informatics, Middle East Technical University, ٠٦٨٠٠ Çankaya, Ankara, Türkiye

Background/Aim: 17β-estradiol (E2), the major circulating estrogen, plays a central role in the physiology and pathophysiology of many tissues, including the breast. The identification of E2-responsive genes and the functions of their protein products are essential for advancing our understanding of breast cancer initiation and progression. Such insights hold the potential for the development of novel or adjunct therapies.
In our previous studies, we identified CXXC5 as a primary E2–ERα (estrogen receptor alpha) response gene. Although CXXC5 participates in diverse cellular processes, including proliferation and cell death, its mechanisms of action remain largely unclear. Our prior findings demonstrate that CXXC5 binds unmethylated CpG regions and contributes to E2-driven cellular proliferation.
Here, we aimed to elucidate the genome-wide functions of CXXC5 in breast cancer in the context of E2 signaling using temporal expression profiling, genome-wide mapping of CXXC5 chromatin occupancy and DNA methylation profiling.
Materials and Methods: We employed RNA-sequencing in ERα-positive and E2-responsive MCF7 cells following siRNA mediated knockdown of CXXC5 in the absence or presence of E2 treatment for 6 or 12 hours. We performed diffferentially expressed gene expression analysis using DESeq2 followed by gene ontology enrichment analyses.
Results: E2-mediated gene expressions either in the presence or absence of CXXC5 indicate a strong proliferative gene expression profile enriched in DNA replication and cell cycle. CXXC5 depletion advances and further expands E2-mediated transcriptional program with many genes implicated in DNA repair, checkpoint, and homologous recombination processes being upregulated by E2 only in the absence of CXXC5.
Conclusion: Overall, these findings suggest that CXXC5 functions as a transcriptional constraint on E2-driven gene expression programs during G1/S transition and appears to play a critical role in maintaining balanced replication dynamics by restraining excessive E2-driven proliferative transcription and safeguarding genome integrity during cell cycle progression in ER-positive breast cancer cells.

Keywords: estrogen, CXXC5, RNA-Seq, cell cycle, DNA replication

PP091
CRISPR Knock-in Reporter Screening Reveals Epigenetic Regulation of ATP7B

Beyza Tutunculer[1], Baris Sergi[2], Selahattin Can Ozcan[2], Batuhan Altay[1], Ayca Acar[1], Umamaheswar Duvvuri[3], Kirill Kiselyov[4], Ceyda Acilan[5]

[1] Cellular and Molecular Medicine, Koc University, Istanbul, Turkey
[2] Koc University Research Center for Translational Medicine (KUTTAM), Koc University, Istanbul, Turkey
[3] School of Medicine, NYU Grossman, New York City, NY, USA
[4] Department of Biological Sciences, University of Pittsburgh, Pittsburgh, USA
[5] School of Medicine, Koc University, Istanbul, Turkey

Background/Aim: ATP7B is a copper-transporting ATPase essential for copper homeostasis. While reduced ATP7B expression underlies Wilson's disease (WD) and overexpression contributes to platinum resistance in cancer, the chromatin-level mechanisms regulating its transcription remain poorly defined.
Materials and Methods: A CRISPR/Cas9 knock-in reporter model was generated by inserting a firefly luciferase cassette into exon 1 of a single ATP7B allele in HEK293T cells, preserving the endogenous regulatory context. An epigenetic drug library of ~150 small molecules was screened at non-toxic concentrations. Candidate hits were validated by assessing endogenous ATP7B mRNA expression, western blotting of global histone modifications, ChIP-qPCR at the ATP7B promoter, and reproducibility testing in hepatic (Huh7) and non-hepatic (Du145) cell lines.
Results: The screening revealed several compounds that increased ATP7B transcription, but none that consistently inhibited reporter activity. HDAC inhibitors emerged as the strongest activators. Western blot analysis identified Chidamide and MS-275 as the most effective compounds, producing global increases in H3K9ac, H3K18ac, and H3K27ac. These two drugs were selected for ChIP-qPCR experiments, which demonstrated specific enrichment of H3K18ac at the ATP7B promoter. The effects were independent of oxidative stress and validated across multiple cell lines.
Conclusion: This study demonstrates that ATP7B expression is controlled by reversible chromatin states, with HDAC activity acting as repressor. HDAC inhibition relieves this repression, increasing promoter acetylation and transcription. The knock-in reporter model provides a powerful tool to study control of gene expression at native gene loci. Clinically, these findings highlight opportunities to enhance ATP7B expression in WD and modulate its levels in platinum-resistant cancers to improve therapeutic outcomes.

Keywords: ATP7B, CRISPR/Cas9, Epidrug Screening, Epigenetic Regulation, HDAC inhibitors

PP092
CXXC5 Is a Potential Therapeutic Vulnerability Factor in ERα-positive Tamoxifen-Resistant Breast Cancer Model

Büşra Bınarcı[1], Gizem Güpür[1], Gizem Turan Duman[1], Hesna Begüm Akman Tuncer[1], Mesut Muyan[1]

[1] Middle East Technical University, Ankara,Türkiye

Background/Aim: Breast cancer (BC) remains a major burden among women. Luminal A-type BC, with the highest prevalence, relies heavily on estrogen hormone (E2) and estrogen receptor (ERα) signaling (E2-ERα) for tumorigenesis. Tamoxifen (TAM), a widely used endocrine therapy, blocks this E2-ERα signaling in BC; however, prolonged exposure to TAM results in resistance, relapse, and metastasis. Existing tamoxifen-resistant models are established in the absence of E2 and ERα, thereby failing to mimic patients' physiological conditions. To address this, we generated an ERα+ tamoxifen-resistant cell line (MCF7-TamR) from luminal A-type MCF7 cells in the presence of E2. We aim to characterize them to uncover clinically relevant resistance mechanisms and targetable vulnerabilities.
Materials and Methods: MCF7-TamR cells were established by culturing parental MCF7 cells in increasing concentrations of TAM up to 5μM for 15 months. RNA-Seq was conducted on resistant and parental cells. Key altered targets were validated using qPCR and Western Blot. One of them was studied functionally with an siRNA approach.
Results: MCF7-TamR cells exhibit reduced ERα gene and protein levels, mirroring clinical patterns observed in resistant patients. Consequently, the transcriptomic analysis showed downregulated E2-ERα signaling and primary E2-ERα response genes (TFF1, GREB1, and CCND1), alongside significant upregulation of alternative survival pathways, including PI3K, MAPK, and Jak/Stat. Importantly, despite the loss of E2-ERα signaling, levels of CXXC5 - which is a primary E2-ERα response gene that codes for a zinc finger protein that is critical for cellular proliferation and differentiation - remained stable, which is consistent with elevated CXXC5 expression in the resistant patient cohort. Additionally, silencing CXXC5 significantly impaired proliferation compared to parental cells, indicating a distinct vulnerability factor for resistance.
Conclusion: While MCF7-TamR cells downregulate E2-ERα targets, CXXC5 expression is sustained, likely driven by alternative pathways upregulated in resistance. CXXC5 could represent a therapeutic vulnerability to overcome tamoxifen resistance in BC.

Keywords: Breast Cancer, Tamoxifen Resistance, E2-ERα signaling, CXXC5, Vulnerability Factor

PP093
Molecular Effects of Crispr/Cas9-Mediated Stat3 Silencing In U87-Mg Glioblastoma Cells

Semiral Tuana Şülekoğlu[1], Evren Önay Uçar[2]

[1] Istanbul University
[2] Istanbul University

Background/Aim: Glioblastoma (GBM) is an aggressive central nervous system tumor characterized by extensive molecular heterogeneity and therapeutic resistance. Signal transducer and activator of transcription 3 (STAT3) regulates multiple processes involved in GBM progression. This study aimed to investigate the molecular consequences of STAT3 silencing in U87-MG glioblastoma cells across survival, apoptosis, autophagy, cellular stress, inflammation, and immune-evasion pathways.
Materials and Methods: STAT3 was silenced in U87-MG cells using a lentiviral CRISPR/Cas9 system. Following puromycin selection, STAT3 suppression was confirmed by Western blotting. All experiments were performed at least in triplicate, with $p<0.05$ considered statistically significant. Protein expression levels of AKT, ERK, cyclin D1, p21, caspase-3, Bax, Bcl-2, Beclin-1, LC3A, Hsp70, Hsp90, IL-6, IL-10, and PD-L1 were comparatively evaluated in control and STAT3-silenced cells.
Results: STAT3 protein expression was reduced by 87–97%. STAT3 silencing decreased AKT by approximately 50%, ERK by 30–40%, and p21 by approximately 80%, whereas cyclin D1 showed no significant change. Caspase-3 decreased, while Bax and Bcl-2 increased without altering the Bax/Bcl-2 ratio, indicating no clear activation of classical apoptosis. Beclin-1 decreased by approximately 90%, whereas LC3A increased nearly threefold, demonstrating divergent regulation of autophagy-associated markers. Hsp70 decreased by 30–40%, while Hsp90 remained unchanged. Notably, IL-6 and IL-10 increased approximately 12-fold and 30-fold, respectively. Both glycosylated and non-glycosylated PD-L1 forms showed an increasing trend, although the changes were not statistically significant.
Conclusion: STAT3 silencing suppresses major survival-associated signaling in U87-MG cells but simultaneously induces complex adaptive responses involving autophagy-related proteins and inflammatory/immune-regulatory mediators. These findings indicate that STAT3-dependent signaling in GBM is embedded within compensatory molecular networks and support the evaluation of STAT3-targeted strategies in combination with complementary pathway-directed approaches.

Keywords: Glioblastoma, STAT3, CRISPR/Cas9, Knockout

PP094

Polydopamine Particles as Potential Drug Delivery Vehicles

Doğa Postacı[1], Aydan Torun[2], Aleyna Aslan[3], Sreeparna Banerjee[2], İrem Erel-Göktepe[3]

[1] Graduate Program in Polymer Science and Technology, Graduate School of Natural and Applied Sciences, Orta Doğu Teknik Üniversitesi, Ankara, Türkiye
[2] Department of Biological Sciences, Orta Doğu Teknik Üniversitesi, Ankara, Türkiye
[3] Department of Chemistry, Orta Doğu Teknik Üniversitesi, Ankara, Türkiye

Background/Aim: Polydopamine (PDA) is a bioinspired polymer that is derived from dopamine. PDA particles are promising drug delivery systems due to their biocompatibility and spontaneous generation in alkaline solutions without the use of any noxious chemicals. In this study, we evaluated whether the addition of tannic acid (TA), a plant-derived polyphenol, to PDA can generate PDATA particles that can serve as drug carriers.
Materials and Methods: PDATA particles were synthesized by polymerization of dopamine with or without TA in alkaline Tris buffer containing sodium periodate and loaded with or without the model drug Doxorubicin (DOX). Particle size and morphology were characterized by scanning electron microscopy (SEM). The effects of temperature and Tris concentration on particle formation were analyzed. The cytotoxic effects of PDATA and DOX-loaded PDATA (PDATA-DOX) were tested in HeLa cells via a cell viability assay.
Results: PDATA particles showed a small size distribution (100-500 nm) and a spherical morphology whereas PDA particles were larger (300-1000 nm) and more aggregated. Among the tested synthesis conditions, 0.03 M Tris buffer generated well defined PDATA particles with least aggregation. Cytotoxicity experiments showed that PDATA at 50 mg/mL and 100 mg/mL had minor effects on the viability of HeLa cells while PDATA-DOX decreased cell viability at these concentrations comparable to free DOX.
Conclusion: Addition of TA to polydopamine improved the size distribution, uniformity and stability of the particles and significantly decreased their aggregation. PDATA itself showed low cytotoxicity, indicating that it is suitable as a drug carrier. Thus, PDATA can be a potent biocompatible drug delivery vehicle that is easy to synthesize with minimal harm to the environment.

Keywords: polydopamine, tannic acid, doxorubicin, drug delivery, nano particles

PP095
Molecular Effects of Regorafenib and Propolis Treatments on Caco-2 Intestinal Cancer Cells

Seda Kuşoğlu Gültekin[1, 2], Nazlı Arda[1, 3]

[1] İstanbul University, Institute of Graduate Studies in Sciences, Department of Molecular Biology and Genetics, ٣٤٤٥٢ Fatih, İstanbul, Türkiye
[2] Üsküdar University, Faculty of Engineering and Natural Sciences, Department of Molecular Biology and Genetics, 34662 Üsküdar, İstanbul, Türkiye
[3] Istanbul University, Faculty of Science, Department of Molecular Biology and Genetics, ٣٤١٣٤, Fatih, Istanbul, Türkiye

Background/Aim: In colorectal cancer treatment, combined approaches with natural products are attracting attention to increase the efficacy of chemotherapeutic agents and reduce their unwanted side effects. The aim of this study is to determine the *in vitro* molecular effects of water-based propolis and Regorafenib (REG), an anticancer drug, administered alone and in combination on Caco-2 intestinal cancer cells.
Materials and Methods: The cytotoxicity of propolis and REG on Caco-2 and healthy control HUVEC cells was evaluated by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay with a 48-hour treatment. The dose exhibiting a synergistic effect (250 µg/mL propolis + 10 µM REG) was determined by combination index analysis. Cell migration and colony formation were examined using *in vitro* scratch and colony formation assays, respectively. The expressions of proteins related to apoptosis, multidrug resistance, and inflammation [Bax, Bcl2, caspase-3, β-catenin, Nuclear Factor kappa B p65 (NF-κB p65), p-glycoprotein (P-gp), heat shock proteins (Hsp90 and Hsp70)] were analyzed by western blot methods.
Results: It was determined that the identified propolis+REG treatment dose exhibited a synergistic cytotoxic effect on Caco-2 cells; while in HUVEC cells, propolis reduced REG-induced toxicity. The combined treatment significantly suppressed Caco-2 migration and colony formation compared to single treatments. The combined treatment was observed to increase proapoptotic Bax and caspase-3 protein levels while decreasing the antiapoptotic Bcl-2 level in Caco-2 cells. Furthermore, the expressions of β-catenin, NF-κB p65, P-gp, Hsp90, and Hsp70 proteins, which are associated with multidrug resistance, cell survival, and inflammation, were found to significantly decrease after the combined treatment.
Conclusion: The obtained data indicate that propolis synergistically increases the anticancer efficacy of REG by inducing apoptosis and suppressing resistance mechanisms. This first study examining the combined application of propolis and REG in colorectal cancer cells provides a strong foundation for new therapeutic approaches.

Keywords: Cancer, propolis, regorafenib, Synergistic effect, apoptosis

PP096
Investigating the Impact of Matrix Stiffness on Tumor Plasticity in EGFR-Mutant NSCLC

Aslinur Ulucay Gul[1, 2], Zelal Duru Seyhan[1, 2], Ece Ozturk[2, 3]

[1] Graduate School of Health Sciences, Koc University, Istanbul, Turkey
[2] Research Center for Translational Medicine (KUTTAM), Koc University, Istanbul, Turkey
[3] School of Medicine, Medical Biology, Koc University, Istanbul, Turkey

Background/Aim: Tumor progression is accompanied by altered remodeling of the extracellular matrix (ECM), resulting in increased tissue stiffness that reshapes cancer cell behavior, cellular signaling and therapeutic response. Yet, the contribution of matrix stiffness to tumor plasticity in Epidermal Growth Factor Receptor (EGFR)-mutant non-small cell lung cancer (NSCLC) remains poorly understood. Our study aims to demonstrate the role of ECM stiffness as a regulator of tumor associated phenotypes in EGFR mutant NSCLC.
Materials and Methods: Here, we developed a bioengineered three-dimensional human lung tumor model with tunable mechanical properties designed to recapitulate healthy- and tumor-like lung tissue stiffness. The model comprises double-network hydrogel platform composed of alginate and decellularized lung ECM. Soft and stiff matrices were constructed by modulating calcium content as the ionic crosslinker for alginate. The mechanical properties of the hydrogels were characterized by rheological measurements. EGFR-mutant NSCLC cell line H1975 were encapsulated within the hydrogels, and the effects of matrix stiffness on cell viability and growth were investigated using the CellTiter-Glo assay. Stiffness-associated changes in epithelial–mesenchymal plasticity was assessed by RT-qPCR analysis of epithelial and mesenchymal markers. Morphological changes to mesenchymal phenotype was assessed by confocal microscopy imaging and results were analyzed with Fiji.
Results: Our preliminary results indicate that increased matrix stiffness is associated with changes in EMT-related gene expression and causes a shift toward a more mesenchymal phenotype.
Conclusion: These findings suggest that matrix stiffness may contribute to the regulation of tumor plasticity in EGFR-mutant NSCLC. Future studies will determine whether stiffness-induced phenotypic changes influence sensitivity and resistance to third-generation EGFR tyrosine kinase inhibitors. This bioengineered model may provide a physiologically relevant platform for investigating biomechanical regulation of tumor behavior and developing more predictive preclinical models for precision oncology.

Keywords: Non-Small Cell Lung Cancer , 3D tumor model, Matrix Stiffness, Epithelial-mesenchymal transition

PP097
Optimization of High-Titer Lentiviral Vector Production and Purification Strategies for Cell and Gene Therapies

Alp Ertunga Eyüpoğlu[1], Betül Çakıcı[2], Elif Çelik[3], Batu Erman[4], Tolga Sütlü[4]

[1] Boğaziçi University, Institute for Graduate Studies in Science and Engineering, Department of Molecular Biology and Genetics
[2] Acıbadem Mehmet Ali Aydınlar University, Graduate School of Natural and Applied Sciences, Department of Molecular Biology and Genetics
[3] Acıbadem Mehmet Ali Aydınlar University, Graduate School of Health Sciences, Department of Translational Medicine
[4] Acıbadem Mehmet Ali Aydınlar University, Faculty of Engineering and Natural Sciences, Department of Molecular Biology and Genetics

Background/Aim: Clinical translation of cell and gene therapies, especially CAR-T, requires robust lentiviral vector (LVV) transduction efficiency. However, generating sufficient infectious particles for large-scale transduction of patient-derived primary cells remains a major bottleneck. Furthermore, transfection of packaging cell lines during LVV production causes critical impurities: Host cell proteins and DNA carry immunogenic and oncogenic risks, bacterial endotoxins from plasmid DNA cause unintended activation and premature T cell exhaustion, xenogenic serum (FBS) may inhibit viral entry and residual transfection reagents may induce severe cytotoxicity. Therefore, optimizing upstream production for increased LVV titer and downstream purification for enhancing efficiency and safety is essential for a scalable, clinical-grade manufacturing platform.
Materials and Methods: On a high-capacity multilayer vessel platform, LVV production efficiency was maximized by optimizing plasmid ratios and using sodium butyrate, an HDAC inhibitor that epigenetically upregulates viral transcription. For downstream processing, following FBS reduction and Benzonase-mediated nucleic acid degradation, supernatants were subjected to two-step chromatographic purification: anion-exchange (Capto DEAE) for initial viral particle enrichment followed by core-shell multimodal polishing (Capto Core 700) to clear contaminants while strictly preserving viral infectivity.
Results: Upstream optimization achieved 5-fold increase in LVV titer in HEK293FT cells. Consequently, two-step chromatography minimized purification losses, achieving an overall recovery exceeding 50% with exceptional purity. In functional assays using patient-derived primary T cells, these purified vectors demonstrated enhanced transduction efficiency over standard protocols.
Conclusion: This optimization study successfully bridges the gap between high-titer viral vector production and clinical-grade purity. By providing the exceptional purity and high viral concentrations required to effectively engineer primary T cells, this project establishes a highly adaptable solution for viral vector-based therapies.

This project was funded by TÜBİTAK grant 20AG006

Keywords: Lentiviral vector purification, multimodal chromatography, primary T cell therapy

PP098
Unraveling The Role of Receptor Kinases in Neuroblastoma Drug Resistance and Metastatic Progression

Ilhan Mert Ozalp[1, 2], Tugce Batur[1], Ezgi Batu[1, 2], Ece Sule Zangur[1, 2], Nisanur Gulmez[1, 2], Evin İscan[1, 2], Ugur Ozbek[1]

[1] Izmir Biomedicine and Genome Center, Izmir, Turkey
[2] Izmir International Biomedicine and Genome Institute, Izmir, Turkey

Background/Aim: Neuroblastoma (NB) is a highly heterogeneous pediatric malignancy arising from immature neural cells. High-risk NB is characterized by drug resistance and metastatic potential, which significantly limit treatment success. Although receptor kinases (RKs) have been implicated in tumor progression, their roles in NB drug resistance and metastasis remain poorly understood. Here, we aim to investigate the contribution of RKs to drug resistance and metastatic potential in NB.
Materials and Methods: We investigated publicly available transcriptomes of NB cell lines, both resistant SH-SY5Y and metastatic NB, to evaluate 70 RKs. Differential expression analysis identified 33 candidate RKs. To further investigate RK expression in metastatic NB, we analyzed single-cell RNA-sequencing data from NCBI BioProject SRP558154, focusing on these candidates. An Adriamycin (ADR) resistant SH-SY5Y cell line was generated to investigate RKs associated with long-term resistance, while short-term drug responses were assessed following 24h and 72h ADR and vincristine exposure. Selected RKs were evaluated by RT-qPCR across these conditions.
Results: Single-cell transcriptomic analysis revealed distinct and heterogeneous RK expression patterns across tissues. Metastatic samples displayed a more extensive RK expression landscape, with increased expression of multiple RKs compared with adrenal gland and primary neuroblastoma tissues. In qPCR analysis, AXL, BMPR1A, BMPR2, and DDR2 were upregulated following short-term drug treatment in resistant cells, consistent with their increased expression in liver metastasis. MET, which showed moderate expression in liver metastasis, was upregulated across all treatment groups, whereas LMTK2 and EPHB2 expression remained relatively stable.
Conclusion: RK expression patterns were divergent across tissues. Transcriptomic analysis revealed a more extensive RK expression landscape in metastatic tissue, suggesting a potential association with metastatic progression. Consistent expression patterns were also observed in drug-treated and resistant cells. Shared activation of multiple RKs may indicate their potential involvement in both metastasis and drug resistance mechanisms in NB.

Keywords: Neuroblastoma, Receptor Kinase, Chemoresistance, Metastasis, Transcriptomics

PP099
Revealing the Role of LEDGF in Regulation of Resistance Mechanisms in Castration-Resistant Prostate Cancer (CRPC)

Beyza Nur Çelik[1], İpek Bulut[2], Buse Cevatemre[2, 3], Hamzah Syed[3], Ceyda Açılan[2, 3]

[1] Koç University Graduate School of Health Sciences, İstanbul, Turkey
[2] Koç University Research Center for Translational Medicine (KUTTAM) İstanbul, Turkey
[3] Koç University School of Medicine, İstanbul, Turkey

Background/Aim: Prostate cancer is primarily treated with radiotherapy and androgen-deprivation therapy (ADT). Castration-resistant prostate cancer (CRPC) arises after ADT, and docetaxel (Dtx) remains the standard-of-care chemotherapy despite frequent resistance. Our preliminary data demonstrate that depletion of lens epithelium-derived growth factor (LEDGF) sensitizes resistant cells to Dtx and supresses Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling, suggesting a functional link. We therefore aimed to determine the roles of LEDGF and JAK/STAT signalling in Dtx-resistance, and to assess the therapeutic potential of combining JAK/STAT inhibitors with Dtx.
Materials and Methods: Dtx-resistant CRPC cell models were generated by stepwise dose escalation, and LEDGF was knocked out using CRISPR-Cas9. Transcriptomic changes and enriched pathways were profiled by RNA-seq, and genome-wide LEDGF binding sites were mapped by cleavage under targets and release using nuclease (CUT&RUN) sequencing. Proliferation and viability were compared between parental and resistant lines, and clinically relevant JAK/STAT inhibitors were tested for restoration of Dtx sensitivity.
Results: Analysis of clinical datasets revealed LEDGF overexpression in resistant cells is associated with poor patient prognosis and correlating positively with IL6/JAK/STAT pathway genes across prostate cancer patients. LEDGF knockout impaired proliferation of resistant cells relative to parental cells and downregulated key IL6/JAK/STAT components at the transcript level. CUT&RUN confirmed LEDGF binding at IL6R, STAT3, and related genes. Pharmacological inhibition of JAK/STAT signalling resensitized resistant cells to Dtx.
Conclusion: Our data suggests that LEDGF may contribute to Dtx-resistance through transcriptional regulation of the JAK/STAT pathway. Therapeutic targeting of this pathway may represent an effective dual-treatment strategy. Ongoing ChIP and upcoming in vivo studies will further define the DNA-binding profile of LEDGF and its contribution to tumorigenicity. Our study highlights LEDGF as a potential epigenetic regulator of JAK/STAT signaling in CRPC and suggests the translational promise of combining JAK/STAT inhibition with standard chemotherapy.

Keywords: Castration Resistant Prostate Cancer, Taxane Resistance, Epigenetics, LEDGF, JAK/STAT

PP100
Anticancer activity of quercetin and berberine against HCT116 human colorectal cancer cells

Berfin ASLAN[1], Zehra Nur GORME[1], Yasemin YILMAZER[1]

[1] Molecular Biology and Genetics Department, Istanbul Sabahattin Zaim University, Istanbul, Türkiye

Background/Aim: Colorectal cancer (CRC) remains one of the leading causes of cancer-related mortality worldwide, highlighting the need for new therapeutic approaches. Quercetin and berberine are plant-derived molecules reported to possess anticancer properties and modulate key signaling pathways involved in tumor progression. Quercetin has been shown to promote apoptosis by regulating β-catenin, Bcl-2, and caspase signaling, whereas berberine suppresses cell proliferation through the inhibition of cyclin-dependent kinase 4 (CDK4) and modulation of cyclin D1. This study investigated the cytotoxic, pro-apoptotic, and anti-migratory effects of quercetin and berberine in HCT116 colorectal cancer cells.
Materials and Methods: Cell viability was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and the concentrations producing 25% (IC25) and 50% (IC50) loss of viability were calculated for both agents. Apoptotic and migratory responses were subsequently characterized using 4′,6-diamidino-2-phenylindole (DAPI) nuclear staining, flow cytometry, and wound-healing assays.
Results: The IC25 and IC50 values were 4.5 and 29.5 µM for quercetin and 2.8 and 9.7 µM for berberine, respectively. DAPI staining revealed marked nuclear fragmentation and apoptotic body formation following treatment with both IC25 and IC50 concentrations of each compound. Wound-healing assays revealed a reduction in migratory capacity compared with untreated controls, with migration decreasing by 6.85% and 7.94% following treatment with IC25 and IC50 concentrations, respectively. Flow cytometric analysis showed a dose-dependent increase in apoptosis, with viable cell populations decreasing to 73.31% at IC25 and 58.56% at IC50.
Conclusion: Both quercetin and berberine induced apoptosis, reduced cell viability, and inhibited migration in HCT116 colorectal cancer cells. Berberine exhibited greater antiproliferative activity than quercetin, as reflected by its lower IC25 and IC50 values. These findings support further investigation of both compounds, particularly berberine, as potential adjunctive therapeutic agents for colorectal cancer.

Keywords: Quercetin; berberine; colorectal cancer; HCT116; apoptosis

PP101
Understanding the Interaction Between Translation Machinery and the Wnt Pathway in Cancer

Mustafa Kaan Kozluca[1], Duygu Kuzuoglu Ozturk[1, 2, 3]

[1] Sabancı University, Istanbul, Turkey
[2] Department of Urology, University of California, San Francisco, San Francisco, CA, USA
[3] Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, San Francisco, CA, USA

Background/Aim: In eukaryotes, translation is initiated by a complex called "Eukaryotic Translation Initiation Complex 4F (eIF4F)". It was previously proven that when the level of the major cap-binding protein, eukaryotic initiation factor 4E (eIF4E), was limited, tumorigenesis was suppressed, hinting that eIF4E is a powerful oncogenic driver. A genome-wide functional screen identified the Wnt signaling pathway as critical for sustaining eIF4E oncogenic activity. This highly conserved pathway, essential for cell proliferation and survival, is frequently hyperactivated in multiple malignancies, including colorectal and prostate cancers.
In this study, we investigate the interaction between eIF4E and the Wnt pathway by focusing on how eIF4E modulates Wnt pathway activity in cancer.
Materials and Methods: All experiments were performed on the colon cancer cell line HCT116, in which eIF4E expression is either 100% ($eIF4E^{+/+}$) or 50% ($eIF4E^{+/-}$). A luciferase reporter assay was performed to assess Wnt pathway activity. Protein levels of β-catenin were measured by Western blotting. Immunofluorescence (IF) was performed to investigate nuclear translocation of β-catenin.
Results: Luciferase reporter assay showed that, upon exogenous activation of the Wnt pathway with LiCl, when eIF4E levels were limited, luminescence signals were lower, indicating that reduced eIF4E levels decrease Wnt pathway activation. To understand mechanistically, we first investigated the levels of β-catenin and its translocation by Western blotting following cell fractionation as well as IF. The results did not show any alteration in the location or expression of β-catenin upon eIF4E depletion.
Conclusion: Building on the findings that implicate a functional link between eIF4E and Wnt signaling, we confirmed this crosstalk through independent luciferase-based reporter assays. Crucially, our initial mechanistic analyses reveal that this interaction is not driven by fluctuations in β-catenin levels, but rather operates through other key members of the Wnt cascade.

Keywords: cancer, translation machinery, wnt pathway

PP102

Chemotherapy-associated Coagulation–hypoxia–inflammation Reprogramming Reveals a Coordinated Malignant-cell State in High-grade Serous Ovarian Cancer

Emir Fatih Kaya[1], Duygu Sari Ak[2]

[1] Department of General Surgery, Division of Surgical Oncology, University of Health Sciences Türkiye, Bakırköy Dr. Sadi Konuk Training and Research Hospital, Istanbul, Türkiye
[2] Department of Medical Biology, Hamidiye International School of Medicine, University of Health Sciences Türkiye, Istanbul, Türkiye

Background/Aim: Platinum resistance remains a major clinical challenge in high-grade serous ovarian cancer (HGSOC). We investigated chemotherapy-associated transcriptional reprogramming in malignant cells and explored its cellular origin, underlying mechanisms, relationship with platinum resistance, and potentially targetable regulatory axes.
Materials and Methods: Public single-cell RNA-sequencing data from GSE165897 were analyzed using 11 patient-matched pre- and post-neoadjuvant chemotherapy samples comprising 51,786 cells, including 8,806 malignant cells. Patient-level pseudobulk expression, module scoring, paired Wilcoxon tests, exact sign-flip tests, Hallmark gene-set enrichment analysis, cellular-compartment comparisons, and malignant-subtype selection-versus-induction analyses were performed. GSE98230 was used as an independent platinum-resistant ovarian cancer cell-line dataset. Target-set enrichment was integrated with clinical-development evidence to identify candidate therapeutic opportunities.
Results: Post-treatment malignant cells showed significant enrichment of TNF-α/NF-κB signaling (normalized enrichment score [NES]=2.85; false discovery rate [FDR]<0.001), coagulation (NES=2.29; FDR<0.001), hypoxia (NES=2.25; FDR<0.001), complement (NES=1.74; FDR=0.002), and TGF-β signaling (NES=1.68; FDR=0.005). These coordinated changes were strongest in malignant epithelial cells. The EOC_C7 malignant-cell state expanded from 2.9% to 16.7% after treatment (FDR=0.023), while coagulation also increased within multiple matched malignant states, supporting contributions from both cellular selection and within-state induction. A composite adaptation score increased in all 11 patients (exact sign-flip p=0.00098). In GSE98230, platinum-resistant cells demonstrated significant reactive oxygen species pathway enrichment (FDR=0.045) and a positive coagulation trend (FDR=0.119). PPARD, CEBPB, and RELA target sets emerged as hypothesis-generating regulatory candidates, while TGF-β, hypoxia, and coagulation-associated axes demonstrated potential therapeutic relevance.
Conclusion: Neoadjuvant chemotherapy was associated with a robust, tumor-cell-intrinsic coagulation–hypoxia–inflammation state in HGSOC. Targetability mapping highlighted PPARD, CEBPB, RELA/NF-κB, TGF-β, hypoxia, and coagulation-associated axes as candidate therapeutic opportunities requiring experimental validation.

Keywords: High-grade serous ovarian cancer, neoadjuvant chemotherapy, single-cell RNA sequencing, coagulation, platinum resistance

PP103
Development and Optimization of EGFRvIII-Targeted VLPs for Functional Cargo Delivery

Yasemin Holly Özoran[1, 2], Canan Bayraktar-Odabas[1, 2], İbrahim Şahin Ozyıildiz[1, 2], Tugba Bagci-Onder[1, 2, 3]

[1] Koç University Graduate School of Health Sciences, Medicine, Istanbul, Turkey
[2] Koç University Research Center For Translational Medicine, Istanbul, Turkey
[3] Koç University School of Medicine, Istanbul, Turkey

Background/Aim: EGFRvIII is a tumor-specific receptor expressed in glioblastoma (GBM), making it a promising therapeutic target. Virus-like particles (VLPs) provide a non-replicative platform for targeted protein delivery without genomic integration. We aimed to optimize EGFRvIII-targeted VLP production and evaluate receptor-specific cargo delivery, including Cas9 RNP-mediated targeting of RPL9 for functional cell killing.
Materials and Methods: A Gag-GFP fusion construct served as a reporter for VLP-mediated protein delivery, and flow cytometry characterized GFP delivery kinetics across time points. VLP concentration methods, including polyethylene glycol (PEG) precipitation and protamine sulfate (PS) aggregation, were compared based on GFP delivery. An EGFRvIII-specific single-chain variable fragment (scFv) was displayed on VLPs to assess receptor-specific delivery by flow cytometry. RPL9-targeting Cas9 ribonucleoprotein (RNP) was delivered as functional cargo, and MTT assays assessed targeted cell killing.
Results: Flow cytometric analysis identified detection windows for transient VLP-mediated GFP delivery. PS aggregation resulted in more efficient GFP delivery than PEG precipitation. EGFRvIII-targeted VLPs demonstrated selective delivery to receptor-expressing cells. Delivery of RPL9-targeting Cas9 RNP resulted in targeted cell killing, demonstrating functional activity of the delivered cargo.
Conclusion: Our findings establish an optimized workflow for EGFRvIII-targeted VLP production and functional cargo delivery. PS-based concentration improved GFP delivery, while EGFRvIII targeting enabled selective cargo delivery. Delivery of RPL9-targeting Cas9 RNP resulted in targeted cell killing, demonstrating the functional potential of this platform for targeted therapeutic delivery in GBM.

Keywords: EGFRvIII, Glioblastoma, Virus-like particles, Targeted delivery, Cas9 RNP

PP105
Characterization and Genome Informatics of a Novel Lytic Stutzerimonas Stutzeri Phage vB_SstS_STP9, Suitable for Biocontrol or Phage Therapy

Sani Sharif Usman[1], Evangeline Christina[2]

[1] Department of Biochemistry and Molecular Biology, Faculty of Science, Federal University of Kashere, P.M.B. 0182, Gombe, Nigeria
[2] School of Biosciences, Engineering and Technology, VIT Bhopal University, Kothri-Kalan, Bhopal-Indore Highway, Madhya Pradesh, Sehore, 466114, India

Background/Aim: In this study, a novel lytic phage vB_SstS_STP9 targeting Stutzerimonas stutzeri strain STP9 was isolated and purified from the untreated site of Sewage Treatment Plant of Lovely Professional University, India.
Materials and Methods: Enrichment, plaque assays, and 2 M ZnCl2 were recruited for phage susceptibility and stability testing. HR-TEM, PCI/SDS, 1% agarose gel-electrophoresis, flourometer, RNase A, DNase I, QIAseq FX DNA Library Kit for Illumina, and Illumina NovaSeq 6000 NGS platform were employed for morphological, molecular, and structural characterization of lytic phage vB_SstS_STP9.
Results: Morphological characterization of phage vB_SstS_STP9 showed an icosahedral capsid of ~ 68 nm in diameter with a long, contractile tail of ~ 97 nm. The genome of vB_SstS_STP9 is a linear, dsDNA harbouring ٣٩,٩٠٩ bp in size with a G+C content of ٪٥٥,٧٧ containing ٥٣ open reading frames (ORFs). The ٥٣ predicted ORFs encoded proteins that partake in infection, DNA entry, replication, gene expression, packaging, assembly, immunity, lysis, among others. Functional annotation of phage vB_SstS_STP٩ genome revealed the absence of tRNAs, temperate markers, toxins genes, virulence factors, antimicrobial resistance genes (ARGs) and CRISPR arrays. BLAST and phylogeny results indicated that phage vB_SstS_STP٩ is genetically akin to Escherichia phage C٢_١٣٠ with a highest percentage identity of ٪٩٥,٦٥. Based on HR-TEM and WGS with bioinformatics analysis, lytic phage vB_SstS_STP٩ is myovirus morphotype devoid of integrase gene, ARGs, CRISPR arrays, virulence factors and toxins genes, showing stability and viability at wide range of pH (3-11) and temperature (–20 °C to 70 °C).
Conclusion: Altogether, lytic phage vB_SstS_STP9 belongs to the class Caudoviticetes, and should be a member of family Zobellviridae which appeared to be 100% virulent and suitable for biocontrol or phage therapy. In tandem with bibliometrics and nucleotide data availability in GenBank, this appears to be the third report of a phage targeting S. stutzeri.

Keywords: Bioinformatics, Transmission electron microscopy, Lytic bacteriophage, Stutzerimonas stutzeri, Next-generation sequencing

PP106
Protego: An Integrated Tool for Complex Mass Spectrometry-based Data Analysis and Visualization

Bora Yazıcıoğlu[1], Fulya Türker[1, 2]

[1] Department of Molecular Biology and Genetics, Bilkent University, Ankara
[2] Department of Neuroscience, Bilkent University, Ankara

Background/Aim: Proteomics data analysis often requires multiple disconnected tools for preprocessing, statistical testing, visualization, biological interpretation, and reporting. This fragmented workflow can reduce reproducibility and make parameter tracking difficult, particularly for label-free quantification and data-independent acquisition datasets. ProteGO was developed as an interactive application to give users an integrated, transparent, and user-friendly environment for proteomics analysis.
Materials and Methods: ProteGO was implemented as an R/Shiny-based application that can be run locally from source on Windows and macOS, with planned web-based deployment for browser access, and supports common proteomics input formats, including MaxQuant, FragPipe, Spectronaut, DIA-NN, Proteome Discoverer-derived tables, TMT, SILAC, SAINT interaction analysis outputs, and generic Excel files. The workflow helps users upload data, define sample groups, filter contaminants and decoys, filter peptide and MS/MS evidence, normalize data, impute missing values, perform differential abundance analysis, and apply multiple-testing correction. Statistical options include limma-based empirical Bayes testing, Permutation FDR (s0 Regularized t-test), SAMr, DEqMS, and standard t-tests. The application also helps users annotate or filter low-confidence proteins using evidence-aware methods, generate interactive volcano and MA plots, heatmaps, and quality-control visualizations, explore pathway enrichment and literature search, map structures, and create HTML/PDF reports for reproduction.
Results: ProteGO enables users to process raw protein intensity matrices, define experimental groups, compare statistical workflows, and generate publication-ready visualizations within a single interface. Evidence-aware annotations help users identify proteins whose apparent differential abundance may be influenced by partial-dropout imputation or insufficient group-level MS/MS support. Users can then retain these proteins with labels or exclude them before or after statistical analysis, enabling a conservative sensitivity assessment.
Conclusion: ProteGO provides an integrated and reproducible platform for proteomics data analysis, visualization, and interpretation. By combining statistical flexibility with evidence-aware quality control and automated reporting, it helps users evaluate proteomics results more transparently and supports downstream biological insight.

Keywords: Proteomics Data Analysis, R/Shiny Application, Bioinformatics

PP107
Characterization of Splicing and Gene/Transcript Level Expression Alterations in Alzheimer's Disease Using Long-Read Transcriptome Sequencing

Tunahan Çakır[1], Hilal Ateş[1]

[1] Department of Bioengineering, Bioinformatics and Systems Biology Program, Gebze Technical University, Kocaeli

Background/Aim: The molecular structure of Alzheimer's disease (AD) is highly complex and triggers mechanisms like abnormal gene expression and alternative splicing. Traditional short-read sequencing (SRS) methods are insufficient for assembling full-length transcript structures. Long-read sequencing (LRS) offers a powerful solution for discovering novel isoforms, identifying full-length transcripts and characterizing alternative splicing events. However, most of the studies in the literature using LRS technology rely on standard cohort-level comparisons rather than patient-specific changes. This project aims to fill this gap by developing an integrated computational workflow that captures expression and splicing changes at both group and individual levels using raw LRS data.
Materials and Methods: We implemented a comprehensive bioinformatics workflow using raw transcriptomic LRS data obtained from AD brain tissue. The workflow was designed to perform differential expression analysis at the gene and transcript levels, discover uncharacterized transcripts and find alternative splicing events. Importantly, in addition to standard group-level differential comparisons, we integrated a personalized medicine framework by individually evaluating each patient sample against a reference group of healthy controls.
Results: Our analytical approach facilitated the discovery of abnormal splicing events and novel RNA isoforms while successfully identifying group-level differential markers such as novel transcripts in AD-related genes (e.g. CASP9, CLUAP1, PSENEN, NDUFV3) and abnormal brain morphology related pathways driven by upregulated transcripts. Furthermore, by integrating sample-specific analyses, the process yielded unique molecular differences for individual patients, revealing critical transcriptomic variations hidden in standard cohort analyses.
Conclusion: Combining cohort-level differential analysis using LRS data with a patient-specific perspective allowed for a broader assessment of the AD transcriptome. Integrating expression and alternative splicing analyses under this framework deepens our understanding of the precise molecular mechanisms driving AD heterogeneity, opening new avenues for identifying specific biomarkers and therapeutic targets unreachable with traditional methods.

Keywords: Alzheimer's disease, long-read sequencing, transcriptome data, personalized medicine

PP108
A Homozygous GHR c.723 C>T p.(Gly241=) Variation Causing Laron Syndrome

Ahmet Arman[1], Bilgesu Saraydaroglu[2], Oyku Idil Kocoglu[3], Iremnur Kansu[1], Durmus Durmaz[4], Zehra Yavas Abalı[5]

[1] Department of Medical Genetics, School of Medicine, Marmara University, Istanbul, Turkey
[2] Department of Medical Biology and Genetics, Istanbul University, Istanbul, Turkey
[3] School of Medicine, Marmara University, Istanbul,Turkey
[4] Department of Medical Genetics, Prof. Dr. Asaf Ataseven Hospital, Istanbul,Turkey
[5] Department of Pediatric Endocrinology, School of Medicine, Marmara University, Istanbul, Turkey

Background/Aim: Growth Hormone (GH) is involved in growth, fat and carbohydrate metabolism. GH shows its activities through growth hormone Receptor (GHR) necessary for production of insulin-like growth factor I (IGF-I). Mutations in the GHR cause severe postnatal growth failure and the disorder is an autosomal recessive genetic disease resulting in GH insensitivity called Laron Syndrome. Our aim was to characterize children with Laron syndrome.
Materials and Methods: Patient was brought for severe short stature, he showed normal GH and low IGF-I level.Genomic DNA was isolated from blood of the patient with LS. Exon 2-10 of GHR of the patient were amplified by polymerase chain reaction (PCR) using the specific primers. The amplified PCR products of GHR exons for the patient were purified and sequenced with direct sequencing of the DNA Cycle sequencing System (ABI Prism kit) with the dideoxychain termination method and applied on an auto sequencer (ABI Prism 377 DNA sequencer). Subsequently, segregation analysis of the detected variant was carried out in family members. Functional analysis experiments for this variant are going.
Results: Exon 3 deletion polymorphism and a homozygous c.723 C>T p.(Gly241=) variation in the GHR gene were detected in the patient. In silico analyses of synonymous alteration indicate that the variant may lead to a splicing defect. The variant is observed in very low frequency in population databases and has been reported in homozygous. Due to its consistency with current clinical findings, the variant has been classified as probable pathogenic according to ACMG criteria. Segregation analysis confirmed that both parents were heterozygote carriers.
Conclusion: Biallelic pathogenic variant (c.723 C>T p.(Gly241=)) in the GHR gene are associated with autosomal recessive Laron syndrome. This result is consistent with the phenotype of the case.

This project was supported by GenEra Diagnostics

Keywords: Laron, Growth Hormone Receptor, Short Stature, GHI

PP110
Ki67 Exhibits Dynamic Terminus- and Cell Cycle-Dependent Proximity Interactomes

Asiyenur Üner[1], Büşra Aytül Kırım[1], Nurhan Özlü[1], Nazan Saner[1, 2]

[1] Koç University, İstanbul
[2] Acıbadem University, İstanbul

Background/Aim: Ki67 is a widely used proliferation marker in cancer research. The recent findings indicate that Ki67 organizes the heterochromatin and mitotic chromosome periphery. Its expression and cellular localization are dynamic throughout the cell cycle. Ki67 localizes to nucleoplasmic foci and nucleolar rim during interphase, and to the surface of chromosomes during mitosis. However, how its protein interaction network changes across the cell cycle progression remains elusive. Furthermore, Ki67 is a large protein (359 kDa) and was shown to undergo conformational elongation in mitosis, suggesting that its N- and C-termini may engage in distinct interaction networks. Collectively, this study aims to identify the dynamic proximity interactome of Ki67 at both N- and C-termini in a cell cycle-dependent manner to gain deeper insight into its molecular function.
Materials and Methods: We performed miniTurbo-based proximity labeling coupled with mass spectrometry to map Ki67 interactome. HEK293T cells expressing either miniTurbo-Ki67 or Ki67-miniTurbo were synchronised to interphase or mitosis and subjected to proximity-dependent biotinylation. The labelled proteins were affinity purified and analysed using LC-MS/MS.
Results: A total of 1338 proteins were identified as interaction partners of Ki67. Among these, 392 were mitosis-specific and 213 were interphase-specific. In addition, 475 proteins were uniquely associated with the N-terminus, whereas 244 were specific to the C-terminus of Ki67. We also identified and characterized an interaction between Ki67 and INCENP, a subunit of the chromosome passenger complex (CPC).
Conclusion: Our proteomic analyses reveal that the interaction networks of Ki67 vary not only between interphase and mitosis, but also between N- and C- termini.

Keywords: Ki67, cell cycle, miniTurbo, proximity labeling, proteomics

PP111
Whirly-associated Molecular, Physiological and Biochemical Responses of Common Bean to Heavy Metal Stress

Meltem Büyüktepe[1], Melisa Sıla Tombul[1], Kağan Dönmezoğlu[1], F. Şeyma Gökdemir[2, 3], Füsun Eyidoğan[1, 2]

[1] Başkent University, Faculty of Science and Letters, Department of Molecular Biology and Genetics, Ankara, Türkiye
[2] Başkent University, Institute of Food, Agriculture and Livestock Development, Ankara, Türkiye
[3] Başkent University, Kahramankazan Vocational School, Department of Food Processing, Ankara, Türkiye

Background/Aim: Heavy metal contamination of agricultural environments threatens crop productivity and food safety because toxic elements may accumulate in edible plant tissues and enter the food chain. Lead (Pb) and cadmium (Cd) are non-essential toxic metals, whereas copper (Cu) becomes phytotoxic at excessive concentrations. WHIRLY proteins are plant-specific DNA-binding proteins associated with organellar functions and stress responses. This study aimed to compare the physiological and biochemical responses of Phaseolus vulgaris L. to heavy metal stress and investigate the potential involvement of PvWHY genes in metal-specific stress adaptation.
Materials and Methods: Göynük٩٨- and Önceler٩٨- cultivars were grown under identical controlled conditions and exposed separately to ٥٠ and ١٠٠ µM Pb, ٢٠ and ٥٠ µM Cu, or ٢٠ and ٥٠ µM Cd, together with untreated controls. Morphological responses, relative water content (RWC), chlorophyll pigments, carotenoid and anthocyanin contents, and malondialdehyde (MDA) accumulation were evaluated. In silico analyses of PvWHY١, PvWHY٢, and PvWHY٣ included promoter cis-element profiling and subcellular targeting prediction. Gene expression profiles are being assessed by quantitative real-time polymerase chain reaction.
Results: Heavy metal treatments induced concentration- and cultivar-dependent responses. Cu exposure reduced plant vigor and water status and increased lipid peroxidation, particularly at the higher concentration. At 100 µM Pb, RWC was 86.95% in Önceler-98 and 90.82% in Göynük-98, while MDA increased from 5.82 to 17.92 and from 8.79 to 17.04, respectively. Cd exposure also altered water status. Promoter analysis identified 52 cis-regulatory elements across PvWHY genes, including W-box, MBS, HSE, ARE, and TC-rich repeats. PvWHY1 and PvWHY3 showed preferential mitochondrial targeting, whereas PvWHY2 was predominantly chloroplast-targeted.
Conclusion: Heavy metal exposure generated distinct genotype- and concentration-dependent stress signatures in common bean. Integrating physiological and biochemical responses with PvWHY regulatory architecture and organelle targeting helps distinguish shared and metal-specific mechanisms and evaluate WHIRLY genes as candidate determinants of heavy metal tolerance.

Keywords: Phaseolus vulgaris, Heavy Metal Stress, WHIRLY, Oxidative Stress, Food Safety

PP112
Comparative Analysis of Peptidoglycan-Derived Muropeptides Accommodation by Bd3459 from Bdellovibrio bacteriovorus

Ceren Bahar KAPÇAK[1], Senem UYLAŞ[1]

[1] Department of Genetics and Bioengineering, Faculty of Engineering, Alanya Alaaddin Keykubat University, Alanya, Antalya, Türkiye

Background/Aim: Bdellovibrio bacteriovorus is a predatory Gram-negative bacterium that remodels prey peptidoglycan (PG) during bdelloplast formation. Bd3459 is a predation-specific PBP4/DacB-like DD-endo/carboxypeptidase lacking Domain III, giving a more open catalytic cleft proposed to accommodate diverse PG substrates. Its ability to accommodate soluble PG-derived muropeptides remains uncharacterized. This study computationally evaluated the accommodation of monomeric and cross-linked muropeptides by Bd3459 and compared their predicted binding poses.
Materials and Methods: The crystal structure of Bd3459 (PDB: 3V39) was prepared for docking. Monomeric muropeptides (MuroTri, MuroTetra, MuroPenta) and cross-linked dimers TriTri (3→3), TetraTri, TetraTetra and TetraPenta (4→3) were studied. Conformers were generated with RDKit, converted to PDBQT with Meeko, and docked using AutoDock Vina. Poses were analyzed in PyMOL and interactions characterized with PLIP.
Results: All ligands docked into the catalytic pocket, with predicted binding energies from −7.394 to −6.146 kcal/mol. MuroTri gave the most favorable score, whereas cross-linked dimers showed comparable affinities. PLIP identified 12–24 hydrogen bonds, 1–4 salt bridges, and 1–7 hydrophobic contacts. All complexes interacted with Ser70 and Lys73, while SXN- and KTG-motif interactions varied among ligands. TetraTri showed the most hydrogen bonds and TetraPenta the most hydrophobic contacts, indicating no direct correlation between interaction number and predicted affinity.
Conclusion: These analyses provide the first systematic structural assessment of soluble muropeptide accommodation by Bd3459, indicating that its Domain III–deficient, open catalytic cleft can geometrically accommodate both monomeric and 3→3 and 4→3 cross-linked muropeptides. All complexes shared a conserved Ser70–Lys73 anchor, whereas SXN- and KTG-motif engagement varied with muropeptide architecture, suggesting a common catalytic anchor with ligand-dependent secondary positioning. The narrow energy range indicates that docking scores alone cannot discriminate substrate preference; specificity and catalytic competence remain to be established. This work provides a structural framework to guide molecular dynamics simulations and experimental validation.

Keywords: Bdellovibrio bacteriovorus, Bd3459, peptidoglycan, muropeptide, molecular docking

PP113
Multi-Cohort Transcriptomic Convergence in Autism, with a Four-Method Test of Circadian Dysregulation

İlayda Keleş[1]

[1] İzmir International Biomedicine and Genome Center

Background/Aim: Autism spectrum disorder (ASD) is genetically and molecularly heterogeneous, making reproducible molecular signatures difficult to identify with conventional gene-level transcriptomics. This study investigated whether convergent molecular changes are resolved more effectively at the transcript level, and whether circadian dysregulation forms a detectable transcriptomic signature, across multiple independent post-mortem brain datasets.
Materials and Methods: Six publicly available post-mortem brain RNA-sequencing datasets (167 samples) were uniformly reprocessed through a harmonized pipeline comprising gene- and transcript-level differential expression, differential transcript usage (DTU), isoform switching, pre-ranked gene-set enrichment, weighted gene co-expression network analysis, and supervised machine learning that integrated multi-layered transcriptomic features to recover curated ASD (SFARI) gene annotation. Circadian involvement was assessed by four complementary approaches, including the clock correlation distance.
Results: Gene-level differential expression was sparse, with no gene recurring across cohorts, whereas transcript-level and isoform-switching analyses recovered substantially more signal. Direct feature-level replication remained limited (21 transcripts, 13 DTU genes, and one isoform-switch gene shared across cohorts), but pathway- and network-level convergence was strong: nineteen pathways were concordantly down-regulated in at least three cohorts, and major co-expression modules were well preserved. The strongest single predictor of SFARI annotation was transcript-level differential-expression significance (AUC = 0.80), while integrated XGBoost and Random Forest models achieved similar discrimination (AUC ≈ 0.79–0.80); gene-level features were far less informative.
Conclusion: Transcript-level analyses capture ASD molecular signatures more effectively than conventional gene-level methods, with convergence emerging at the pathway and network levels. Despite strong behavioural evidence, no circadian signature was detectable in static post-mortem transcriptomes. The integrative framework prioritised novel candidate genes not yet catalogued in ASD databases, including *SMARCC1*, as targets for future functional study.

Keywords: Autism spectrum disorder, circadian rhythm, transcriptomics, machine learning, systems biology

PP114

Evaluation of GM130 Gene Expression in SMN-Deficient Motor Neuron-Like Spinal Muscular Atrophy Model

Fulya Şahin[1], Yasemin Göktürk [1], Hayat Erdem Yurter[1], Gamze Bora Akoğlu [1]

[1] Hacettepe Unıversity, Department of Medical Biology, Faculty of Medicine, Ankara, Turkey

Background/Aim: Spinal muscular atrophy (SMA) is a rare neurodegenerative disease caused by the mutations in survival of motor neuron 1 (SMN1) gene, resulting in reduced levels of SMN protein. SMN loss leads to multiple cellular perturbations, and we and others have previously shown Golgi fragmentation in SMN-deficient cells. Although fragmentation has been associated with the Golgi stress response, it has not been previously investigated in SMA. In this study, we analyzed the expression of GM130, a structural Golgi protein widely used in studies of Golgi fragmentation and stress in a in vitro SMA model.
Materials and Methods: SMN gene was knocked down in motor neuron-like NSC34 cells by siRNA-mediated gene silencing approach to generate an in vitro SMA model. Total RNA was isolated from SMN siRNA and scrambled siRNA transfected cells, and then quantitative real-time PCR (RT-qPCR) was performed to analyze GM130 gene expression.
Results: RT-qPCR analysis revealed that SMN level was downregulated by %92 in SMN siRNA-transfected cells. However, we did not detect a significant alteration in GM130 gene expression in SMN-depleted NSC34 cells compared to controls.
Conclusion: Our findings indicate that SMN deficiency does not alter GM130 transcript level in this in vitro model. To better evaluate Golgi stress in SMA, other stress-related genes should be investigated. Studies are ongoing to understand the relationship between SMN deficiency, Golgi fragmentation and stress response.
This study was supported by the Hacettepe University Scientific Research Projects Coordination Unit (Project No. THD-2025-22354, TYL-2025-21568).

Keywords: Spinal muscular atrophy, Golgi fragmentation, GM130 Gene Expression

PP115

Disease-specific Remodeling of BAFF-associated Extracellular Vesicle Programs Across Hematologic Malignancies: A Multi-cohort Bioinformatics Analysis

Sevil Nasırova[1], Bahadır Batar[2]

[1] ١ Department of Medical Genetics, Faculty of Medicine, Tekirdağ Namık Kemal University, Tekirdağ, Türkiye
[2] ٢Department of Medical Biology, Faculty of Medicine, Tekirdağ Namık Kemal University, Tekirdağ, Türkiye

Background/Aim: B-cell activating factor (BAFF/TNFSF13B) regulates B-cell survival and immune signaling; however, its relationship with extracellular vesicle (EV) biology remains unclear. We investigated whether BAFF-associated EV programs show uniform or disease-specific patterns across malignancies.
Materials and Methods: Microarray datasets of diffuse large B-cell lymphoma (DLBCL; GSE10846, n=420), acute myeloid leukemia (AML; GSE37642, n=422), multiple myeloma (MM; GSE31161, n=780), and chronic lymphocytic leukemia (CLL; GSE22529, n=41) were analyzed across eight EV functional modules. Comparisons included breast cancer (GSE42568, n=104), lung adenocarcinoma (GSE31210, n=226), colorectal cancer (GSE39582, n=566), and metastatic melanoma (GSE19234, n=44) cohorts. BAFF–EV associations were assessed by Spearman correlation. Hematologic–solid correlation differences ($\Delta\rho=\rho_{hematologic}-\rho_{solid}$) were evaluated using bootstrap confidence intervals and Benjamini–Hochberg false discovery rate (FDR) correction. Gene set enrichment analysis and ExoCarta/Vesiclepedia cross-referencing were additionally performed.
Results: In DLBCL, BAFF was associated with EV markers ($\rho=0.504$), lysosomal routing ($\rho=0.368$), and Endosome/MVB ($\rho=0.343$). BAFF-associated transcriptional profiles were enriched for interferon-γ response (NES=3.75), TNFα/NF-κB (NES=2.95), IL6/JAK/STAT3 (NES=2.74), and protein secretion (NES=2.36). Hematologic–solid comparisons revealed marked module-specific differences. DLBCL showed stronger EV-marker (mean $\Delta\rho=0.41$; 4/4 FDR-significant) and Endosome/MVB associations ($\Delta\rho=0.26$; 3/4), whereas MM showed stronger EV-release association ($\Delta\rho=0.35$; 3/4). Cargo-loading associations were lower across all four hematologic malignancies, with 12/16 comparisons FDR-significant. Membrane-fusion associations were consistently lower in DLBCL, AML, and MM (12/12 FDR-significant comparisons). CLL displayed a distinct profile, particularly for lysosomal routing (mean $\Delta\rho=-0.49$). Overall, 60/128 comparisons remained FDR-significant, while 72/128 bootstrap 95% confidence intervals excluded zero. ExoCarta and Vesiclepedia supported 40/41 (97.6%) EV module-gene memberships.
Conclusion: BAFF-associated EV patterns differ between hematologic and solid malignancies and vary among hematologic disease types, supporting shared and disease-specific programs rather than uniform EV activation. However, as these analyses are transcriptomic and correlation-based, the findings do not establish causal BAFF–EV relationships or direct changes in EV production or cargo and require experimental validation.

Keywords: BAFF, Hematolojik malignancies, Extracellular vesicles, Bioinformatics

PP116
Cancer-type-specific Divergence of PPP2CA and MIEF1 Coupling Between PML-I and PML-IV

Zeynep Aydın Sinirlioğlu[1]

[1] Istanbul Sabahattin Zaim University

Background/Aim: The promyelocytic leukemia (PML) protein recruits the AKT phosphatase PP2A into the PML nuclear body, while MIEF1/MiD51 serves as an outer-membrane adaptor for the fission GTPase DRP1. PML is transcribed into multiple isoforms with distinct partners, yet cancer transcriptomic studies almost universally analyse it as a single gene. We previously linked PML-IV to a PP2A/MIEF1 module in glioblastoma (GBM); however, it remains to be investigated whether this association is isoform-specific or restricted to glioma.
Materials and Methods: Isoform-level TOIL RSEM quantification (UCSC Xena) of 22 PML transcripts was analyzed in 8,275 primary tumors across 23 TCGA cancer types, in which PML-IV (ENST00000395135, NM_002675) exceeded the detection floor in ≥50% of samples. Transcript abundances were summed in linear space before log transformation. Isoform preference was defined as Δρ (PML-IV minus PML-I; ENST00000268058), with each isoform–target partial Spearman correlation conditioned on the summed other 21 transcripts. Benjamini–Hochberg correction was used. Robustness of PML-IV coupling to global expression was tested by conditioning on PML-I and a leave-one-target-out median of the remaining 53 genes, with correction across 46 cancer-type–target tests.
Results: Coupling met both-model criteria in 8 of 23 cancer types. PML-I preference occurred in clear-cell renal cell carcinoma (PPP2CA/MIEF1 Δρ −0.29/−0.34), head and neck squamous cell carcinoma (−0.24/−0.21) and prostate adenocarcinoma (−0.18/−0.27; all $q<0.05$). PML-IV preference was significant only for MIEF1 in GBM (Δρ +0.328, 95% CI 0.090–0.534, $q=0.018$) and ovarian serous cystadenocarcinoma (+0.182, $q=0.012$); GBM PPP2CA difference (+0.234) was nonsignificant ($q=0.064$). In GBM, PPP2CA and MIEF1 ranked first and second among 54 mitophagy genes for PML-IV (partial $\rho=0.417$ and 0.378; panel median 0.105), but only 30th and 44th for PML-I.
Conclusion: PPP2CA and MIEF1 transcriptional coupling differ between PML isoforms in a cancer-type-specific manner. GBM shows the strongest preferential PML-IV coupling, a pattern obscured by gene-level analyses.

Keywords: PML isoforms, glioblastoma, cancer transcriptomics, mitocondrial fission, MIEF1

PP117
Discovering Subtypes in Alzheimer's Disease from RNA-seq Data

Timur Çakmakoğlu[1], Tunahan Çakır[1]

[1] Gebze Tehnical University, Kocaeli, Türkiye

Background/Aim: Alzheimer's Disease (AD) is a heterogeneous neurodegenerative disease that is challenging to profile with molecular data. Most studies either don't stratify the patients or rely on disease-severity scoring systems such as Braak staging or CERAD scores, which have high false-positive and false-negative rates even in distinguishing AD samples from controls. To our knowledge, only one prior study has used RNA-seq data from brain tissue to identify putative AD subtypes. We aim to gain further insights into AD subtypes using a deep learning-based clustering approach.
Materials and Methods: We combined RNA-seq data from four cohorts (ROSMAP, MSBB, MAYO Clinic, and GSE125583) to train an autoencoder, then applied deep-embedded clustering (DEC) using the encoder extracted from the pre-trained autoencoder. Because the batch effects across datasets could not be fully eliminated, clustering was performed within each dataset individually. We used the Kruskal-Wallis test to identify genes significantly associated with cluster differences, then intersected significant gene sets across the datasets and performed enrichment analysis to assess disease relevance.
Results: Given the lack of ground-truth subtype labels or a known optimal cluster number, we validate clustering solutions using internal metrics such as silhouette scores and clustering stability across multiple trials. This work is ongoing; we are finalizing results and comparing our findings with the prior subtyping study to evaluate overlap and divergence.
Conclusion: Identifying robust AD subtypes may help clarify disease heterogeneity and inform more targeted diagnostic and therapeutic strategies.

Keywords: RNA-seq, Alzheimer's Disease, Disease subtyping, Deep learning, Clustering

PP118

Integrative Proteomic Prioritization Of Extracellular Vesicle-Associated Biomarker Candidates for Molecular Subtyping Of Diffuse Large B-Cell Lymphoma

Furkan Ozben[1], Emine Sümeyra Turalı Emre[1]

[1] Boğaziçi University, Institute of Biomedical Engineering, Istanbul, Türkiye
[2] Boğaziçi University, Institute of Biomedical Engineering, Istanbul, Türkiye

Background/Aim: Diffuse large B-cell lymphoma (DLBCL) comprises biologically distinct activated B-cell-like (ABC) and germinal center B-cell-like (GCB) molecular subtypes with different molecular characteristics and clinical behavior. This study aimed to identify reproducible proteomic determinants of ABC/GCB subtype identity and to prioritize translational biomarker candidates through the integrative reanalysis of DLBCL cell-line & patient-tissue proteomes.
Materials and Methods: Proteomic datasets comprising ten DLBCL cell lines, including five ABC and five GCB models analyzed in triplicate, and twenty formalin-fixed paraffin-embedded patient tissues were reprocessed using an updated MaxQuant-based workflow. Normalized Super-SILAC ratios were used for cell-line analyses, whereas light-channel label-free quantification intensities were used for tissue profiling. Protein groups flagged as site-only, reverse/decoy, or potential contaminants were removed. Replicate reproducibility, protein-level detection support, missingness, and global proteomic structure were systematically assessed. ABC/GCB-associated proteins were prioritized using differential abundance, effect-size, correlation, and leave-one-cell-line-out stability analyses. A subtype classifier was constructed exclusively from cell-line data and subsequently applied to patient tissues under a label-withheld design. Final candidates were ranked by cross-dataset concordance, pathway and protein-interaction context, extracellular-vesicle association, and biomarker novelty.
Results: Quality-controlled analysis retained 7,086 protein groups in the cell-line dataset & 9,847 in the tissue dataset. Among cell-line proteins, 2,830 showed ≥2-of-3 replicate detection support across all ten cell lines. Integrative subtype analysis identified a stable panel of ABC/GCB-associated proteins that performed consistently across replicate-aware and leave-one-cell-line-out assessments. The locked cell-line-derived signature was successfully transferred to the label-withheld tissue cohort, enabling molecular subtype assignment independent of published tissue labels. Cross-dataset and systems-level prioritization further refined the resulting candidates according to biological consistency and translational relevance.
Conclusion: Integrative reanalysis of DLBCL proteomes enabled reproducible prioritization of subtype-associated protein signatures and demonstrated the feasibility of transferring cell-line-derived proteomic information to patient tissues. This framework provides a reproducible basis for molecular classification and biomarker development in DLBCL.

Keywords: Extracellular Vesicle-Associated Biomarker Candidates, Diffuse Large B-Cell Lymphoma, Molecular Subtyping, Cancer Proteomics

PP119
OVARI-AI: An explainable multi-omics framework for computational prediction of AKT inhibitor response in ovarian cancer

Emine Kazanc[1], Kubra Cengiz[2]

[1] Bogazici University
[2] Istanbul Technical University

Background/Aim: Ovarian cancer exhibits marked biological heterogeneity, and genomic alterations alone do not fully explain therapeutic response. Because metabolites capture the functional output of signalling and nutrient-use programmes, we investigated whether metabolic state provides biologically relevant information beyond gene expression for predicting response to AKT inhibition.
Materials and Methods: Transcriptomic and metabolomic profiles (225 metabolites) from 600 Cancer Cell Line Encyclopedia models, including 29 ovarian or fallopian-tube lines, were integrated with capivasertib half-maximal inhibitory concentration (IC50) data from the Genomics of Drug Sensitivity in Cancer resource. Leakage-controlled nested cross-validation compared transcriptomics-only, metabolomics-only, early-fusion and late-fusion models. Feature contributions were examined to identify molecular processes associated with drug response, and ovarian-line results were evaluated separately.
Results: Integrating metabolites with transcriptomic profiles increased the Spearman correlation between predicted and observed capivasertib response from 0.44 (95% confidence interval, 0.37-0.51) to 0.47 (0.40-0.53), with a paired improvement of 0.026 (0.004-0.049). Late-fusion analysis assigned complementary contributions to transcriptomics (0.69) and metabolomics (0.31), indicating that drug response was not explained by transcriptional state alone. Four of the 20 highest-contributing features were metabolites. Alpha-glycerophosphocholine showed the strongest metabolic contribution, implicating phosphatidylcholine turnover and membrane-lipid remodelling as candidate determinants of AKT inhibitor sensitivity. The same direction of effect was observed across ovarian models, although uncertainty remained high because of the limited subgroup size.
Conclusion: These findings support a biological link between cellular metabolic state and AKT-pathway dependence in ovarian cancer. OVARI-AI provides an interpretable computational framework for prioritising metabolic-transcriptional features and generating experimentally testable therapeutic hypotheses, rather than serving as a clinical predictor.

Keywords: ovarian cancer, multiomics, metabolomics, precision medicine, system biology

PP120
Developing Cell-Impermeable Proteasome Inhibitors via IEDDA Click Chemistry to Selectively Target Neuronal Membrane Proteasomes

Rohat Erdoğan[1], Fulya Türker[1, 2]

[1] Department of Molecular Biology and Genetics, Bilkent University, Ankara, Türkiye
[2] Department of Neuroscience, Bilkent University, Ankara, Türkiye

Background/Aim: Neuronal membrane proteasomes (NMPs) are specialized proteasome complexes localized to the neuronal plasma membrane that degrade intracellular proteins and release short peptides into the extracellular matrix to regulate neuronal signaling. Dysregulation in these processes plays a significant role in the pathogenesis of neurodegenerative conditions such as Huntington's disease (HD). Currently, the selective study of NMP biology remains highly challenging because standard pan-proteasome inhibitors are membrane-permeable and target the whole proteasome pool. We aim to overcome these limitations by developing novel, cell-impermeable chemical probes derived from two key scaffolds: the well-established proteasome inhibitor Epoxomicin and YU-101, the basis of carfilzomib, to specifically investigate NMP biology.
Materials and Methods: To specifically inhibit NMPs, we propose novel activity-based probes by conjugating hydrophilic, electrostatically repulsive functional groups (e.g., cysteic acid and PEG linkers) to the Epoxomicin and YU-101 backbones. This structural modification is designed to restrict plasma membrane permeability of those inhibitor backbones via passive diffusion. These probes also incorporate a trans-cyclooctene (TCO) handle for a two-step bioorthogonal labeling strategy. Following live-cell treatment, inverse electron-demand Diels-Alder (IEDDA) click chemistry will be performed using tetrazine-conjugated fluorophores and affinity tags for activity-based protein profiling (ABPP).
Results: We have currently designed the predicted structures of the potential candidate inhibitors. The conjugation of cysteic acid and PEG linkers into the Epoxomicin and YU-101 scaffolds is expected to effectively abolish plasma membrane permeability while preserving binding affinity to the catalytic subunits of the proteasome. Consequently, the two-step IEDDA labeling strategy will provide a tagging mechanism that selectively targets for precise visualization and biochemical isolation of NMPs.
Conclusion: The combination of these cell-impermeable probes with the IEDDA two-step labeling method provides specific chemical tools for understanding NMP biology. Ultimately, this approach will help elucidate the role of NMPs in HD and create a strong basis for developing targeted treatments.

Keywords: Neuronal Membrane Proteasomes, Huntington's Disease, Proteasome Inhibitors, Activity-Based Probes, IEDDA Click Chemistry

PP121
Recombinant Human β-glucocerebrosidase Production in Komagataella Phaffii (syn. Pichia Pastoris) Using Different Promoter Systems

Şükran Abik[1], Burcu GÜNDÜZ ERGÜN[1, 2]

[1] Yildiz Technical University, Istanbul, Türkiye
[2] Health Biotechnology Centre of Excellence for Joint Research and Application (SABIOTEK), Yildiz Technical University, Istanbul, Türkiye

Background/Aim: Gaucher disease is an inherited lysosomal storage disorder caused by the deficiency of the lysosomal enzyme β-glucocerebrosidase (GCase). Although enzyme replacement therapy is an effective treatment, the high production costs of therapeutic enzymes limit their accessibility. Therefore, the development of efficient and cost-effective expression platforms for recombinant GCase production is of considerable interest.
Materials and Methods: In this study, *Komagataella phaffii* (syn. *Pichia pastoris*) was used as a host system for recombinant production of human β-glucocerebrosidase. Due to its ability to reach high cell densities, perform eukaryotic post-translational modifications, and produce recombinant proteins at relatively low cost, *K. phaffii* is widely used in biopharmaceutical production. To compare different expression strategies, the human *GBA1* gene was expressed under the control of three different promoter systems: the methanol-inducible alcohol oxidase 1 promoter (P*AOX1*), a modified alcohol oxidase 1 promoter (P*mAOX1*), and the constitutive glyceraldehyde-3-phosphate dehydrogenase promoter (P*GAP*).
Results: Recombinant expression cassettes were integrated into the *K. phaffii* genome and transformants were screened by enzyme activity analyses. Preliminary results revealed differences in β-glucocerebrosidase production among the promoter systems evaluated. In addition, considerable variation in enzyme activity was observed among transformants carrying the same promoter construct. Several high-activity clones were identified and selected for further characterization.
Conclusion: These findings indicate that *K. phaffii* is a promising host for recombinant β-glucocerebrosidase production and that promoter selection significantly influences production performance. Further studies will focus on detailed characterization of selected clones and optimization of production conditions for enhanced enzyme production.

Keywords: Gaucher disease, β-glucocerebrosidase, Komagataella phaffii, recombinant protein production, promoter systems.

PP122
Targeted LNP-mRNA CAR-Treg Combination Therapy for Immune Thrombocytopenia

Asmar Aydamirli[1, 2], Melis Erginsoy[1, 2], Ali Çalışkan[1, 2], Ferdi Oğuz[1, 2], Alişan Kayabölen[1, 2]

[1] Translational Advanced Research on Gene Engineering & Therapeutics (TARGET) Lab, Koç University School of Medicine, Istanbul,
[2] Koç University Translational Medicine Research Center (KUTTAM). Istanbul

Background/Aim: Immune thrombocytopenia (ITP) is an autoimmune disorder characterized by autoantibody-mediated platelet destruction and bone marrow megakaryocyte dysfunction. Current therapies predominantly rely on systemic immunosuppression or thrombopoietin receptor agonists, lacking antigen-specific immune tolerance and harboring high relapse rates. We propose a dual-arm platform that generates chimeric antigen receptor regulatory T cells (CAR-Tregs) directly in vivo via targeted lipid nanoparticles (LNPs), avoiding ex vivo cell manufacture, combined with megakaryocyte-directed mRNA delivery to restore platelet production.
Materials and Methods: Anti-GPIIb/IIIa (CD41/CD61) CAR constructs are engineered through systematic scFv sequence selection and hinge-length optimization, then co-encapsulated with FOXP3 mRNA. These dual payloads are encapsulated into CD25-targeted, dead ApoE (dApoE)-precoated lipid nanoparticles engineered for liver detargeting and selective in vivo regulatory T cell delivery. In parallel, CD41-targeted LNPs encapsulating p45-NF-E2 mRNA are produced for megakaryocyte-specific delivery to restore proplatelet formation. Following physicochemical characterization (NTA, encapsulation efficiency, stability) and in vitro binding/suppression assays, therapeutic efficacy, kinetics, and immune tolerance will be evaluated in mouse models of ITP.
Results: Preliminary work demonstrated that programmable LNP platforms achieve cell-selective mRNA delivery, while dApoE precoating reduces liver signal by up to 90%. Furthermore, targeted delivery to glioblastoma cells via CD44-LNPs and bone marrow megakaryocytes via CD41-LNPs in vivo highlights the platform's versatility across distinct biological targets. Anti-CD41/CD61 CAR constructs have been designed and cloned, with surface expression and binding evaluations ongoing to prepare for subsequent in vivo functional studies.
Conclusion: Preliminary data confirming selective in vivo megakaryocyte targeting support the feasibility of this platform. By combining antigen-specific CAR-Treg generation with megakaryocyte-directed platelet restoration, our approach uniquely targets both pathological axes of ITP simultaneously. Unlike existing cytotoxic, ex vivo CAR-based strategies, our infusion-free, in vivo platform offers a more scalable, cost-effective route to antigen-specific tolerance, with broader applicability to other autoimmune cytopenias and transplant rejection.

Keywords: Immune Thrombocytopenia, CAR-Treg, Lipid Nanoparticles, Megakaryocytes, mRNA Delivery

PP123
Prime Editing-Mediated Disruption of GFP Expression: A Targeted Approach in HEK293T Cells

Mustafa Sami Ozaydin[1, 3], Hande Ozkan Coskun[1, 2], Gozde Korkmaz[1, 2]

[1] Koç University, Research Center for Translational Medicine (KUTTAM), Istanbul, Türkiye.
[2] School of Medicine, Koc University, Sariyer, İstanbul, Türkiye
[3] Istanbul University Aziz Sancar Institute of Experimental Medicine, Genetics, Istanbul, Türkiye.

Background/Aim: The Prime Editing (PE) system is a CRISPR-based precision genome editing platform composed of the Cas9 nickase (Cas9n) enzyme, a reverse transcriptase (RT) enzyme, and a prime editing guide RNA (pegRNA). This system introduces specific genetic changes directly at the target site without inducing double-strand DNA breaks. As a result, it facilitates precise point mutations, insertions, and deletions, while reducing the frequency of unwanted indels compared to genome editing platform methods. Recent studies have developed multiple PE variants by modifying the structural features of the RT enzyme within the PE system. The editing efficiencies of these variants differ significantly. This study involved the design, optimization, and functional analysis of the prime editing system using a model that introduced a stop codon into the GFP chromophore region.
Materials and Methods: Multiple PE variants, including PEmax, PE6a, PE6b, PE6c, PE6d, PE7, PE8max, and PE8c, were compared, and editing conditions were systematically optimized. Quantitative assessment of fluorescence reduction following editing was performed using flow cytometry, a plate reader, and fluorescence microscopy. Editing efficiency and cellular cytotoxicity were evaluated across the different PE variants.
Results: This research established a visual and quantitative reporter platform to systematically investigate the design parameters influencing prime editing efficiency.
Conclusion: The findings offer methodological advancements for modeling pathogenic nonsense mutations and for the development of precise genome editing strategies.

Keywords: Prime editing, stop codon, precision genome editing

PP124
Statistical Shape Analysis of Silica and Gold Nanoparticles: Relevance to Nanomedicine

Devrim Bilgili[1], Mayssam Najib [2], E. Yegan Erdem[2]

[1] University of North Florida
[2] Bilkent University
[3] Bilkent University

Background/Aim: Silica (SiO_2) and gold (Au) nanoparticles are widely investigated as platforms for molecular imaging, biosensing, targeted delivery, and other nanomedicine applications. Their biological performance can depend on morphology because particle size and shape influence surface characteristics and interactions with biomolecules. This study aimed to develop a direct statistical framework for characterizing nanoparticle morphology from transmission electron microscopy (TEM) images and to demonstrate its relevance to biological and molecular shape-analysis problems.
Materials and Methods: TEM images of silica and gold nanoparticles were analyzed using functional data analysis and statistical shape analysis. Particle contours were represented through the square-root velocity function (SRVF) framework. Geodesic distances were calculated between individual nanoparticles and between image frames. These distances were used to cluster nanoparticles according to morphological similarity, while deformation paths described the transformations required to align one particle boundary with another.
Results: The framework generated particle-level and frame-level measures rather than reducing each sample to a single indirect size estimate. Geodesic distances quantified morphological dissimilarity, clustering identified groups of similarly shaped particles, and deformation paths showed how one morphology changed into another. The image-based approach retained irregular and agglomerated particles that may be overlooked by conventional spectrometric summaries. The underlying geometric framework also provides a methodological connection to broader biological shape-analysis problems, including comparisons of molecular or protein conformations, although such biological structures were not analyzed in the present study.
Conclusion: SRVF-based shape analysis provides a direct and interpretable method for characterizing silica and gold nanoparticles. By quantifying morphology at the individual-particle level, the approach can support quality assessment of nanoparticle systems intended for biosensing, imaging, delivery, and other nanomedicine applications.

Keywords: Nanomedicine, Molecular imaging, Shape analysis, Gold nanoparticles, Targeted drug delivery

PP125
Modulatory Effects of Synthetic Bioregulatory Peptides Epithalon and Vilon on Bacterial Growth and Ultrastructural Morphology

Rimsha Kanwal[1, 2], Bilal Jawed[1, 2], Syed Khuram Zakir[1, 2], Maria Fortunato[2], Roberta Rossi[4], Matteo Botteghi[3], Pietro Guida[5], Giusi Romanazzi[5], Angela Linzalone[5], Stefano Martinotti[2], Elena Toniato[1]

[1] Centre of Advanced Studies and Technology, Department of Innovative Technology in Medicine and Dentistry, G. D'Annunzio University, ٦٦١٠٠ Chieti, Italy
[2] Unit of Clinical Pathology and Microbiology, Miulli General Hospital, ٧٠٠٢١ Acquaviva Delle Fonti, Bari, Italy
[3] Experimental Pathology Research Group, Department of Clinical and Molecular Sciences, Università Politecnica Delle Marche, 60126 Ancona, Italy
[4] University of Bari Aldo Moro, Bari, Italy
[5] Regional General Hospital "F. Miulli", ٧٠٠٢١ Acquaviva Delle Fonti, Bari, Italy

Background/Aim: Synthetic bioregulatory peptides modulate diverse cellular processes and have been investigated for their regulatory activities in mammalian systems; however, their interactions with microorganisms and potential effects on bacterial physiology remain poorly understood. Exploring peptide–bacteria interactions may provide new insights into how these molecules influence microbial growth and cellular behavior. This study aimed to investigate the concentration-dependent effects of Epithalon and Vilon on Gram-positive and Gram-negative bacteria and evaluate peptide-associated morphological and ultrastructural alterations.
Materials and Methods: Clinical isolates of Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Enterococcus faecium were exposed to Epithalon or Vilon (50–500 ng/mL), with physiological saline (0.9% NaCl) as the vehicle control. Bacterial growth was assessed by colony-forming unit (CFU) enumeration after 24 h in triplicate experiments. Treatment effects and concentration-dependent associations were evaluated using one-way analysis of variance (ANOVA) and Spearman's rank correlation, respectively. Morphological and ultrastructural changes were evaluated by scanning electron microscopy (SEM) and transmission electron microscopy (TEM) following bacterial exposure to 500 ng/mL Epithalon or Vilon for 1, 4, and 8 h.
Results: Significant differences among treatment groups were observed for E. coli ($p<0.0001$) and S. aureus ($p=0.0006$), but not for P. aeruginosa or E. faecium. Epithalon concentration positively correlated with growth of E. coli ($\rho=0.62$, $p=0.0059$) and S. aureus ($\rho=0.60$, $p=0.007$). Vilon also positively correlated with E. coli growth ($\rho=0.50$, $p=0.0197$). Preliminary TEM analysis of E. coli revealed time-dependent morphological and proliferative changes, with Vilon associated with reduced cell length and increased cell counts and division events at 8 h.
Conclusion: Epithalon and Vilon exhibited species- and concentration-dependent effects on bacterial growth, suggesting differential modulation of bacterial proliferation and physiology. These findings highlight previously underexplored peptide–microbe interactions. Ongoing quantitative TEM and SEM analyses will further characterize peptide-associated morphological and ultrastructural changes and help elucidate the mechanisms underlying these responses.

Keywords: Synthetic Peptides, Bacterial Growth, Peptide microbe interaction, Vilon, Epithalon

PP126
Screening Adhiron Molecules That Bind the BTB Domains of ZBTB Transcription Factors

Ilgın Akkaya[1], Görkem Odabaş[1], Batu Erman[1, 2]

[1] ١. Acibadem Mehmet Ali Aydinlar University, Graduate School of Natural and Applied Sciences, Department of Molecular Biology and Genetics, Istanbul, Türkiye
[2] ٢. Acibadem Mehmet Ali Aydinlar University, Faculty of Engineering and Natural Sciences, Department of Molecular Biology and Genetics, Istanbul, Türkiye

Background/Aim: ZBTB proteins are transcription factors that control critical biological processes. This highly conserved protein family is associated with multiple malignancies and a rare disease known as Immunodeficiency, Centromeric Instability and Facial Anomalies (ICF) syndrome resulting from the deficiency of ZBTB24. Deciphering molecular interactions of these ZBTB proteins can unravel on developmental processes in humans and pave the way of novel diagnosis and therapies. Adhirons are derived from pytocystatins and their small size, and thermostability make them attractive bio-reagents. By using molecular engineering and library selection, we aimed to develop adhirons with high binding specificity against ZBTB proteins that can be used for imaging, detection or molecular inhibition.
Materials and Methods: An adhiron library with randomized loops was transformed into Saccharomyces cerevisiae in a yeast surface expression vector. We screened this Adhiron library to find novel binders against the BTB domain of ZBTB24, using magnetic selection followed by cell sorting. We sequenced the binding clones and cloned them into a bacterial expression vector to produce and purify anti-ZBTB24 adhiron proteins. We performed surface plasmon resonance (SPR) experiments to confirm binding affinities.
Results: We screened and identified a novel clone binding to the ZBTB24 protein from sorted yeast populations. SPR experiments with Adhirons produced in E.coli and ZBTB24 confirmed a binding affinity around 50-600nM. We also screened and identified 5 yeast clones binding to C reactive protein (CRP) and will be producing and conducting SPR experiments to analyze binding affinities of these Adhirons against CRP.
Conclusion: In vitro selection of yeast display libraries can effectively and rapidly produce specific Adhirons binding diverse target proteins.
This project was funded by TÜBİTAK Grant 123Z458 (project no). Ilgın Akkaya is granted scholarship from TÜBİTAK BİDEB 2211A (application no: 1649B032408832).

Keywords: ZBTB Transcription Factors, ICF2, Adhiron, CRP

PP127
Establishment of a Recombinant Human PVRIG Platform for the Evaluation of PVRIG-Targeting Binder Proteins

Gizem Bulut[1], Alp Ertunga Eyüpoğlu[2], Ahmed Selim Üzüm[3], Türkan Haliloğlu[3], Batu Erman[1, 4]

[1] Department of Molecular Biology and Genetics, Graduate School of Natural and Applied Sciences, Acibadem Mehmet Ali Aydinlar University, İstanbul, Türkiye
[2] Department of of Molecular Biology and Genetics, Faculty of Arts and Sciences, Bogazici University, Istanbul, Türkiye
[3] Polymer Research Center, Bogazici University, İstanbul, Türkiye
[4] Department of Molecular Biology and Genetics, Faculty of Engineering and Natural Sciences, Acibadem Mehmet Ali Aydinlar University, İstanbul, Türkiye

Background/Aim: PVRIG (CD112R) is an inhibitory immune checkpoint receptor expressed on T and NK cells that negatively regulates antitumor immunity through interactions with the ligands CD112 and CD155. Together with TIGIT, CD96, and DNAM-1 (CD226), it contributes to the fine-tuning of immune responses within the tumor microenvironment. Given its immunosuppressive function, PVRIG has emerged as a promising target for cancer immunotherapy. This study aimed to generate computationally designed PVRIG-binding proteins and evaluate their interactions with recombinant PVRIG as potential immune check-point inhibitors.
Materials and Methods: PVRIG cDNA was amplified from Jurkat cells, and the extracellular domain (ECD) was cloned into the pcDNA3-IgG1 expression vector. The sequence-verified construct was transfected into HEK293 cells. Single-cell clones were screened by surface plasmon resonance (SPR) following Protein G capture, and recombinant protein was purified by Protein G affinity chromatography. The biological activity of the recombinant antigen was verified through its interaction with CD112 by SPR. DNA fragments encoding designed PVRIG binders were synthesized and cloned into the pET-GFP expression vector. Verified constructs were transformed into E. coli Rosetta (DE3) cells, and recombinant protein expression was induced with IPTG. Binder proteins were purified from lysates by immobilized metal affinity chromatography (IMAC) and are being evaluated for binding to recombinant PVRIG using SPR.
Results: Two stable HEK293 single-cell clones producing recombinant PVRIG-Fc were successfully established. Purified PVRIG-Fc retained its ability to bind CD112, confirming preservation of ligand-binding activity. In addition, computationally designed PVRIG-targeting binder proteins were successfully expressed in E. coli and purified for downstream characterization. Binding analyses are currently underway to evaluate their interaction with recombinant PVRIG-Fc.
Conclusion: The successful establishment of recombinant human PVRIG-Fc production and binder expression workflows provides a platform for the identification and characterization of novel PVRIG-targeting binders with potential immunotherapeutic applications.
This project was funded by TÜBİTAK grant 324S695

Keywords: PVRIG, Immune Checkpoint, De Novo Design Proteins, Cancer Immunotherapy, Surface Plasmon Resonance

PP128
Recombinant Production, Purification, and Structural Analysis of Enoyl-CoA Carboxylase/Reductase Enzyme of Ruminococcus champanellensis

Ece Akyüz[1], Hasan Demirci[1, 2, 3]

[1] Department of Molecular Biology and Genetics, Faculty of Science, Koç University, Istanbul, Türkiye
[2] Koç University Isbank Center for Infectious Diseases (KUISCID), Koç University, Istanbul, Türkiye
[3] Stanford PULSE Institute, SLAC National Laboratory, Menlo Park, CA, USA

Background/Aim: In May 2026, approximately 428.73 ppm of carbon dioxide (CO2) was measured over marine surface sites. It is 1.69 ppm higher than May 2025. Nature has various CO2 fixation mechanisms already exist, including Calvin-Benson cycle (CBC), the reductive tricarboxylic acid cycle, and the ethylmalonyl-CoA pathway. RuBisCO is one of the enzymes that works in CBC to convert non-biodegradable CO2 to biologically active carbon. Despite fully understood mechanism of RuBisCO, there is a need to develop and improve new mechanisms that are both efficient and fast. For this reason, the Enoyl-CoA carboxylase/reductase (ECR) protein family has attracted attention for its rapid mechanisms. However, ECR's mechanisms remain poorly understood. Understanding the structures of the proteins involved in the mechanism will shed light on this mechanism. The aim of the project is to recombinantly express, crystallize, and analyze the structure of the ECR protein from Ruminococcus champanellensis (ECR-RC).
Materials and Methods: Recombinant expression of ECR-RC was performed in E. coli, and ECR-RC protein was purified by affinity chromatography. In addition to these experiments, the oligomeric state of the ECR-RC was analyzed. More than 3000 conditions were tested in different concentrations, temperatures, and co-crystallization conditions. In addition, predicted ECR-RC structures were produced by using the AlphaFold and analyzed in silico.
Results: Recombinant ECR-RC production and purification were performed successfully. Despite crystallization trials, no ECR-RC crystal can be formed. From SEC experiments, ECR-RC can be found in both dimeric and tetrameric forms. Residual differences between other deposited ECR enzymes from different spices were compared.
Conclusion: ECR-RC exists in both dimeric and tetrameric forms, in contrast to reported ECRs in the literature, which exhibit only tetrameric or dimeric conformations. Understanding the structures of ECR enzymes from different species can improve our understanding of their mechanisms. By the way, new industrial carbon dioxide capturing mechanisms can be discovered.

Keywords: Carboxylase,, Carbon Dioxide,, Structural Anaylses

PP129
Recombinant Production, Purification, and Structural Analysis of Enoyl-CoA Carboxylase/Reductase Enzyme of Streptomyces Filamentosus

Necati Atalay[1], Hasan Demirci[1, 2, 3]

[1] Department of Molecular Biology and Genetics, Faculty of Science, Koç University, Istanbul, Türkiye
[2] Koç University Isbank Center for Infectious Diseases (KUISCID), Koç University, Istanbul, Türkiye
[3] Stanford PULSE Institute, SLAC National Laboratory, Menlo Park, CA, USA

Background/Aim: Use of fossil fuels and deforestation, which began with the Industrial Revolution, are increasing atmospheric CO2 levels. Rising CO2 levels are causing global warming. Although various measures are being taken to reduce CO2 levels, no significant decrease has been observed. Therefore, the need to investigate new methods has emerged. Enoyl-CoA carboxylase/reductase (ECR) enzymes were discovered in the last decade and are the CO2 fixation enzymes with the highest known turnover rate. These enzymes catalyze the reduction of enoyl-CoA derivatives to alkylmalonyl-CoA esters. ECRs operate on average 10 times faster than RuBisCO, which performs CO2 fixation in plants. The mechanisms underlying the high speed and activity of ECRs remain poorly understood. ECRs are classified into two categories: primary and secondary. Primary ECRs have high turnover rates and provide substrates for energy production pathways. Secondary ECRs operate more slowly and contribute to polyketide production. This study aims to purify, crystallize, and analyze the structure of a secondary ECR enzyme from Streptomyces filamentosus (ECR^{Sf}).
Materials and Methods: ECR^{Sf} sequence was cloned into the pET-16b vector with an N-terminal HisTag and expressed recombinantly in E. coli. It was purified using Ni-NTA affinity chromatography. Size-exclusion chromatography (SEC) was performed to determine its oligomeric state. Sitting-drop microbatch under-oil crystallization screening was performed for apo and cofactor-bound states across nearly 3000 conditions at various temperatures. Since crystals could not be obtained, ECR^{Sf} structure was predicted via AlphaFold 3 Multimer and analyzed.
Results: ECR^{Sf} was obtained with high purity. SEC analysis showed that it forms a tetrameric assembly. Structural analysis revealed differences at critical residues involved in substrate specificity, binding, and stabilization.
Conclusion: Crystallization attempts should be made with the substrate, as some regions cannot be stabilized without a substrate. Structures of different ECR enzymes should be solved and analyzed to clarify their mechanisms.

Keywords: CO2, Enoyl-CoA Carboxylase/Reductases, Structural Biology

PP130

LNP-Mediated Programming of Peripheral Factory Cells to Deliver Engineered Extracellular Vesicles Across the Blood-Brain Barrier for Spinal Muscular Atrophy

Melis Erginsoy[1, 2, 3], Ali Çalışkan[1, 2, 3], Ferdi Oğuz[1, 2, 3], Asmar Aydamirli[1, 2, 3], Aleyna Kaya[1, 2, 3], Alişan Kayabölen[1, 2, 3]

[1] Translational Advanced Research on Gene Engineering & Therapeutics (TARGET) Lab, Koç University School of Medicine, ٣٤٤٥٠ Istanbul, Turkey
[2] Koç University Research Center for Translational Medicine (KUTTAM), ٣٤٤٥٠ Istanbul, Turkey
[3] Koç University, Rumelifeneri Yolu, 34450 Sarıyer, İstanbul, Türkiye

Background/Aim: Spinal muscular atrophy (SMA) is a rare neurodegenerative disease caused by SMN1 mutation, leading to motor neuron loss; current FDA-approved treatments cost millions of dollars annually and it has limited effect. Lipid nanoparticles (LNPs) offer non-viral delivery but are limited by hepatic tropism, especially across the blood-brain barrier (BBB). This project aims to enable therapeutic protein delivery to motor neurons by reprogramming accessible cells into in vivo "factories": LNP-transfected cells express engineered exosomal fusion constructs secreting engineered extracellular vesicles (eEVs) carrying therapeutic mRNAs, allowing sustained production and biological barrier bypass. We identify receptor–ligand pairs enhancing BBB transcytosis, then engineer exosomal release constructs for neuronal targeting.
Materials and Methods: T7-promoter plasmids encoding exosome-enriched scaffolds (Lamp2b, CD9, CD63) were designed, fused extracellularly with BBB-targeting peptides (RVG, TAT, TAT-HA2, Angiopep-2, TGN). Cytosolic domains were optimized using GFP and Cre recombinase, with therapeutic fusions incorporating cleavable linkers (BACE1, mMaple3). All combinations were cloned and encapsulated into LNPs via in vitro transcribed mRNA.
Results: CD44- and CD71-antibody-conjugated LNPs substantially increased BBB endothelial cell targeting relative to untargeted controls. In SH-SY5Y neuroblastoma cells, RVG-conjugated LNPs produced modest uptake, while Angiopep-2-conjugated LNPs showed significantly higher uptake. RVG-fused exosomal constructs (Lamp2b, CD9, CD63-GFP) were expressed in HEK293T cells, and collected exosomes were applied to U87 glioblastoma, undifferentiated, and RA-differentiated SH-SY5Y cells (n=3). The RVG-CD63-GFP construct achieved the highest uptake among scaffolds, outperforming CD9 and Lamp2b variants. Uptake trends were consistent across glioblastoma and neuronal-lineage cells, suggesting broad applicability of the CD63-based platform for CNS-directed exosome delivery.
Conclusion: These findings establish a modular, scaffold-optimized platform for BBB-targeted eEV delivery. Future work will incorporate SMN-directed therapeutics and validate transport in transwell and in vivo SMA models.

Keywords: Lipid Nanoparticles, Targeted Delivery and Therapy, Extracellular Molecules, Spinal Muscular Atrophy,

PP131
Suppression of the G2/M–E2F Mitotic Program Constrains Neuronal Differentiation in SMN-Deficient Neural Progenitor Cells

Zeynep Büşra Aksoy[1], Ekin Doğan[2], Manolya Gündoğdu[2], Recep Uyar[3], Ebru Kocakaya[4]

[1] Ankara University, Stem Cell Institute, Ankara, Turkey
[2] Ankara University, Institute of Health Science, Ankara, Turkey
[3] Ankara University, Genetic Department, Institute of Forensic Medicine, Ankara, Turkey
[4] Ege University, Graduate School of Natural and Applied Sciences, Department of Biotechnology, İzmir, Turkey
[5] Ege University, Department of Bioengineering, İzmir, Turkey

Background/Aim: Spinal muscular atrophy (SMA) is a progressive motor neuron disease; however, current findings indicate that transcriptional dysregulation begins much earlier during the neural progenitor cell (NPC) stage. This study aimed to comprehensively elucidate how SMA disrupts the proliferative program and alters the differentiation trajectory of these progenitor cells.
Materials and Methods: To investigate these early disruptions, we performed a comprehensive differential expression analysis between iPSC-derived SMA and healthy control NPCs. This was integrated with Gene Ontology and transcription factor target enrichment analyses. Additionally, rMATS alternative splicing analysis was conducted to identify splicing defects, and RT-qPCR profiling of a stage-specific neural marker panel was utilized to validate transcriptomic findings and assess developmental alterations.
Results: Analysis revealed that cell cycle genes were strongly and directionally affected; the mitotic (G2/M) module was coordinately repressed, with significant down-regulation of 14 out of 23 detected mitotic regulators, including cyclins (CCNB1, CCNB2), CDC20, and mitotic kinases (PLK1, AURKA). Conversely, G1 cyclins (CCND1, CCND2) were upregulated while RB1 decreased, indicating a pathological redistribution away from mitotic progression. Enrichment analyses confirmed a significant loss of E2F and TP53-driven proliferative outputs, while NFkB-linked stress and inflammation targets (TNF, TNFRSF10A, FAS) were actively induced. Furthermore, rMATS identified mis-splicing in key cell cycle regulators (e.g., RBL2, ATM, ATR, CDK6, CDK7), reflecting a broader genome-wide spliceopathy. RT-qPCR validation demonstrated a marked reduction in the self-renewing marker SOX2 and mature pan-neuronal markers (TUBB3, MAP2), alongside an abnormal increase in early lineage-specification markers (PAX6, OLIG2, ChAT), while the baseline marker Nestin remained unchanged.
Conclusion: SMA NPCs do not merely undergo a simple proliferative shutdown. Instead, they exhibit a mitotically restricted, NFkB-primed progenitor state that becomes trapped in an initiated but ultimately incomplete defective differentiation program. This pathological trajectory directly compromises the orderly expansion and successful maturation of the neural progenitor pool.

Keywords: Spinal Muscular Atrophy (SMA), Cell Cycle Dysregulation, Neural Progenitor Cells (NPCs), Differentiation Trajectory, Spliceopathy

PP132
Mitochondrial Targeted Therapy Rescues Bioenergetic Deficits Prior to Motor Neuron Degeneration in Spinal Muscular Atrophy

Ekin Doğan[1, 2], Zeynep Büşra Aksoy[1], Manolya Gündoğdu[1, 2], Ebru Kocakaya[3], Kamil Can Akçalı[4]

[1] Ankara University Stem Cell Institute
[2] Ankara University Institute of Health Science
[3] Ege University Department of Biotechnology
[4] Ankara University Faculty of Medicine Department of Biophysics

Background/Aim: Spinal muscular atrophy (SMA) is a genetic neuromuscular disorder characterized by survival motor neuron (SMN) protein deficiency followed by motor neuron degeneration, loss, and severe muscle atrophy. While motor neurons are the primarily affected cell type in SMA, mitochondrial defects start during neuroectodermal development. This study aims to investigate early metabolic deficits in induced pluripotent stem cell (iPSC)-derived neural progenitor cells (NPCs) and to evaluate the therapeutic potential of the FDA-designated drug, mitochondria-stabilizing peptide Elamipretide (SS-31).
Materials and Methods: We analyzed the transcriptomic profiles of iPSC-derived NPCs generated from SMA patients and controls using bulk RNA sequencing. SMA NPCs were treated with Elamipretide to investigate its capacity to alleviate bioenergetic deficits by quantifying changes in ROS generation, mitochondrial membrane potential (MMP), mitochondrial dynamics (fission/fusion), fatty acid oxidation, and autophagy.
Results: SMA NPCs exhibited severe differentiation deficits, accompanied by marked downregulation of key neural progenitor (SOX1, PAX6, NES) and neuronal differentiation markers (MAP2, TUBB3) compared to controls. Bulk RNA-seq analysis unveiled significant bioenergetic impairment in SMA NPCs, defined by compromised fatty acid oxidation (CPT1A, ACADL), elevated glycolysis (PFKP), and marked mitochondrial stress. Functional evaluation confirmed this bioenergetic deficit, characterized by severe MMP dissipation and increased ROS production. Elamipretide treatment reversed this dysfunction, restoring mitochondrial capacity in SMA.
Conclusion: These results indicate that metabolic and bioenergetic defects emerge early during neurogenesis in SMA models, prior to mature motor neuron degeneration. Overall, Elamipretide rescues early bioenergetic defects in SMA neurogenesis, highlighting its therapeutic potential.

Keywords: Spinal Muscular Atrophy (SMA), Neural Progenitor Cells (NPCs), Mitochondrial Dysfunction, Transcriptomics

PP133
Investigation of Impaired Lipid Metabolism and Insulin Resistance in SMA IPSC-Derived Hepatocytes

Manolya Gündoğdu[1, 2], Ekin Doğan[1, 2], *Zeynep Büşra Aksoy[1]

[1] Ankara University, Stem Cell Institute, Ankara, Turkey
[2] Ankara University, Institute of Health Science, Ankara, Turkey

Background/Aim: Spinal muscular arophy (SMA) is a progressive neuromuscular disease; however, metabolic and hepatic abnormalities, are increasingly recognized. This study aimed to show lipid metabolism abnormalities and status of insulin response in induced pluripotent stem cell (iPSC)-derived hepatocyte like cells (iHEPs).
Materials and Methods: We differentiated control and SMA iPSC into mature iHEPs as an in vitro model, and examined lipid accumulation, and insulin-response. To assess lipid accumulation, iHEPs were examined by qRT-PCR (PLIN2-SPAST) and BODIPY-C11 staining. Cellular ROS levels were measured by CellRox assay. PPARα, LPL, PPARY, and C/EBPα expression levels were assessed to show impaired lipid metabolism. To eliminate, ER-stress-mediated lipid accumulation, PERK, IRE1, and ATF4 expression were examined. FOXO1, PCK1, and G6PC gene expressions were analysed by qRT-PCR.
Results: We confirmed successful hepatic differentiation in both groups, with downregulation of pluripotency markers (SOX2, NANOG, OCT4) and upregulation of hepatocyte markers (ALB, SERPINA1, HNF4A, AFP). PLIN2 expression was significantly higher and SPAST expression was significantly lower in SMA iHEPs. SMA iHEPs showed increased ratio of non-oxidized: oxidized lipids and increased levels of cellular ROS. ER-stress markers did not show any expressional changes between groups. SMA iHEPs also exhibited reduced PPARα mRNA and decreased LPL, PPARg, and C/EBPα protein levels, indicating impaired lipid metabolism. The differential expression changes of FOXO1, PCK1, and G6PC genes point the autonomous insulin resistance in SMA iHEPs.
Conclusion: SMA iHEPs demonstrated impaired lipid metabolism, lipid accumulation, and insulin resistance. As, ER-stress markers did not change, we eliminate the possible ER-stress-mediated lipid accumulation. The possible PPARα-mediated impaired lipid metabolism, and disrupted insulin response of SMA hepatocyte models were firstly showed in this study. This iPSC-based model successfully recapitulates the dysregulated lipid metabolism in SMA.

Keywords: Spinal Muscular Atrophy (SMA), iPSC, Hepatocyte Differentiation, Lipid Accumulation, Insulin Signaling

PP134
Unraveling the Role of NLRP7 Deficiency in Modulating Cellular Response to Genotoxic Insults

Ayşe Hülya Şanal[1], İlke Süder[2], Tamer Önder[3], Nesrin Erkol[4]

[1] Department of Molecular Biology and Genetics, Bogazici University, Istanbul, Turkey
[2] Department of Molecular Biology and Genetics, Bogazici University, Istanbul, Turkey
[3] School of Medicine, Koç University, Istanbul, Turkey
[4] Department of Molecular Biology and Genetics, Bogazici University, Istanbul, Turkey

Background/Aim: NLRP7 is a maternal-effect gene in which biallelic mutations of NLRP7 cause hydatidiform mole (HM), a gestational disease characterized by excessive trophoblast proliferation in the absence of proper embryonic lineages [1][2]. KHDC3L, another gene implicated in HM, functions in genomic stability, raising the possibility that NLRP7 acts through a similar mechanism [3]. Early human embryos are frequently aneuploid; as lineages segregate, aneuploid cells are eliminated via p53-mediated apoptosis in the epiblast (EPI) and primitive endoderm (PE), whereas the trophectoderm (TE) is relatively resistant [4]. We hypothesized that NLRP7 deficiency impairs genomic stability surveillance in early embryos, and investigated whether TE is selectively favored while EPI/PE are eliminated by apoptosis, or whether EPI and TE fail to form altogether.
Materials and Methods: Wild-type (WT) and NLRP7-mutant, patient-derived HM induced pluripotent stem cells (iPSCs) were used as an in vitro model. Genomic instability was induced using the DNA-damaging agents etoposide and hydroxyurea, complementing prior doxorubicin data. DNA damage and apoptotic responses were assessed by Western blot for phosphorylated γH2AX (p-γH2AX), phosphorylated p53 (p-p53), p53, cleaved caspase-3, and cleaved PARP1, and confirmed by immunofluorescence (IF) for p53 and p-γH2AX.
Results: With doxorubicin, WT cells upregulated p53 and underwent apoptosis, while HM cells upregulated p53 without apoptosis. Etoposide treatment increased p-γH2AX and p-p53 levels more prominently in HM than WT cells by Western blot, while cleaved caspase-3 and PARP1 cleavage were lower in HM cells compared to WT cells, indicating impaired apoptosis despite greater DNA damage. Immunofluorescence analysis confirmed stronger p53 and p-γH2AX signals in HM cells. Hydroxyurea treatment reproduced these findings.
Conclusion: NLRP7-deficient cells accumulate DNA damage and activate p53 but fail to execute apoptosis, supporting a role for NLRP7 in genomic stability surveillance during early embryogenesis, potentially underlying TE survival and abnormal lineage composition in HM.

Keywords: NLRP7,, hydatidiform mole,, genomic instability,, p53,, early embryogenesis

PP135
SIK2-Mediated Regulation of Self-Renewal in Human Adipose-Derived Stem Cells

Kaan GÜRKAN[1], Mehmet Soner TÜRKÜNER[1], Ferruh ÖZCAN[1]

[1] Department of Molecular Biology and Genetics, Gebze Technical University, Kocaeli, Turkey

Background/Aim: Adipose-derived stem cells (ADSCs) are multipotent progenitor cells that play an essential role in maintaining the balance and regeneration of adipose tissue. Self-renewal of ADSCs is regulated by many signalling pathways, such as PI3K/AKT, Wnt/β-catenin, Notch, and Hippo pathways. Salt-inducible kinase 2 (SIK2) is a serine/threonine kinase that has been widely reported to have functions in energy metabolism, cell cycle, and cytoskeleton rearrangement. Given the active crosstalk between SIK2 and these major signalling pathways, SIK2 might act as an integrative regulator of self-renewal potential of ADSCs. Therefore, this study aims to investigate the possible engagement of SIK2 in ADSC self-renewal and stemness.
Materials and Methods: Human ADSCs will be genetically modified using lentiviral vector systems for obtaining SIK2 knockdown, overexpression, and also its kinase inactive forms. In parallel, the pharmacological inhibition of endogenous SIK2 with its specific inhibitor HG-9-91-01 will be conducted. SIK2 modifications and its activity will be assessed by specific antibodies using immunoblotting assays. Cellular proliferation rate and Cell Cycle profile will be estimated using Flow Cytometry analysis and EdU incorporation assay, respectively.
Results: The successful development of genetic and pharmacological SIK2-regulated ADSC models will be proven. Modulation of SIK2 expression is expected to change fundamental proliferation ability, influence DNA replication rate as shown by EdU, and control protein markers responsible for stem cells maintenance.
Conclusion: Identification of multiple regulatory functions of SIK2 in ADSCs will contribute to our knowledge about mechanisms of stem cells maintenance. This research can draw attention to SIK2 as a new target for tissue engineering and obesity-induced regeneration disorders.

Keywords: SIK2,, ADSC,, Self-Renewal,, Cell Cycle

PP136
Investigating the Contribution of YAP/Hippo Signaling to BMP4-driven Trophoblast Differentiation in an NLRP7-mutant Hydatidiform Mole Model

Hatice Serra Berilgen[1], İlke Süder[1], Ferhat İlbasmış[1], Nesrin Erkol[1]

[1] Department of Molecular Biology and Genetics, Boğaziçi University, Istanbul, Türkiye

Background/Aim: Hydatidiform mole (HM) is a gestational trophoblastic disease characterized by excessive trophoblast development and abnormal embryogenesis. Loss-of-function mutations in the maternal-effect gene NLRP7 are a major cause of recurrent biparental complete HM. Our previous studies using NLRP7-mutant induced pluripotent stem cells (iPSCs) demonstrated heightened BMP4 pathway activation, resulting in accelerated trophoblast differentiation. However, the upstream mechanism responsible for BMP4 activation remains unclear. Since YAP/Hippo signaling regulates trophectoderm specification and shares downstream targets with BMP4 signaling, we investigated whether it contributes to BMP4 pathway activation in HM.
Materials and Methods: Wild-type and NLRP7-mutant iPSCs were differentiated toward the trophoblasts via inhibition of activin/nodal and FGF pathways. YAP–TEAD interaction was inhibited with Verteporfin or TED-347 following dose optimization. Trophoblast differentiation was evaluated by qPCR and western blot analyses of pluripotency (OCT4) and trophoblast (CDX2 and KRT7) markers, together with morphological assessment. In addition, immunofluorescence staining was performed to examine YAP1 subcellular localization during differentiation.
Results: YAP inhibition modestly reduced the expression of trophoblast markers, particularly CDX2, but did not prevent trophoblast differentiation. OCT4 expression progressively decreased under all differentiation conditions, while morphological and protein analyses confirmed continued trophoblast commitment. Immunofluorescence suggested increased nuclear YAP1 accumulation in HM cells during differentiation; however, nuclear localization became evident only after ~24 h, whereas BMP4 pathway activation has previously been reported within the first 6–8 h. This temporal dissociation suggests that BMP4 activation precedes YAP1 nuclear translocation.
Conclusion: Pharmacological inhibition of YAP–TEAD interaction was insufficient to block trophoblast differentiation in NLRP7-mutant iPSCs. Together with the delayed nuclear localization of YAP1 relative to BMP4 activation, our findings suggest that YAP/Hippo signaling is unlikely to initiate BMP4 pathway activation and may instead function downstream of BMP4 during trophoblast differentiation. These findings may guide future studies investigating early signaling events and the role of YAP/Hippo signaling in NLRP7-associated disease.

Keywords: Hydatidiform Mole, NLRP7, BMP4 pathway, YAP/Hippo Signaling

PP137
Investigation of the Role of ING1 in Neuroectoderm Differentiation of Mouse Embryonic Stem Cells

Deniz AK[1], Nihal TERZİ ÇİZMECİOĞLU[1]

[1] Middle East Technical University

Background/Aim: Neuroectoderm (NE) differentiation is essential during embryonic development. Although epigenetic regulation plays an essential role in stem cell differentiation, the functions of many epigenetic factors remain unclear. In a previous CRISPR/Cas9 screen, Inhibitor of Growth 1 (ING1) was identified as a candidate gene required for the NE differentiation of mouse embryonic stem cells (mESCs). ING1 has been implicated in chromatin regulation through SIN3A/HDAC complex as well as DNA damage response, apoptosis, and cell-cycle regulation from cancer studies. However, its role during early neural differentiation remains unknown, and whether observed phenotype is mediated by SIN3A/HDAC-dependent/independent mechanisms has yet to be determined. This study aimed to investigate the role and molecular mechanisms of ING1 during NE differentiation.
Materials and Methods: ING1 knockout (ing1Δ) mESC line was generated using CRISPR/Cas9. Wild-type and ing1Δ mESCs were differentiated toward NE and endoderm lineages. To investigate SIN3A/HDAC-independent functions of ING1, protein levels of p53, proliferating cell nuclear antigen (PCNA), cleaved caspase-3, and γ-H2AX were analyzed by Western blot at pluripotency and during NE differentiation. Furthermore, interactions between ING1 and HDAC1/2 proteins were examined by co-immunoprecipitation (Co-IP).
Results: ING1 loss impaired NE differentiation, whereas endoderm differentiation remained unaffected. Western blot analyses revealed no significant differences in p53, PCNA, cleaved caspase-3, γ-H2AX levels between wild-type and ing1Δ cells, suggesting that DNA damage response, apoptosis, and p53 stabilization are unlikely to explain the phenotype. Co-IP experiments demonstrated interactions between ING1, SIN3A, HDAC1, and HDAC2 in pluripotent mESCs.
Conclusion: Our findings indicate that ING1 is required for proper NE differentiation of mESCs. The differentiation defect observed in ing1Δ cells is unlikely to result from SIN3A-independent functions of ING1 and may instead involve SIN3A/HDAC-dependent mechanisms. Transcriptomic analyses will further elucidate the molecular pathways regulated by ING1 during early neural differentiation. This work was supported by METU GAP-108-2023-11378 and TUBITAK 125Z418.

Keywords: ING1, Neuroectoderm differentiation, Mouse Embryonic Stem Cells, SIN3A/HDAC Complex

PP138
SETD1B-dependent H3K4me3 Dynamics Regulate Neuroectoderm-related Gene Expression During ESC Differentiation

Çağla Almina ÇINAR[1], Emre BALBAŞI[1], Nihal TERZİ ÇİZMECİOĞLU[1]

[1] Middle East Technical University

Background/Aim: SETD1B, a histone methyltransferase within the COMPASS complex, catalyzes histone H3 lysine 4 trimethylation (H3K4me3). Its loss causes neuroectoderm differentiation (NE-DIF) defect. Our RNA-sequencing shows that SETD1B loss causes downregulation of neuroectoderm-related genes. However, how these transcriptomic changes relate to SETD1B-dependent H3K4me3 alterations remains unclear. Therefore, we aimed to determine H3K4me3 dynamics in pluripotent and neuroectoderm-differentiated WT and setd1bΔ ESCs using chromatin immunoprecipitation sequencing (ChIP-seq) and compare it with RNA-seq to associate H3K4me3 alterations with transcriptomic changes.
Materials and Methods: WT and setd1bΔ mESCs were cultured in 2i medium and subjected to NE-DIF. Cells were collected at both pluripotent and NE-DIF stages, and H3K4me3 ChIP-seq was performed. Sequencing data were processed using a standard ChIP-seq analysis pipeline to identify H3K4me3-enriched regions. ChIP-seq findings were validated by ChIP-qPCR using primers targeting H3K4me3 peaks, while transcription end sites (TES) were used as negative controls to test the success of ChIP. Differentially expressed genes (DEGs) obtained from RNA-seq were compared with differential ChIP-seq peaks through Venn diagram analyses to assess overlap between chromatin and transcriptomic changes.
Results: ChIP-seq quality was confirmed by expected enrichment of H3K4me3 at transcription start sites relative to TESs, and peaks were validated by ChIP-qPCR. Setd1b loss caused a locus-specific, rather than genome-wide, reduction in H3K4me3 levels. 439 H3K4me3 differential peaks were identified as unique to WT neuroectoderm differentiated cells. Also, 27% of these differential H3K4me3 peaks overlapped with DEGs. Importantly, gene ontology analysis of the overlapping genes revealed significant enrichment for neuroectoderm differentiation-related processes, including brain development and neuron differentiation. This overlap supports a link between SETD1B-dependent H3K4me3 deposition and the transcriptional defects observed during NE-DIF.
Conclusion: These findings suggest that SETD1B plays a critical role in neuroectoderm differentiation by regulating the expression of neuroectoderm-related genes through H3K4me3 deposition. This project was supported by TÜBA-GEBİP.

Keywords: SETD1B, H3K4me3, Neuroectoderm differentiation, ChIP-seq, Embryonic stem cells

PP139
Identifying the Role of SETD1B in Neuroectoderm Differentiation of Mouse Embryonic Stem Cells

Emre Balbaşı[1], Dersu Sezginmert[1], Ahmet Emre Özdemir[1], Burçak Otlu Sarıtaş[1], Nihal Terzi Çizmecioğlu[1]

[1] Middle East Technical University, Ankara

Background/Aim: Embryonic stem cells (ESC) provide a valuable platform for the research of early mammalian development due to their pluripotency and self-renewal properties. They can differentiate to primary germ layers in vivo and in vitro under strictly controlled conditions. Epigenetic control is one of the major governing mechanisms of early ESC differentiation. SETD1B, a H3K4 methyltransferase, is identified through CRISPR/Cas9 pooled screening as one of the important epigenetic/chromatin factors for neuroectoderm differentiation of mouse ESCs (mESCs). SETD1B is associated with several neurodevelopmental malignancies in humans including mental retardation, epilepsy, and autism. However, the role of SETD1B in neurodevelopment remains elusive.
Materials and Methods: CREPI, a custom single guide RNA (sgRNA) library containing more than 300 chromatin and epigenetic factors, was employed to determine the factors important for neuroectoderm differentiation of mESCs. SETD1B, a H3K4 methyltransferase of the COMPASS complex, was identified. The role of SETD1B in neuroectoderm differentiation of mESCs was investigated by CRISPR/Cas9 mediated deletion of Setd1b and subsequent RNA-sequencing during neuroectoderm differentiation time-course. Transcriptomic changes were analyzed to yield affected biological processes and pathways.
Results: Loss of SETD1B leads to decreased neuroectoderm marker gene expression. Time-course RNA-sequencing revealed that the loss of SETD1B results in aberrant expression of endoderm markers during neuroectoderm differentiation, but endoderm differentiation remains unaffected.
Conclusion: Our data indicate that SETD1B may play an important role for the lineage choice between neuroectoderm and endoderm. This work was supported by L'Oréal-UNESCO "For Women in Science" program, TUBİTAK (ARDEB-1001 Project, 123Z952), and METU-BAP (TEZ-D-108-2023-11379).

Keywords: SETD1B, mESC, neuroectoderm, endoderm, differentiation

PP140
The Role of Divergent Disordered Domains in the Developmental Activity of Bicoid

Dilara Temizkan[1], Dilan Akdoğan[1], Demir Erensoy[1], Pınar Önal[1]

[1] İhsan Doğramacı Bilkent University, Ankara, Turkey

Background/Aim: Transcription factors are composed mostly of structured and conserved DNA binding domains and activator domains, which are usually disordered with limited conservation. Their functions vary and are not completely defined. Bicoid (Bcd) is a well-known transcription factor of Drosophila that is composed of a DNA binding Homeodomain and several disordered activator domains with different chemical composition. Even though Bcd and its Homeodomain are thoroughly investigated, how these divergent disordered domains contribute to embryonic development and DNA binding specificity remains poorly understood.
Materials and Methods: To investigate the role of disordered domains on development, we generated several mutant flies expressing truncated Bcd variants. We evaluated their roles on development using quantitative phenotypic analyses, molecular analyses of the Bcd-target gene expression patterns of the early mutant embryos and thermal stress survival assays with viable mutants.
Results: Our findings demonstrate that different activator domains have distinct developmental functions. Some deletion mutants showed a wild-type-like phenotype at mild temperatures and gene expression patterns suggesting redundancy within the disordered activator domains. However, they showed decreased survival rates at elevated temperatures, implying that some domains confer robustness. Most deletions resulted in maternal-effect caused lethality, where homozygous females produced non-viable offspring with diverse larval phenotypes. Some showed severe head defects, others lacked thoracic or abdominal segments, and there were some that displayed combined defects.
Conclusion: Our results indicate that disordered activator domains are essential for transcriptional regulation and robust development conferred by Bcd. Additionally, the functional redundancy observed in some of the mutants suggests that these disordered regions provide the structural plasticity necessary to drive transcription factor evolution forward. Taken together, these findings emphasize the role that divergent disordered domains play in transcriptional activation during development, pointing out that they may function as specific regulatory modules across transcription factors in general.

Keywords: Bicoid, Drosophila melanogaster, transcriptional regulation, IDRs

PP141
Development of Chemical-Induced Pluripotent Stem Cell Production Methods

Hasan Barışhan Çolak[1, 2], Tamer Önder[1, 2]

[1] Koç University School of Medicine, Istanbul, Turkey
[2] Koç University Research Center for Translational Medicine, Istanbul, Turkey

Background/Aim: Induced pluripotent stem cells (iPSCs) are derived from somatic cells through reprogramming, and hold potential for the treatment of degenerative diseases. However, current reprogramming methods are not efficient and have genetic risks. Generating iPSCs through chemicals could address these limitations, but the epigenetic barriers that constrain such approaches remain poorly understood. This project seeks to establish a high-efficiency, safe, chemical route to iPSC generation by systematically mapping and overcoming these epigenetic obstacles, with particular attention to BRD9. This project is funded by Tubitak 1001 grant 125Z332.
Materials and Methods:
Chemical reprogramming of fibroblast cells was performed using a three-stage rapid CR protocol. The role of BRD9 as a reprogramming barrier was assessed pharmacologically and genetically. Reprogramming efficiency was monitored by TRA-1-60 flow cytometry and gene expression analysis across defined time points was examined by RT-qPCR. Furthermore, a genome-wide epigenetics-focused CRISPR/Cas9 screen (EPIKOL library) will be performed to identify genes restricting chemical reprogramming.
Results: The effect of chemical reprogramming on gene expression in fibroblast cells revealing that cells underwent mesenchymal-to-epithelial transition (MET), expressed intermediate plastic state genes and showed increased expression of pluripotency genes during CR. Subsequent TRA-1-60 flow cytometry analysis confirmed the generation of iPSCs following CR. Protocol optimization further identified the most favorable conditions for reprogramming. Additionally, the effect of BRD9 depletion on genetic reprogramming was examined and compared with its effect on chemical reprogramming.
Conclusion: Chemical reprogramming of fibroblasts proceeded through the expected stages toward pluripotency, and the resulting iPSCs were confirmed by flow cytometry under optimized protocol conditions. BRD9 depletion enhanced chemical reprogramming efficiency, consistent with its role as a barrier in genetic reprogramming, suggesting that BRD9 acts as a obstacle across reprogramming strategies. The planned CRISPR screen will help identify further regulators of chemical reprogramming, moving toward safer and more cost-effective iPSC production protocols.

Keywords: Stem Cells, Chemically Induced Pluripotent Stem Cells (ciPSC), Reprogramming, BRD9, CRISPR Screen

PP142
Identifying the Cell Death Status of 8-Cell-Like Cells and Their Maintenance

Eren Ozturk[1, 2], Arda Odabas[1, 2], Tevfik Tamer Onder[1, 2]

[1] Koç University School of Medicine, Istanbul, Turkey
[2] Koç University Research Center for Translational Medicine, Istanbul, Turkey

Background/Aim: Rare cells resembling human 8-cell blastomeres have been identified within naïve human pluripotent stem cell (PSC) cultures and are referred to as 8-cell-like cells (8CLCs). They comprise only 0.5–1% of naïve PSC cultures and are characterized by activation of 8-cell/ZGA-associated genes, including TPRX1 and LEUTX, as well as cleavage-stage transposable elements. However, 8CLCs are extremely transient, difficult to maintain, and rapidly lost after prospective isolation. Therefore, this study aimed to investigate alterations in cellular stress responses and cell death mechanisms during 8CLC induction and identify conditions that support their maintenance in a homogeneous, self-renewing state.
Materials and Methods: A naïve human PSC line was engineered to express GFP downstream of the endogenous TPRX١ gene and was used to identify and monitor ٨CLCs. To investigate whether reduced cell viability of the ٨CLCs could be rescued, we tested pharmacological inhibitors targeting major cell death pathways. In addition, CRISPR/Cas-٩mediated knockout of TP٥٣ was established to investigate the contribution of p-٥٣dependent mechanisms. Western blot analysis was performed to determine apoptosis-related markers.
Results: 8CLC viability was not rescued by pharmacological inhibition of necroptosis, ferroptosis or parthanatos. In contrast, the pan-caspase inhibitors Q-VD-OPh or Emricasan, as well as the JNK inhibitor JNK-IN-8, significantly improved viability while preserving TPRX1 positivity, an effect similarly observed upon TP53 depletion. Moreover, Western blot analysis confirmed the induction of apoptosis by demonstrating the cleavage of caspase-3 and PARP1.
Conclusion: These findings identify caspase-dependent cell death as a major barrier to 8CLC maintenance, although they do not yet establish which upstream pathway induces it. Defining the cellular stress responses associated with 8CLC induction may help identifying the signals responsible for caspase activation. Targeting these upstream mechanisms could provide a strategy to improve 8CLCs survival and facilitate their maintenance in a more stable and homogeneous state.

Keywords: 8-cell-like cells, totipotency, cell death

PP143
PTU as an Active Modifier of Sodium Fluoride–Induced Developmental Toxicity in Zebrafish

Begüm Yaşar[1], Nuray Söğünmez Erdoğan [2]

[1] Boğaziçi University
[2] Kadir Has University

Background/Aim: Fluoride exposure is widespread through drinking water, foods, beverages, infant formula prepared with fluoridated water, and dental products. Although beneficial for caries prevention, excessive exposure during development can damage mineralized tissues and has raised concern about neurodevelopmental outcomes in children. Zebrafish embryos are sensitive models for these effects, yet pigmentation is routinely suppressed with 0.003% PTU, which inhibits thyroid hormone synthesis and activates autophagy. As these pathways also contribute to fluoride toxicity, we investigated whether PTU modifies NaF-induced developmental and cardiac toxicity in a timing-dependent manner.
Materials and Methods: Embryos were exposed from 1–5 dpf to 50–200 mg/L NaF alone or with 0.003% PTU, alongside controls. Survival and heart rate were monitored to 5 dpf. Survival was analyzed by Fisher's exact tests with Holm correction. Pilot qPCR assessed sp7 expression. At 125 mg/L NaF, exposure conditions were changed at 72 hpf by NaF washout, PTU removal after earlyco-exposure, or late PTU addition.
Results: NaF significantly reduced survival from 125 mg/L onward. At matched doses, PTU improved survival at 125, 150, and 200 mg/L, with odds ratios of 2.58, 27.58, and 20.41, respectively (Holm-adjusted $p \leq 0.0002$). NaF reduced heart rate, whereas PTU co-treatment preserved it, with a significant treatment-by-dose interaction ($p < 0.01$). In pilot qPCR experiments, NaF+PTU treatment restored sp7 expression relative to NaF treatment, resulting in an approximately 1.92-fold increase compared with NaF-treated embryos. Among 90 embryos exposed to 125 mg/L NaF, survival was 8.9% after 3 dpf NaF washout, 6.7% after 3 dpf PTU removal, and 0% after 3 dpf PTU addition.
Conclusion: PTU modified NaF toxicity, improving survival and cardiac function only during concurrent exposure. Its timing-dependent effects and transcriptional changes support modulation through shared pathways, identifying PTU as an active floride-toxicity modifier rather than an inert depigmenting agent.

Keywords: Sodium floride toxicity, propylthiouracil (PTU), zebrafish, developmental biology

PP144

Pharmacological Inhibition of Porcupine by LGK-974 Attenuates WNT-Driven Fibrotic and Inflammatory Signaling in Human Fibroblast and Fibrosarcoma Cells

Dilara Nur Kılıç[1], Yunus Güngörmez[1], Ayşe Koçak Sezgin[1]

[1] Kütahya Health Sciences University

Background/Aim: Aberrant activation of WNT signaling is a key molecular event in the initiation and progression of tissue fibrosis by promoting fibroblast activation, extracellular matrix accumulation, and persistent inflammatory responses. Porcupine inhibitors suppress the secretion of WNT ligands and have emerged as promising therapeutic candidates for the treatment of fibrotic diseases. However, the molecular effects of LGK-974 on apoptosis, inflammatory mediators, TGF-β signaling, and WNT ligand expression remain incompletely characterized.
This study aimed to investigate the effects of LGK-974 on cell viability, apoptosis-associated genes, inflammatory cytokines, TGF-β production, and WNT ligand expression in different fibroblast-related cell models.
Materials and Methods: IC_{50} values of LGK-974 were determined in WEHI, CCD-1135, and HT1080 cells using a cell viability assay. Cells were subsequently treated with the selected inhibitory concentration of LGK-974. The mRNA expression levels of BCL2, BAX, TP53, WNT1, WNT3, WNT5A, WNT7A, WNT7B, WNT10A, and WNT10B were quantified by quantitative real-time PCR. Secreted levels of IL-6, IL-1β, and TGF-β were measured using ELISA. Statistical analyses were performed to compare treated and untreated groups.
Results: Treatment with LGK-974 significantly reduced inflammatory mediator production in fibroblast and fibrosarcoma cell models, as evidenced by decreased IL-6 and IL-1β levels. A concomitant reduction in TGF-β secretion further supported suppression of profibrotic signaling. Expression analysis demonstrated consistent downregulation of multiple WNT ligands, including WNT1, WNT3, WNT5A, WNT7A, WNT7B, WNT10A, and WNT10B, confirming effective inhibition of WNT pathway activity. Although LGK-974 induced changes in the expression of apoptosis-related genes (BCL2, BAX, and TP53), these alterations were not statistically significant. Collectively, LGK-974 primarily modulated WNT-dependent inflammatory and fibrotic signaling rather than apoptosis.
Conclusion: LGK-974 effectively attenuated WNT-dependent inflammatory and profibrotic signaling in fibroblast and fibrosarcoma cells through suppression of WNT ligand expression and TGF-β production. These findings support Porcupine inhibition as a promising therapeutic strategy for WNT-driven fibrotic disorders.

Keywords: fibrosarcoma, WNT signaling, LGK-974

PP145
Immunological Basis of Tuberculosis Development in Older Adults

Anastasia Dmitrieva[1], Elizaveta Asekritova[1], Olga Chelnokova[1], Natalia Skrypnik[1]

[1] Federal State Budgetary Educational Institution of Higher Education "Yaroslavl State Medical University" Of the Ministry of Healthcare of the Russian Federation, Yaroslavl, Russia

Background/Aim: Aging is associated with immunosenescence and inflammaging, characterized by impaired immune regulation and declining functional reserve. These changes may impair control of Mycobacterium tuberculosis and facilitate endogenous reactivation of latent infection. Frailty and comorbidity may further aggravate immune and hematopoietic dysfunction, contributing to severe disease and reduced diagnostic sensitivity of skin-based immune tests. This study aimed to investigate immunological and geriatric factors associated with tuberculosis progression in adults aged over 60 years and compare the diagnostic value of the recombinant tuberculosis allergen skin test and T-SPOT.TB.
Materials and Methods: A prospective observational study included patients aged over 60 years with tuberculosis. Clinical, radiological, laboratory and geriatric parameters were assessed, including tuberculosis form, bacteriological status, diagnostic delay, post-tuberculosis changes, calcifications, frailty and hematological indices. The skin test was performed in 195 patients, while T-SPOT.TB was performed in 20 newly diagnosed patients.
Results: Disseminated and infiltrative tuberculosis and tuberculoma predominated. Bacteriological positivity was observed in 45.9% of patients, while diagnostic delay exceeding one month occurred in 59.1%. Frailty was associated with a more severe course: bacteriological positivity occurred in 61.5% versus 41.7% of patients without frailty, and subtotal pulmonary involvement in 62.5% versus 2.9%, respectively. Frail patients more frequently had leukopenia (35.3% vs. 7.2%), lymphopenia (35.3% vs. 7.2%), thrombocytopenia (15.7% vs. 5.2%) and anemia (70.6% vs. 30.8%), indicating reduced hematopoietic and immune reserve. The skin test was positive in 33.3% of patients, whereas T-SPOT.TB was positive in all tested patients. Negative skin-test results increased with age.
Conclusion: The interaction of immunosenescence, inflammaging, frailty and reduced hematopoietic reserve may contribute to tuberculosis reactivation and severe disease. The high frequency of negative skin-test results may reflect age-related physiological immunodeficiency associated with immunosenescence. T-SPOT.TB may provide greater diagnostic value in older adults.

Keywords: Tuberculosis, immunosenescence, inflammaging, frailty, T-SPOT.TB

PP146
Priority of Molecular Genetic Methods for Mycobacterium Tuberculosis Identification in the Aging Organism

Elizaveta Asekritova[1], Elizaveta Andreeva [1], Anastasia Dmitrieva [1], Olga Chelnokova [1], Natalia Skrypnik [1]

[1] Federal State Budgetary Educational Institution of Higher Education "Yaroslavl State Medical University" Of the Ministry of Healthcare of the Russian Federation, Yaroslavl, Russia

Background/Aim: Despite global improvements in the tuberculosis epidemiological situation, the proportion of patients aged over 60 years is increasing. Tuberculosis in older adults is frequently diagnosed late, with advanced disease and bacteriological positivity. Age-related physiological immunodeficiency, comorbidity and geriatric syndromes may complicate timely diagnosis. This study aimed to evaluate the role of molecular genetic methods (MGMs), including polymerase chain reaction (PCR), in tuberculosis diagnosis among patients aged over 60 years.
Materials and Methods: A prospective observational study included 300 patients aged over 60 years with tuberculosis. Clinical, radiological, laboratory and geriatric parameters were assessed, including tuberculosis form, bacteriological status, bacillary load and frailty. The diagnostic value of MGMs was evaluated in the context of delayed tuberculosis detection and age-related clinical features.
Results: Disseminated (32.7%) and infiltrative (26.7%) pulmonary tuberculosis predominated. Bacteriological positivity was detected in 45.9% of patients. Among bacteriologically confirmed cases, PCR-based MGMs identified Mycobacterium tuberculosis in 100% of cases, compared with 67.6% by microscopy and 94.1% by culture. A high bacillary load was observed in 62.1% of patients of senile age. Frailty was associated with a more severe course: bacteriological positivity occurred in 61.5% versus 41.7% of patients without frailty, while subtotal pulmonary involvement occurred in 62.5% versus 2.9%, respectively ($p<0.01$).
Conclusion: MGMs, including PCR, play a leading role in tuberculosis diagnosis in older adults. Their use may be particularly informative in early disease and low bacterial burden, when microscopy may be insufficiently sensitive. MGMs should be considered for tuberculosis screening in older adults, especially frail and mobility-limited patients.

Keywords: tuberculosis, molecular genetic methods, Mycobacterium tuberculosis, aging, polymerase chain reaction

PP147
Investigation of the Effects of the Combined Use of Finerenone and Exenatide on Liver Tissue in Diabetic Nephropathy

Sevim Buse KARAOĞLU[1], Meliha KOLDEMİR GÜNDÜZ[2], Güllü KAYMAK[2], Elif AYDIN[3], Ayşe KOÇAK SEZGİN[2]

[1] Afyon Kocatepe University
[2] Kütahya Health Sciences University
[3] Ağrı İbrahim Çeçen University

Background/Aim: Type 2 diabetes mellitus (T2DM) is associated with liver injury characterized by oxidative stress, inflammation, and fibrosis. Finerenone, a selective mineralocorticoid receptor antagonist, and Exenatide, a glucagon-like peptide-1 (GLP-1) receptor agonist, exhibit anti-inflammatory, anti-fibrotic, and metabolic regulatory effects. However, their combined impact on diabetic liver injury remains unclear. This study aimed to investigate the molecular effects of Finerenone and Exenatide, administered alone and in combination, on liver tissue in a streptozotocin (STZ)-induced T2DM rat model.
Materials and Methods: Ethics committee approval has been obtained for this study (Ethics committee number: 2025.10.01). T2DM was induced in male Wistar albino rats using STZ. Animals were divided into control and treatment groups receiving Finerenone and/or Exenatide. Liver tissues were collected for biochemical and molecular analyses. Oxidative stress was assessed by measuring total antioxidant status (TAS) and total oxidant status (TOS). Gene expression levels of NRF2, TGF-β, a-SMA, collagen, adiponectin, SIRT1 were analyzed using quantitative real-time PCR (qPCR). Hepatic enzyme activities (ALP, ACP) were analyzed spectrophotometric assay. Cytokine levels of IL-1β, IL-6 and TNF-a protein experessions were determined using enzyme-linked immunosorbent assay (ELISA).
Results: STZ-induced diabetes caused significant hepatic oxidative stress, inflammation, and fibrosis, as evidenced by decreased TAS and increased TOS, ALP, ACP, IL-1β, IL-6, and TNF-α levels. Diabetic rats also showed increased TGF-β, α-SMA, and collagen expression together with reduced NRF2, SIRT1, and adiponectin expression. Both Finerenone and Exenatide ameliorated these alterations, while the combined treatment produced the greatest improvement by restoring antioxidant defenses, suppressing inflammatory cytokines, and reducing profibrotic gene expression compared with either monotherapy.
Conclusion: Combined Finerenone and Exenatide therapy provided greater hepatoprotection than either treatment alone by restoring antioxidant capacity, suppressing inflammatory responses, and inhibiting fibrotic signaling. These findings suggest that dual targeting of oxidative stress and fibrosis may represent a promising therapeutic approach for diabetic liver injury.

Keywords: T2DM, Liver Fibrosis, Finerenone, Exenatide

PP148
Phosphorylation-gated Nuclear Localization of the Inflammasome Adaptor Asc/pycard Defines a Non-canonical, Compartment-specific Function

Enes Sivri[1], Efe Elbeyli[1], Nesrin Erkol[1]

[1] Department of Molecular Biology and Genetics, Center for Life Sciences and Technologies, Bogazici University, Istanbul, Turkey

Background/Aim: ASC/PYCARD (apoptosis-associated speck-like protein containing a CARD) is classically defined as a cytoplasmic inflammasome adaptor. Although a nuclear pool has been reported, the signals governing its nucleocytoplasmic distribution and its inflammasome-independent function remain undefined. We aimed to map the residues and post-translational signal controlling ASC nuclear localization and to determine whether nuclear ASC forms a distinct subnuclear structure.
Materials and Methods: A panel of GFP-tagged ASC point mutants targeting the putative PYD nuclear export signal (L27, L32), a phospho-license residue (S157), and a CARD phosphosite (S164) was expressed in HEK293 cells and analyzed by confocal immunofluorescence for compartment and morphology. Nuclear export dependence was probed with an NES-insertion series. Subnuclear identity was assessed by co-staining with the nuclear body marker PML. In parallel, phospho-S164 behavior was analyzed in the CPTAC breast-cancer phosphoproteome (Mertins et al.) with Benjamini-Hochberg correction and single-site pathway enrichment.
Results: ASC nuclear accumulation required two independent inputs behaving as an AND-gate: a disabled NES (L32) together with a phospho-license (S157D), consistent with a dual-lock model. NES-insertion experiments revealed a position-specific nuclear-anchoring function separating L32 from L27. The CARD phosphomimetic S164D localized to the nucleus and formed discrete nuclear puncta that did not colocalize with PML, defining a PML-independent nuclear structure. In CPTAC, phospho-S164 was consistently elevated across tumors in a uniformly one-directional manner, with the strongest site-specific pathway associations (reduced WNT, elevated innate-immune signatures) at S164 relative to neighboring phosphosites.
Conclusion: ASC nuclear entry is governed by a phosphorylation-gated dual-lock mechanism, and phospho-S164 drives a PML-independent nuclear pool associated with a distinct pathway signature. These findings define a compartment-specific, inflammasome-independent dimension of ASC biology with potential relevance to tumor signaling.

Keywords: ASC/PYCARD; nucleocytoplasmic shuttling; phosphorylation; nuclear puncta; inflammasome-independent function

PP149
SH3-Interaction Network in ASC Protein: A Molecular-Level Analysis of Inflammation, Cytoskeletal, and Infection Dynamics

Ramazan Emre Terim[1], Efe Elbeyli[1], Nesrin Erkol[1]

[1] Department of Molecular Biology and Genetics, Boğaziçi University, Istanbul, Türkiye

Background/Aim: ASC is a central adaptor of inflammasome signaling that regulates inflammation and programmed cell death. However, the regulatory roles of PTMs at key ASC residues, particularly T154, Y144, and S195, remain poorly understood. The T154 region contains SH3-binding motifs and multiple potential phosphorylation sites, suggesting a role in cytoskeletal regulation and protein–protein interactions. This project aims to investigate how PTMs at these residues modulate ASC interactions with SH3 domain-containing proteins how these interactions affect inflammasome activation, host defense, and cytoskeletal dynamics.
Materials and Methods: gRNAs targeting the *PYCARD* gene were designed and cloned into lentiviral vectors. Lentiviral particles were produced by transient transfection of HEK293T cells and used to transduce Cas9-expressing THP-1 cells. GFP-positive cells were isolated by FACS, and successful ASC knockout was confirmed by Western blot. To generate ASC reconstitution constructs, ASC-mCherry was cloned into a miniTurbo expression vector. Site directed mutagenesis (SDM) was performed to generate ASC mutants. Following sequence verification, lentiviral particles carrying wild-type or mutant ASC-miniTurbo constructs will be produced and transduce ASC-knockout THP-1 cells, generating stable cell lines. Biotin-based proximity labeling will be performed, followed by immunoprecipitation of biotinylated proteins and mass spectrometry (MS) analysis to identify ASC-associated protein interaction networks.
Results: ASC-targeting gRNAs cloned and lentiviruses containing gRNA were produced and lentiviruses were transduced. Transduced THP-1 CAS9 cells were selected via FACS. ASC-knockout THP-1 cells, confirmed by Western blot analysis. ASC-mCherry cloning into the miniTurbo vector was confirmed by sequencing and transfection, and ASC mutants were generated by SDM.
Conclusion: This project provides a comprehensive framework to investigate phosphorylation-mediated regulation of ASC and its interaction networks. The findings are expected to reveal novel mechanisms linking inflammasome signaling, cytoskeletal dynamics, and host defense. Improved understanding of ASC-dependent regulatory pathways may contribute to the development of new diagnostic and therapeutic strategies for inflammatory, autoimmune, infectious, and cancer-related diseases.

Keywords: ASC/PYCARD, Inflammasome, Post-translational Modifications, Proximity Labeling, Protein–Protein Interactions

PP150
Investigation of the Effects of Helicobacter pylori Outer Membrane Vesicles on Proliferation and Cytokine Secretion of Splenocytes and Bone Marrow Derived Macrophages

Eliz Önbaş[1], Minel Horozlar[1], Pelin Su Gelmez[1], Zeynep Nur Şentürk[1], Ayşegül Ekmekçioğlu[1], Sinem Öktem Okullu[2], Ayça Sayı Yazgan[1, 3]

[1] Molecular Biology-Genetics and Biotechnology Department, Istanbul Technical University, Istanbul, Turkiye
[2] Faculty of Medicine, Department of Medical Microbiology, Acıbadem Mehmet Ali Aydınlar University, Istanbul, Turkiye
[3] Department of Biosciences, Brunel University of London, London, United Kingdom

Background/Aim: Helicobacter pylori (H. pylori) infection is a primary cause for gastritis, peptic ulcers, and gastric adenocarcinoma, with prevalence reaching approximately 80% within the Turkish population. Outer membrane vesicles (OMVs), which transport various biomolecules including bacterial proteins and lipopolysaccharides (LPS), represent a multifaceted platform for the development of novel immunization strategies. In this study, we isolated OMVs from clinical isolates obtained from Turkish patients, and evaluated their in vitro immunomodulatory effects on cellular proliferation and cytokine profiles in murine splenocytes and bone marrow-derived macrophages (BMDMs).
Materials and Methods: OMVs were purified from Turkish H. pylori clinical isolates via differential ultracentrifugation. Primary splenocytes, derived from C57BL/6 mice, and BMDMs were subjected to H. pylori OMV (HP1) stimulation for 24 and 48 hours, respectively. Adjuvant properties were assessed using K-CpG (0.3 and 1.0 μM), with LPS (10 μg/ml) as the positive control. Lymphocyte proliferation was determined through Carboxyfluorescein-succinimidyl-ester (CFSE) labeling and flow cytometric analysis. Concentrations of secreted cytokines, specifically interleukin-6 (IL-6), interferon-gamma (IFN-γ), and tumor necrosis factor-alpha (TNF-α), were measured using enzyme-linked immunosorbent assay (ELISA).
Results: KCpG stimulation induced robust splenocyte proliferation, reaching 84.9%, while significant increases were also observed following treatment with LPS and HP1 OMV. In BMDMs, all experimental stimulants (KCpG, LPS, HP1) elicited significant TNF-α secretion. Furthermore, LPS and low-dose KCpG (0.3 μM) significantly enhanced IL-6 secretion compared to the negative control. Within the splenocyte population, KCpG stimulation resulted in the highest IL-6 levels. Notably, while LPS, KCpG, and low-dose HP1 induced moderate IFN-γ production, high-dose HP1 (10 μg/ml) triggered a statistically significant elevation in IFN-γ secretion.
Conclusion: These findings suggest that LPS-enriched or adjuvant-supplemented OMVs possess significant potential as immunotherapeutic agents against H. pylori. Comprehensive in vivo investigations are required to further validate the therapeutic efficacy of these vesicle-based platforms.
This project was supported by TÜBİTAK. (Project Number: 22AG077)

Keywords: Outer Membrane Vesicles,, Helicobacter pylori,, Immune Response

PP151
What Are the Anti-Inflammatory Effects of Glp-1 Receptor Agonists for Weight Management in Non-diabetic Patients: A Systematic Review

Faisal Rashid Said Abusharkh[1], Maein Mare Mohammed Alshaykhi[1], Abdulrahman Alshawaf[1], Abdulaziz Qasem Ali Aljaboobi[1], Mohamed Salah Aldine Al Halabi[1], Yahya Almelibari[2], Abdullah Musaad Almatraf[3], Awatif jubran Hazazi[4]

[1] Istinye University,Istanbul.
[2] Ibn Sina National College. Saudi Arabia.
[3] King Abdulaziz University. Saudi Arabia
[4] University of Jeddah. Saudi Arabia.

Background/Aim: Obesity involves chronic low-grade inflammation, but whether glucagon-like peptide-1 receptor agonists (GLP-1 RAs) exert anti-inflammatory effects beyond weight loss remains uncertain. To synthesize evidence on inflammatory, immune, endothelial, and thrombotic responses to GLP-1–based therapy in obesity.
Materials and Methods: We narratively synthesized the supplied table's 23 abstracts (2008–2026). Ten reports described randomization; one additional report analyzed placebo-controlled trials, and most others were uncontrolled or observational studies. Populations included obesity with varied metabolic and inflammatory comorbidities. Outcomes included C-reactive protein (CRP), cytokines, adipokines, endothelial and fibrinolytic markers, proteomics, and immune-cell function. Search and risk-of-bias methods were unavailable.
Results: Samples ranged from 7 to a combined 4,225 participants. Exercise plus liraglutide reduced high sensitivity CRP by 43% versus placebo ($P = 0.03$), whereas liraglutide alone did not. Semaglutide showed 25%–43% high-sensitivity CRP reductions versus placebo, significant only at 0.2 and 0.4 mg/day and nonsignificant after weight-change adjustment. CRP decreased during uncontrolled liraglutide studies in hidradenitis suppurativa (4.5 to 3.0 mg/L; $P = 0.04$) and psoriasis (3.9 to 1.8 mg/L; $P < 0.0001$). Perioperative GLP-1 use was associated with smaller postoperative increases in CRP, interleukin-6, fibrinogen, D-dimer, and plasminogen activator inhibitor-1. Other reports described lower monocyte chemoattractant protein-1, selectins, or clot lysis time. Direct endothelial-function measures generally did not improve; growth differentiation factor-15 was unchanged in two studies; and a short-term semaglutide cohort found no changes in interleukin-6, tumor necrosis factor-α, resistin, or plasminogen activator inhibitor-1. Small mechanistic cohorts reported altered inflammatory proteomic pathways and improved natural killer-cell function. Results varied by population, drug, duration, comparator, and biomarker. Most outcomes were surrogate.
Conclusion: GLP-1–based therapy may improve selected inflammatory and atherothrombotic markers in obesity, but inconsistent findings do not establish a direct effect independent of weight loss. Larger randomized trials using standardized biomarkers and mediation analyses are needed.

Keywords: GLP - receptor agonist

PP152

Investigation of the Effects of Helicobacter ylori Outer Membrane Vesicles on the Viability and IL-10 Secretion of Splenocytes and Bone Marrow-Derived Macrophages

Minel Horozlar[1], Eliz Önbaş[1], Pelin Gelmez[1], Zeynep Nur Şentürk[1], Ayşegül Ekmekçioğlu[1], Sinem Öktem Okullu[2], Ayça Sayı Yazgan[1, 3]

[1] Istanbul Technical University, Department of Molecular Biology and Genetics, Istanbul, Türkiye
[2] Acıbadem Mehmet Ali Aydınlar University, Faculty of Medicine, Department of Basic Medical Sciences, Department of Medical Microbiology, Istanbul, Türkiye
[3] Brunel University of London, Department of Biosciences, Uxbridge UB٣ ٨PH, London, England

Background/Aim: Helicobacter pylori (H. pylori) is a Gram-negative pathogen critical in chronic gastritis, peptic ulcers, and gastric cancer [1]. With an 80% prevalence in the Turkish population, an effective clinical vaccine is lacking. Outer membrane vesicles (OMVs), 100-300 nm nanoparticles secreted by Gram-negative bacteria, carry bioactive cargos like proteins and lipopolysaccharides [2]. Serving as mediators of bacterial communication and virulence, OMVs offer a versatile platform for therapeutic applications due to their immunostimulatory properties [2]. In this study, OMVs were purified from an H. pylori strain obtained from Turkish patient isolates, and the immunomodulatory effects of these vesicles on splenocyte's and bone marrow derived macrophage's (BMDM) viability and interleukin 10 (IL-10) cytokine secretion were investigated in vitro.
Materials and Methods: OMVs were isolated via ultracentrifugation from Turkish H. pylori isolate. Splenocytes and BMDMs from C57BL/6 IL-10-EGFP mice were incubated with isolated OMVs (0.4-2-10 µg/ml for splenocyte, 0.4 µg/ml for BMDM) for 48 hours, using 10 µg/ml LPS as a positive control. Following incubation, cell viability and IL-10 production were analyzed via flow cytometry using 7-AAD, Zombie Aqua staining, and evaluating IL-10-GFP signals. The IL-10 cytokine secretion was further confirmed using ELISA.
Results: Splenocyte examination revealed a significant 24% increase in cell viability following stimulation with HP1 OMV. IL-10 assessments via flow cytometry and ELISA yielded comparable outcomes, demonstrating that HP1 administration increased IL-10 GFP production by approximately 10%. In the case of BMDM cells, however, the OMVs did not affect cell viability but increased IL-10 secretion by nearly 5%.
Conclusion: Findings indicate that lipopolysaccharide-containing OMVs from H. pylori can serve as potential therapeutic tools. These promising in vitro results should be validated through comprehensive future in vivo studies.

Keywords: Helicobacter pylori,, Outer membrane vesicles,, İmmune response,, Interleukin-10

PP153
Development of an ex vivo Expansion and Genetic Modification Process for Human Natural Killer Cells

Beyza Nur Uysak[1], Betül Çakıcı[1], Elif Çelik[2], Alp Ertunga Eyüpoğlu[3], Tolga Sütlü [1, 2, 4]

[1] Acıbadem Mehmet Ali Aydınlar University, Graduate School of Natural and Applied Sciences, Department of Molecular Biology and Genetics, Istanbul
[2] Acıbadem Mehmet Ali Aydınlar University, Graduate School of Health Sciences, Department of Translational Medicine, Istanbul
[3] Boğaziçi University, Institute for Graduate Studies in Science and Engineering, Department of Molecular Biology and Genetics, Istanbul
[4] Acıbadem Mehmet Ali Aydınlar University, Faculty of Engineering and Natural Sciences, Department of Molecular Biology and Genetics, Istanbul

Background/Aim: Natural Killer (NK) cells hold massive potential for cellular immunotherapies due to their human leukocyte antigen (HLA)-independent cytotoxicity and serial killing capabilities. However, their clinical application is frequently blocked by two major bottlenecks: low cell count and resistance to genetic modification. This study aims to overcome these limitations by developing an efficient protocol for the ex vivo expansion and lentiviral transduction of primary NK cells.
Materials and Methods: To enhance ex vivo proliferation, various K562 feeder cell lines were genetically engineered from wild-type (WT) K562 cells to express distinct costimulatory ligands (CD70, CD80, CD86) and membrane bound cytokines (IL-15, IL-21). From healthy donors, primary human NK cells were isolated and co-cultured with these engineered feeders in the presence of different cytokine combinations (interleukin [IL]-2, IL-15, and IL-21). Concurrently, multiple lentiviral gene delivery parameters such as small molecule inhibitors ((5Z)-7-Oxozeanol [OXO] and BX-795), different multiplicity of infections (MOI), and feeder cell support were systematically assessed to optimize transduction efficiency.
Results: Co-culturing NK cells with CD70- and CD80-expressing feeder cells, combined with all three cytokines, resulted in significantly higher expansion rates over a 14-day culture period compared to alternative combinations. In lentiviral transduction optimization assays, increasing MOI above 10 provided no significant benefit to transduction efficiency. Furthermore, utilizing OXO in place of BX-795 drastically affected transduction rates. Evaluating post-transduction feeder supplementation revealed that CD70-expressing K562 cells yielded the most efficient transduction among the tested feeders.
Conclusion: Combining specific engineered feeder cells and cytokine cocktails with an optimized lentiviral transduction protocol successfully enables high-yield ex vivo expansion and efficient transgene expression in primary NK cells, overcoming key barriers to their clinical utility.
Acknowledgements: This project is supported by 2247 - A National Leading Researchers Program (Project No: 123C511).

Keywords: Natural killer cells, ex vivo Expansion , Lentiviral Transduction , K562 feeder cells, Cancer immunotherapy

PP154
Scalable Production of Bioactive Recombinant Human Interleukin-2 in E. coli Using an Industrial Pipeline

Erdem Bozluolcay[1, 6], Elif Çelik[2, 5], Beyza Nur Uysak[1], Alp Ertunga Eyüpoğlu[4], Pelinsu Albey[5], Tolga Sütlü[1, 2, 3]

[1] ١. Acıbadem Mehmet Ali Aydınlar University, Graduate School of Natural and Applied Sciences, Department of Molecular Biology and Genetics, Istanbul, Turkiye
[2] ٢. Acıbadem Mehmet Ali Aydınlar University, Graduate School of Health Sciences, Department of Translational Medicine, Istanbul, Turkiye
[3] ٣. Acıbadem Mehmet Ali Aydınlar University, Faculty of Engineering and Natural Sciences, Department of Molecular Biology and Genetics, Istanbul, Turkiye
[4] ٤. Boğaziçi University, Institute for Graduate Studies in Science and Engineering, Department of Molecular Biology and Genetics, Istanbul, Turkiye
[5] ٥. Epsilon Elektronik San. Ve Tic. A.Ş., Istanbul, Turkiye
[6] ٦. MNT Tanı Ve Tedavi Hizmetler A.Ş., Istanbul, Turkiye

Background/Aim: Recombinant human Interleukin-2 (rhIL-2) is a critical cytokine required for the ex vivo expansion of NK and T cells for cell therapy. To establish a scalable manufacturing pipeline for research-grade rhIL-2, we optimized an upstream fed-batch bioprocess and downstream purification pipeline for His-tagged rhIL-2 expressed in Escherichia coli.
Materials and Methods: High-density fed-batch fermentation was conducted in a 10 L bioreactor with isopropyl -D-1-thiogalactopyranoside (IPTG) induction at stationary phase. After 22 hours, biomass was disrupted at 12,000 bar via high-pressure homogenization and treated with magnesium chloride () and deoxyribonuclease I (DNase I) to reduce deoxyribonucleic acid (DNA) contamination and viscosity. Inclusion bodies (IBs) were isolated and washed four times with 20 mM Tris-HCl (pH 8.0), 1% Triton X-100, 2M urea, and 1 mM dithiothreitol (DTT). IBs were solubilized using 6 M guanidine-HCl with 5 mM DTT, filtered (), diluted 1:1 to 3 M guanidine, and loaded onto HisTrap immobilized metal affinity chromatography (IMAC) column. On-column refolding was achieved via a 60-minute gradient removing guanidine-HCl before 500 mM imidazole was used for elution. Polishing and buffer exchange into phosphate-buffered saline (PBS) was performed via size-exclusion chromatography (SEC). Identity, purity, and function were evaluated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), Western blotting, starved NK-92 cell proliferation assays, cytotoxic T-lymphocyte line-2 (CTLL-2) potency testing, and enzyme-linked immunosorbent assay (ELISA) yield quantification.
Results: Fermentation achieved an optical density at 600 nanometers () of ~45 and wet biomass exceeding 50 grams per liter (g/L). The IMAC refolding and SEC cascade yielded soluble rhIL-2 with purity matching Proleukin. Functional assays demonstrated NK-92 proliferation and colony formation equivalent to Proleukin.
Conclusion: We established a scalable 10 L bioprocess combining high-pressure lysis, IB processing, on-coloumn refolding, and SEC polishing to yield bioactive rhIL-2.

Keywords: Interleukin-2, Inclusion body, Bioprocess engineering, On-column refolding, Affinity chromatography

PP155
Generating A Reporter Cell Line Carrying Mutant IL-1 Receptor

Fatma Sude Albayrak[1], Alp Ertunga Eyupoglu[2], Batu Erman[3]

[1] Acibadem Mehmet Ali Aydinlar University, Graduate School of Natural and Applied Sciences, Department of Molecular Biology and Genetics, Istanbul, Turkiye
[2] Bogazici University, Institute of Science, Departmant of Molecular Biology and Genetics, Istanbul, Turkiye
[3] Acibadem Mehmet Ali Aydinlar University, Faculty of Engineering and Natural Sciences, Department of Molecular Biology and Genetics, Istanbul, Turkiye

Background/Aim: The interleukin-1 (IL-1) signaling pathway orchestrates innate immunity through IL-1 receptor type I (IL-1RI) activation. A missense mutation (K131E) in the IL1RI gene replaces a positively charged lysine with a negatively charged glutamic acid, severely disrupting ligand interaction. Consequently, patients carrying this mutation fail to suppress inflammatory signaling using their endogenous IL-1 receptor antagonist (IL-1Ra) or its recombinant therapeutic counterpart, anakinra. This study aims to model this mutation in vitro using a reporter cell line to evaluate anakinra's reduced efficacy and investigate the therapeutic potential of a novel nanobody previously identified to bind a distinct epitope on IL-1RI.
Materials and Methods: The K131E mutation was introduced into HEK-Blue reporter cells using CRISPR-Cas9 and homology-directed repair. Following mutant clone validation, Quanti-Blue assays were performed to assess downstream signaling responses to IL-1β induction. We then evaluated and compared the inhibitory capacities of anakinra and our unique inhibitory nanobody, which recognizes different epitopes on the receptor.
Results: Mutated HEK-Blue cells successfully activated downstream signaling upon IL-1β induction. However, their response to anakinra was severely altered; as anticipated, anakinra failed to inhibit the signaling cascade due to the mutation at its primary binding site. Conversely, because our novel nanobody targets a distinct epitope on IL-1RI, it remained completely unaffected by the K131E mutation and successfully blocked receptor activation. This places our nanobody as a potential candidate for further therapeutic approaches even in patients with mutated IL1RI receptors.
Conclusion: Overall, the mutant reporter cell line effectively models clinical anakinra resistance. These findings demonstrate that alternative epitope targeting with nanobodies can overcome mutation-induced therapeutic limitations, supporting their potential as novel treatments for IL1RI-associated autoinflammatory diseases.

This project was funded by TÜBİTAK grant 20AG007 and TÜSEB grant 46728. The presenter is supported by a scholarship from TÜBİTAK under the 2210-E program.

Keywords: interleukin-1, CRISPR, nanobodies, autoinflammatory diseases

PP156
Comparative Single-Cell RNA Sequencing Analysis of Thymic and Peripheral Blood B-Cell Populations in Patients with Myasthenia Gravis

Elif Çelik[1], Betül Çakıcı[2], Alper Bülbül[3], Cemre Aktaş[4, 5], Salih Duman[6], Arman Çakar[7], Hacer Durmuş[7], Yeşim Parman[7], Berker Özkan[6], Tolga Sütlü[1, 2, 9], Güher Saruhan Direskeneli[5]

[1] Acıbadem Mehmet Ali Aydınlar University, Graduate School of Health Sciences, Department of Translational Medicine, Istanbul, Türkiye
[2] Acıbadem Mehmet Ali Aydınlar University, Graduate School of Natural and Applied Sciences, Department of Molecular Biology and Genetics, Istanbul, Türkiye
[3] Acıbadem Mehmet Ali Aydınlar University, Graduate School of Health Sciences, Department of Biostatistics and Bioinformatics, Istanbul, Türkiye
[4] Istanbul Technical University, Graduate School of Science and Technology, Department of Molecular Biology and Genetics, Istanbul, Türkiye
[5] Istanbul University, Istanbul Faculty of Medicine, Department of Physiology, Istanbul, Türkiye
[6] Istanbul University, Istanbul Faculty of Medicine, Department of Thoracic Surgery, Istanbul, Türkiye
[7] Istanbul University, Istanbul Faculty of Medicine, Department of Neurology, Istanbul, Türkiye
[8] Istanbul University, Istanbul Faculty of Medicine, Department of Pathology, Istanbul, Türkiye
[9] Acıbadem Mehmet Ali Aydınlar University, Faculty of Engineering and Natural Sciences, Department of Molecular Biology and Genetics, Istanbul, Türkiye

Background/Aim: Myasthenia gravis (MG) is a chronic autoimmune disease in which B-cell-derived autoantibodies impair neuromuscular transmission. Although the thymus plays a central role in MG pathophysiology, transcriptomic differences between thymic and peripheral blood B cells, and their signatures relative to healthy reference atlases, remain undefined. This study aims to map tissue-specific B-cell heterogeneity in MG patients at single-cell resolution.
Materials and Methods: Paired thymus and PBMC samples were obtained from four acetylcholine-receptor-antibody-positive MG patients undergoing thymectomy. Libraries were prepared with the 10x Genomics Chromium 5' assay and sequenced on NovaSeq 6000. Data were processed in Cell Ranger and analyzed in Scanpy after quality control (≥400 genes/cell, ≤10% mitochondrial reads). Harmony integration and Leiden clustering (resolution 1.0) were applied; B-cell subtypes were annotated by consensus of CellTypist, ScType and SingleR. Patient PBMC were benchmarked against chemistry-matched healthy reference donors from CELLxGENE Census.
Results: Of 45,711 QC-passing cells, 42,026 (92%) were B cells, grouped into 23 clusters. Thymic B cell clusters showed consistently elevated memory, class-switch, germinal-center and interferon-response programs relative to paired PBMC in all four patients. Surface proteins mediating B–T interaction and interferon signaling (CD58, SLAMF1, CD40, CD27, BST2) were reproducibly upregulated in thymic B cell clusters. Patient PBMC composition did not differ meaningfully from the healthy donors. Apparent gene-level differences traced to batch and ambient-RNA artifacts rather than disease biology.
Conclusion: These findings indicate thymic B cells in MG sustain a local germinal-center reaction under interferon tone, supporting the thymus as an active site of autoimmune B-cell activation, whereas peripheral B-cell composition closely resembles healthy individuals. These results point to thymus-derived pathways as candidate biomarkers in MG.
This project was supported by the TÜBİTAK 1001 Program (Project No: 123S090).

Keywords: Myasthenia gravis, B cells, single-cell RNA sequencing

PP157
Immunological Evaluation of STING and TLR9 Agonist-Loaded Polymeric Nanoparticles in Murine Primary Immune Cell Models

Sarehan Akyüz[1], Ebru Nur Ay[2], Rojia Houshang[3], Asmaa Maaz[4], Pınar Çakır Hatır[3,5], Banu Mansuroğlu[1], Günnur Deniz[6]

[1] Department of Molecular Biology and Genetics, Faculty of Arts and Sciences, Yildiz Technical University
[2] Department of Molecular Biology and Genetics, Faculty of Engineering and Natural Sciences, Istinye University
[3] Department of Biomedical Engineering, Faculty of Engineering and Natural Sciences, Istinye University
[4] Department of Biomedical Engineering, Kocaeli University
[5] Nanotechnology and Advanced Materials Research Center, Istinye University
[6] Department of Immunology, Aziz Sancar Institute of Experimental Medicine, Istanbul University, Istanbul, Türkiye

Background/Aim: Activation of the Stimulator of Interferon Genes (STING) and Toll-like receptor 9 (TLR9) pathways represents a promising strategy for immune modulation and cancer immunotherapy. Polymeric nanoparticles enable the intracellular delivery of immunostimulatory agonists. This study aimed to characterize type I interferon responses to polymeric nanoparticles loaded with the STING agonist cyclic di-AMP (c-di-AMP) and the TLR9 agonist CpG oligodeoxynucleotides (CpG ODN) in murine immune cell models.
Materials and Methods: Splenocytes isolated from C57BL/6 mice were treated with free or nanoparticle-loaded c-di-AMP [NP(AMP)] and CpG ODN [NP(CpG)], individually or in selected combinations, at different concentrations and periods. IFN-α and IFN-β levels were evaluated by enzyme-linked immunosorbent assay (ELISA). Bone marrow cells were differentiated into plasmacytoid dendritic cell (pDC)-like populations using Fms-like tyrosine kinase 3 ligand (FLT3L). Cell populations were characterized by flow cytometry using CD11c, B220, and PDCA-1, and cytokine responses after stimulation were evaluated by ELISA.
Results: Splenocytes exhibited treatment- and time-dependent type I interferon responses, with the clearest separation between selected formulations and untreated controls observed at 24 h. Across three independent biological experiments, NP(CpG) at 8 μg/mL resulted in a 1.39 ± 0.23-fold IFN-β response relative to untreated controls at 24 h (mean ± SD). In FLT3L-derived cultures, type I interferon responses varied according to agonist treatment and incubation time, with treatment-dependent IFN-α responses observed at both 12 and 24 h. Flow cytometric analysis identified a pDC-like population, with PDCA-1$^+$CD11c$^+$ cells representing 12.61% of the B220$^+$ cell population.
Conclusion: Both splenocytes and FLT3L-derived pDC-like cells exhibited treatment- and time-dependent type I interferon responses. NP(CpG) at 8 μg/mL produced reproducible IFN-β responses in splenocytes across independent experiments. These findings support further evaluation of nanoparticle-mediated immune responses through lymphocyte proliferation and tumor cell co-culture assays.

Keywords: STING agonist, CpG oligodeoxynucleotide, polymeric nanoparticles, plasmacytoid dendritic cells

PP158

Harnessing 174xCEM.T2 and PBMC-Based Platforms for in vitro Expansion of Antigen-Specific T Cells: A Strategy for TCR Discovery in Cancer Immunotherapy

Serra Özarı[1, 2], Dilara Aslan[4], Tolga Sütlü[1, 3, 4]

[1] Acıbadem Mehmet Ali Aydınlar University, Faculty of Engineering and Natural Sciences, Department of Molecular Biology and Genetics
[2] Boğaziçi University, Institute for Graduate Studies in Sciences and Engineering, Department of Molecular Biology and Genetics
[3] Acıbadem Mehmet Ali Aydınlar University Graduate School of Health Sciences Department of Translational Medicine
[4] Acıbadem Mehmet Ali Aydınlar University Graduate School of Natural and Applied Sciences Department of Molecular Biology and Genetics

Background/Aim: T cell receptor (TCR) gene therapy is a promising immunotherapeutic strategy redirecting the activity of lymphocytes towards tumor antigens. Genes encoding TCR-alpha/beta pairs are introduced into T or NK cells, which are reinfused into patients to achieve clinical responses. In August 2024, FDA approved the first TCR-gene therapy targeting melanoma-associated antigen A4 for synovial sarcoma. This highlights the importance of discovering novel tumor-antigen reactive TCRs to develop targeted immunotherapies.
To discover novel TCRs using peptide-loaded 174xCEM.T2 cells and peripheral blood mononuclear cell (PBMC)-based stimulation systems for in vitro expansion of antigen-specific T cells, followed by paired TCR sequencing of single clones.
Materials and Methods: Transporter associated with antigen processing (TAP1/2)-deficient 174xCEM.T2 cells were loaded with major melanoma-associated and viral peptides. Alternatively, peptide sequences were ectopically expressed in 174xCEM.T2 cells using ER targeting signal sequences. $CD8^+$ T cells were isolated from HLA-A2+ donors. Mitomycin-C-treated, peptide-loaded 174xCEM.T2 cells were co-cultured with primary $CD8^+$ T cells to induce antigen-specific expansion. In parallel, peptide-pulsed PBMCs were used as stimulators in co-cultures with autologous responder PBMCs.
Results: Peptide-loading was confirmed with dose-dependent increases in the surface HLA-A2 expression in 174xCEM.T2 cells. Both T2 and PBMC-based co-cultures induced antigen-specific T cell activation and expansion. Rechallenging conditioned PBMCs with corresponding peptides enhanced $CD8^+$ T cell activation compared to unloaded and irrelevant peptide controls, confirming that recurrent exposure strengthens antigen-specific reactivity. Response magnitudes varied among donors, reflecting diverse baseline T cell repertoires. cDNA libraries from expanded antigen-specific T cell clones were prepared to identify paired TCR alpha/beta sequences.
Conclusion: The use of peptide-loaded 174xCEM.T2 cells alongside PBMC-based stimulation strategies effectively expands antigen-specific T cells, offering a feasible approach for discovering novel TCRs against selected epitopes. This antigen-presenting platform provides a valuable basis for the downstream identification of tumor-reactive TCRs.
(Supported by TÜBİTAK 1003, Project No: 218S897)

Keywords: T cell receptor (TCR), Cancer Immunotherapy, Antigen Presentation

PP159
Predicting Fingolimod Response in MS: The Role of IL7R Splicing and IL-7 Signaling

Meziyet Dilara Reda[1], Derin Kaprol[2], Verda Tekinbay[3], Abdullah ALper Bülbül[4], Eda Tahir Turanlı[1, 2]

[1] Department of Molecular Biology and Genetics, Graduate School of Natural and Applied Sciences, Acibadem Mehmet Ali Aydinlar University, Istanbul, Türkiye
[2] Department of Molecular Biology and Genetics, Faculty of Engineering and Natural Sciences, Acibadem Mehmet Ali Aydinlar University, Istanbul, Türkiye
[3] Department of Pharmacy, Faculty of Pharmacy, Acibadem Mehmet Ali Aydinlar University, Istanbul, Türkiye
[4] Department of Biostatistics and Bioinformatics, Graduate School of Health Sciences, Acibadem Mehmet Ali Aydinlar University, Istanbul, Türkiye

Background/Aim: Multiple Sclerosis (MS) is an immune-driven neurodegenerative disorder of the central nervous system. Treatments like Fingolimod (FTY720) reduce inflammation by causing the internalization of the S1PR1 receptor on T cells, trapping them in lymphoid tissues. Despite its efficacy, patient non-response is a major clinical hurdle, heavily influenced by underlying genetic variations and fluctuating cytokine levels. This study explores the mechanistic relationship between IL-7 signaling, the alternative splicing of the IL7Rgene, and S1PR1 surface expression in T cells. A key focus is how genetic polymorphisms (e.g., rs6897932) influence Exon 6 inclusion, shifting the ratio of the membrane-bound receptor (mIL7R) to the soluble decoy isoform (sIL7R).
Materials and Methods: We analyzed T-cell dynamics at defined experimental intervals. The expression profiles of IL7R variants were quantified using real-time PCR (qPCR), and flow cytometry was used to track changes in S1PR1 surface protein levels following exposure to the IL-7 ligand.
Results: Our findings reveal that exogenous IL-7 functions as a decisive molecular toggle. Exposure to the cytokine drastically reduces the transcription of the soluble isoform (sIL7R) and strongly favors the retention of the full-length, membrane-bound transcript (mIL7R). Simultaneously, IL-7 treatment leads to a profound decrease in cell-surface S1PR1 density.
Conclusion: Cytokine conditions and IL7R splicing behavior directly regulate S1PR1 surface expression. These findings highlight their strong potential as pharmacogenomic biomarkers, providing a clear mechanistic basis for identifying Fingolimod non-responders and tailoring personalized therapies in MS.

Keywords: Multiple Sclerosis, IL7R, Pharmacogenomics, Fingolimod

PP161
Beyond Antiviral Sensing: RIG-I in Hypoxia-Responsive Adaptation of Human Microglia

Yagmur Ozcelebi[1], Esra Kantar[1], Orhan Tat[1], Sueda Tepe[1], Sude Kara[1], Necip Ismet Beydogan[1], Ceren Ciraci[1]

[1] Istanbul Technical University, Faculty of Science and Letters, Department of Molecular Biology and Genetics, İstanbul, Türkiye

Background/Aim: Hypoxia represents a major microenvironmental challenge for microglia, requiring coordinated adaptations in cellular signaling and homeostasis. While the molecular mechanisms underlying hypoxic adaptation have been widely studied, the contribution of innate immune sensors to this process remained largely undefined. Retinoic acid-inducible gene-I (RIG-I) is a cytosolic RNA sensor classically associated with antiviral immunity, yet accumulating evidence suggests that its functions may extend beyond pathogen sensing. Nevertheless, the potential role of RIG-I in modulating hypoxia-induced cellular responses in microglia has yet to be clarified. In this study, we investigated the involvement of RIG-I in hypoxia-responsive signaling and cellular adaptation in human microglial HMC3 cells.
Materials and Methods: Chemical hypoxia was induced using cobalt chloride ($CoCl_2$) in HMC3 cells, and the cellular response was characterized by examining HIF-1α expression, AKT/mTOR signaling, autophagy, and pro-inflammatory cytokine responses. A range of $CoCl_2$ concentrations and exposure times was initially evaluated based on RIG-I and HIF-1α expression, after which an optimal condition was selected for subsequent analyses. To further investigate the contribution of RIG-I, its expression was downregulated using siRNA prior to hypoxic stimulation.
Results: $CoCl_2$ treatment induced HIF-1α expression without substantial loss of cell viability and was associated with suppression of AKT/mTOR signaling and increased autophagy. Notably, downregulation of RIG-I further increased HIF-1α expression under hypoxic conditions, while promoting AKT/mTOR signaling and reducing the autophagy response. It was also associated with alterations in pro-inflammatory cytokine expression.
Conclusion: Together, these findings suggest that RIG-I may contribute to the regulation of microglial adaptation to hypoxic stress through coordinated effects on HIF-1α, AKT/mTOR signaling, autophagy, and inflammatory responses. Collectively these findings provide evidence for a potential role of RIG-I beyond its canonical role in innate immune sensing.

Keywords: Hypoxia, RIG-I, Microglia

PP162
Identification of Cleavage Sites and Investigation of Caspase-8-Mediated Cleavage of NLRP13

Melike Bağlama[1], Elif Öykü Çakır[1], Nesrin Erkol[1]

[1] Department of Molecular Biology and Genetics, Bogazici University, Istanbul, Turkey

Background/Aim: NLRP13 is one of the least characterized members of the NOD-like receptor (NLR) family, and its molecular functions remain largely unknown. Previous studies have suggested that NLRP13 undergoes proteolytic cleavage following activation of caspase-8; however, the precise cleavage site(s) remain unidentified. This study aims to identify cleavage sites of NLRP13 and investigate caspase-8-dependent cleavage using a combination of cellular stimulation, pharmacological inhibition, and site-directed mutagenesis (SDM) approaches.
Materials and Methods: Candidate caspase-8 cleavage sites predicted from the NLRP13 sequence were subsequently targeted by SDM, and mutant NLRP13 constructs were evaluated for their susceptibility to proteolytic cleavage. To establish a controlled model of caspase-8 activation, HEK293FT cells were treated with TRAIL/TNFSF10 protein and pan-caspase inhibitor Z-VAD-FMK or a caspase-8-selective inhibitor. Treatment conditions were optimized by western blot analysis of caspase-8 activation. Following optimization, NLRP13 was expressed in HEK293FT cells and its cleavage was assessed under caspase-8 activation conditions.
Results: TRAIL-induced caspase-8 activation was evaluated in HEK293FT cells using different treatment conditions and pharmacological inhibitors. TRAIL treatment increased the levels of cleaved caspase-8 products, whereas treatment with Z-VAD-FMK or a caspase-8 inhibitor resulted in increased pro-caspase-8 levels and reduced cleaved caspase-8. NLRP13 cleavage will be investigated in THP-1 cells following LPS and nigericin stimulation. Some of the candidate cleavage sites within NLRP13 have not yielded significant results so far, but studies are still ongoing in other candidate cleavage sites.
Conclusion: This study aims to define the molecular mechanism underlying NLRP13 cleavage by caspase-8 and to identify the specific residues required for this proteolytic processing. Characterization of NLRP13 cleavage may provide new insights into the regulation of NLRP13 during inflammatory and cell death-associated signaling.

Keywords: NLRP13, Caspase-8, Proteolytic cleavage, Site-directed mutagenesis, Inflammasome

PP163

Production of nanobodies targeting interleukin-1 receptor type 1

Gizem Çile[1], Alp Ertunga Eyüpoğlu[2], Ahmed Selim Üzüm[3], Elif Güzel[4], Batu Erman[4], Sema Sırma Ekmekçi[5], Ahmet Gül[6], Eda Tahir Turanlı[4]

[1] Acibadem Mehmet Ali Aydinlar University, Graduate School of Natural and Applied Sciences, Department of Molecular Biology and Genetics, Istanbul, Turkey
[2] Bogazici University, Institute of Graduate Studies in Science and Engineering, Department of Molecular Biology and Genetics, Istanbul, Turkey
[3] Bogazici University, Faculty of Science, Department of Molecular Biology and Genetics, Istanbul, Turkey
[4] Acibadem Mehmet Ali Aydinlar University, Faculty of Engineering and Natural Sciences, Department of Molecular Biology and Genetics, Istanbul, Turkey
[5] Istanbul University, Aziz Sancar Experimental Medicine Research Institute, Istanbul, Turkey
[6] Istanbul University, Department of Internal Medicine Sciences, Istanbul, Turkey

Background/Aim: Interleukin-1 receptor type 1 (IL-1R1) is an innate immune receptor that plays a critical role in regulating inflammation. Functional impairments in the IL-1R1 signaling pathway can lead to inflammatory diseases. Currently, competitive inhibitors such as Anakinra (Kineret) are used as anti-inflammatory therapeutics to target this receptor. Nanobodies are recombinant antibody fragments derived from heavy-chain-only antibodies of camelids. They are attractive therapeutic candidates due to their small size, high stability, and specificity. This project aimed to produce nanobodies against IL-1R1 to develop alternative treatments for IL-1R1-mediated inflammatory diseases.
Materials and Methods: B-lymphocyte libraries obtained from llamas immunized with human IL-1R1 (hIL-1R1) were screened using the phage display method. A total of 136 different nanobody clones with the capacity to bind to hIL-1R1 were identified. These nanobodies were expressed in bacterial periplasmic systems and purified using Fast Protein Liquid Chromatography (FPLC). The binding affinities of the purified nanobodies were analyzed using Surface Plasmon Resonance (SPR) technology. Additionally, inhibitory activities of these high-affinity nanobodies were analyzed in cell culture experiments. Finally, as a preliminary step before proceeding to mouse studies, a cell line co-expressing human IL-1R1 and mouse IL-1R accessory protein (IL-1RAcP) was generated to assess the IL-1 response of the human/mouse heterodimer and to determine whether the candidate nanobodies could inhibit this response.
Results: SPR experiments showed that more than ten nanobodies showed picomolar affinity for hIL-1R1. Among these, two nanobodies successfully inhibited hIL-1R1 activation in cell culture and one showed an inhibition profile like Anakinra. Furthermore, this nanobody could also inhibit IL-1 signaling mediated by a human/mouse IL1R1/IL1RAcP heterodimeric receptor.
Conclusion: These results demonstrate that the candidate nanobody effectively suppresses IL-1R1 signaling in vitro. To fully establish its clinical potential, in vivo efficacy studies are being conducted.
Acknowledgements: This project was funded by TÜBİTAK grant 20AG007.

Keywords: nanobody, interleukin-1 receptor type 1, inflammatory diseases

PP165
Establishment of a Polyglutamine Aggregation Model to Investigate the Neuronal Membrane Proteasome in Huntington's Disease

Yağmur İri[1], Fulya Türker[1, 2]

[1] Department of Molecular Biology and Genetics, Bilkent University, Ankara, Türkiye 06800
[2] Department of Neuroscience, Bilkent University, Ankara

Background/Aim: The neuronal membrane proteasome (NMP) is a recently identified, neuron-specific subpopulation of the 20S proteasome localized to the plasma membrane, where it degrades intracellular proteins and releases extracellular signaling peptides. Although proteasomal dysfunction is a hallmark of various neurodegenerative diseases, the role of NMP in Huntington's disease (HD), a polyglutamine (polyQ) repeat expansion disorder, has not been investigated. We aimed to establish HD cell models and determine whether mutant huntingtin (mHTT) polyQ expansion and aggregate formation alter NMP abundance and activity.
Materials and Methods: HEK293 cells were transfected with EGFP-tagged huntingtin exon-1 constructs carrying non-pathogenic (Q23) or expanded (Q74) polyQ repeats, and disease phenotypes were analyzed by flow cytometry, fluorescence microscopy, and immunoblotting to establish an aggregation model. We are extending this model to STHdhQ7/Q7 and STHdhQ111/Q111 cells, which are striatal-derived neuronal cells that express endogenous wild-type or expanded mHTT. We use surface biotinylation, antibody feeding-based fluorescence imaging, and FACS analysis to compare NMP surface levels and activity across both models.
Results: Initial validation in HEK293 cells confirmed that EGFP-Q74 expression led to punctate aggregate formation, while EGFP-Q23 protein remained diffusely distributed. Immunoblotting confirmed dose- and time-dependent expression of the constructs. Building on validated models, we anticipate a profound, disease-state-dependent depletion of NMPs in striatal cells. Comparing STHdhQ7/Q7 and STHdhQ111/Q111 will reveal early-onset NMP dysregulation driven by polyQ toxicity. Crucially, comparing Q23- and Q74-transfected cells will demonstrate how advanced aggregate formation modulates NMP biology.
Conclusion: These findings establish a robust polyQ aggregation model and the experimental framework required to investigate aggregate-dependent alterations in NMP levels and enzymatic activity. We propose that NMP abundance is altered by mHTT and may represent a previously unrecognized, disease-relevant proteostasis pathway meriting further mechanistic investigation in HD to define its therapeutic potential.

Keywords: Huntington's disease, Neuronal membrane proteasome, polyglutamine aggregation, STHdh cells

PP166
Establishment of a Zebrafish Xenograft Assay to Analyze Glioblastoma Cells-Microenvironment Interactions and Therapeutic Resistance

Remziye Nur Akalper[1, 2], Beyza Nur Köseoğlu[1, 2], Ezgi Yağmur Kala Kalkan[2], Tugba Bagci-Onder[2, 3], Emre Yaksi[2, 3]

[1] Koç University, Graduate School of Health Sciences, Cellular and Molecular Medicine, Istanbul ٣٤٤٥٠, Turkey
[2] Koç University, Research Center for Translational Medicine, Istanbul ٣٤٤٥٠, Turkey
[3] Koç University, School of Medicine, Istanbul ٣٤٤٥٠, Turkey

Background/Aim: Zebrafish are emerging as powerful *in vivo* models in cancer research due to the transparency of the embryos, their rapid development, and *in vivo* drug screening. These advantages enable real-time visualization of tumor cell behavior and provide cost-effective and flexible alternative to traditional mammalian models. Zebrafish xenograft system have gained increasing attention for studying tumor-microenvironment interactions in Glioblastoma (GBM), an aggressive primary brain tumor characterized by extensive invasion into surrounding brain tissue and limited response to current strategies. Here, we aimed to utilize a zebrafish xenograft model to analyze GBM cell interactions with the brain microenvironment as well as drug resistance behavior using our paired GBM cell line, U87MG and its Temozolomide-resistant derivative, U87MG-TR.
Materials and Methods: Accordingly, the *in vitro* behavior of the U87MG and U87MG-TR were characterized using cell viability assays and morphological analyses. In parallel, zebrafish injection protocols were optimized in transgenic lines.
Results: Preliminary results revealed that both the U87MG and U87MG-TR cells exhibit adherent, epithelial-like characteristics, and requiring strong cell-cell adhesion for optimal survival. U87MG-TR cells display significantly higher IC50 to Temozolomide, which is DNA alkylating agent and the first-line chemotherapeutic for GBM.
Conclusion: Our current efforts are focused on analyzing the transplantation capacity of both the U87MG and U87MG-TR cells, which are engineered to stably express GFP or mCherry using lentiviral transduction. The *in vivo* behavior of these cells will be monitored in zebrafish larvae and analysis will be performed with confocal microscopy.

Keywords: Glioblastoma (GBM), Glioma cell behavior, Zebrafish xenograft , Drug resistance

PP167
Synthesis and Analysis of Novel Benzofuranone Derivatives for Alzheimer's Disease

Derya YILDIZ[1], Alirıza İSLİM[1], Nehir ÖZDEMİR ÖZGENTÜRK[1], Zerrin ÇALIŞKAN[1], Canan BAĞCI[2], Hüseyin SERVİ[3]

[1] Yıldız Technical University, İstanbul
[2] Bahçeşehir University
[3] İstanbul Yeni Yüzyıl University

Background/Aim: Benzofuranone derivatives hold a significant place in pharmacological research due to their broad spectrum of biological activities. Harboring the chiral α-hydroxy ketone skeleton, a fundamental building block of numerous natural compounds, these derivatives attract considerable attention as novel therapeutic agents against neurodegenerative diseases owing to their high potential. Alzheimer's disease (AD), one of the most prevalent and progressive forms of these diseases, urgently requires the development of novel drug candidates. Accordingly, benzofuranone derivatives stand out as promising molecules for the treatment of AD. In this study, the synthesis, structural modifications, and *in silico* evaluations of novel benzofuranone derivatives with potential therapeutic effects were performed.
Materials and Methods: The benzofuranone skeleton was constructed via an exothermic reaction using a 1,3-cyclohexanedione derivative as the core structure. Subsequently, the target acetoxy and hydroxy derivatives were synthesized through $Mn(OAc)_3$ mediated regioselective acetoxylation and K_2CO_3 mediated hydrolysis reactions, respectively.
Results: Molecular docking studies were employed to investigate the effects of these compounds. In parallel with these results, the cytotoxic effects of the compounds and their impact on cell viability will be evaluated in an Alzheimer's cell line model using the MTT assay.
Conclusion: In light of these findings, these novel derivatives, synthesized for the first time in the literature, hold significant potential to pave the way for future preclinical studies targeting Alzheimer's disease.

Keywords: Alzheimer's Disease, Benzofuranone, Molecular Docking, Cytotoxicity

PP168
Early Indicator of Nervous System Dysfunction in Body-First and Brain-First Animal Models of Parkinson's Disease

Bora Deniz Yılmaz[1, 2], Almina Düzen[1, 2], Ayça Arslan[1]

[1] UNAM - National Nanotechnology Research Center, Bilkent University, Ankara, Turkey
[2] Institute of Materials Science and Nanotechnology, Bilkent University, Ankara, Turkey

Background/Aim: Parkinson's Disease (PD) is characterised by the aggregation of misfolded alpha-synuclein into Lewy bodies. Emerging evidence suggests that α-synuclein pathology can originate either in the brain or in peripheral tissues before spreading to the central nervous system through the peripheral autonomic nervous system. To investigate these distinct pathological pathways, we established novel brain-first and body-first α-synuclein pathology models in zebrafish.
Materials and Methods: To induce the two initial pathological pathways, α-synuclein preformed fibrils (PFFs) were injected intracerebrally and intraperitoneally. The distribution of α-synuclein pathology in the brain and subsequent separation of the body was examined using immunofluorescence (IF) and immunohistochemistry (IHC) assays. Quantitative analysis of dopaminergic neurons was performed to evaluate PD associated neurodegeneration. Furthermore, qPCR and Western blot analyses were conducted to assess the expression of disease-related genes and protein markers in the brain, heart, and gut. Behavioural alterations were observed and analysed using AquaMaze software.
Results: Molecular analyses demonstrate alterations in gene expression and protein levels related to α-synucleinopathy in two distinct models. IHC assays indicate α-synuclein positive regions in peripheral tissues and the brain. Loss of dopaminergic neurons was detected in brain sections in an IF assay. Behavioural analyses revealed an increase in anxiety behaviour, particularly in the body-first PD model.
Conclusion: The findings suggest that the anatomical site of pathology initiation may influence disease progression by inducing distinct molecular, cellular, and behavioural alterations. Furthermore, this study demonstrates that the zebrafish model enables comprehensive investigation of PD associated α-synucleinopathy.
Acknowledgement
This work was supported by the Scientific and Technological Research Council of Türkiye (TÜBİTAK) under Project No. 122N005.

Keywords: Parkinson's Disease, Zebrafish, α-synuclein

PP169
Rational Design and In Silico Investigation of Novel Asetilcolinesterase Inhibitors

Batu Dokuyucu[1], Rabia Ela Ertem[2], Elif Arık Sever[3]

[1] Istanbul Okan University, Faculty of Pharmacy, Istanbul, Türkiye
[2] Istanbul Okan University, Faculty of Health Science, Department of Nutrition and Dietetics, Istanbul, Türkiye
[3] Istanbul Okan University, Faculty of Medicine, Department of Medical Biology, Istanbul, Türkiye

Background/Aim: Acetylcholinesterase (AChE) inhibitors are critical in treating neurodegenerative diseases. This study aimed to rationally design novel, blood-brain barrier (BBB)-permeable AChE inhibitors and evaluate them in silico.
Materials/
Materials and Methods: Using 6,809 ChEMBL220 compounds, two Random Forest QSAR models (descriptor-selection-based and Morgan fingerprint-based) were trained and validated via R^2, Q^2, RMSE/MAE, Golbraikh-Tropsha criteria, Y-scrambling, applicability domain, and SHAP analysis. Their consensus (60% fingerprint/40% descriptor) guided a BRICS-based genetic algorithm to generate candidates satisfying BBB permeability, drug-likeness (QED), and PAINS filters. Candidates were screened for Tox21 toxicity, and 10 were selected — based on applicability domain and synthesizability — for AutoDock Vina docking against AChE (PDB 4EY7), validated by re-docking. The top three candidates and the co-crystallized ligand underwent static single-trajectory MM-GBSA calculations. Novelty was verified against PubChem, ChEMBL, and SureChEMBL.
Results: Both QSAR models showed acceptable validation performance (test $R^2 = 0.71$ and 0.65; Golbraikh-Tropsha and Y-scrambling passed). Of 207 designed candidates, nine of the ten selected were absent from public databases. Three candidates surpassed donepezil's docking score (−11.74 to −13.31 kcal/mol), and two also exceeded it in MM-GBSA binding energy (−45.5 to −46.3 kcal/mol), with consistent per-residue interaction profiles.
Conclusion: These findings suggest the designed candidates are promising AChE inhibitor leads or scaffolds. Combining in silico and de novo design offers a cost-effective, rapid strategy for neurobiological drug discovery. As activity predictions remain computational, future work will include molecular dynamics simulations, synthesis, and in vitro validation.

Keywords: Acetylcholinesterase, QSAR, Molecular docking, MM-GBSA, Generative molecular design

PP170
ALCAM Knockdown Effect on Blood-Brain Barrier Integrity and Neurodegenerative Cascade in Zebrafish

Sena Dedeoğlu[1], Ayça Arslan[2]

[1] Aysel Sabuncu Brain Research Center (ASBAM) & National Magnetic Resonance Research Center (UMRAM), Bilkent University, Ankara
[2] Bilkent University National Nanotechnology Research Center (UNAM), Ankara

Background/Aim: The blood-brain barrier (BBB) is a key structure for homeostasis, selective transport, and protection of the central nervous system. In the case of its disruption, it may cause Parkinson's, Alzheimer's diseases, and Multiple sclerosis (MS), caused by degeneration due to apoptosis of cells or unintended transition of pathogens or toxins to brain parenchyma. Activated leukocyte cell adhesion molecule (ALCAM) is involved in endothelial cell interactions and BBB integrity. However, the effects of ALCAM on the BBB and its relationship to neurodegeneration in vivo remains unclear. Therefore, our purpose is to observe the impact of ALCAM gene knockdown on BBB integrity and neuroinflammatory responses in a zebrafish (Danio rerio) model.
Materials and Methods: Also, it is unclear which alcama or alcamb paralogs of zebrafish formed by gene duplication is more likely to human ALCAM in terms of the relationship with BBB. Thus, alcama and alcamb, and both together, will be knockdown at the one-cell stage of zebrafish embryos by morpholino oligonucleotides. Since BBB formation is observed after 3 days post-fertilization (dpf), microinjections of a fluorescent tracker will be given at 5 dpf, and BBB permeability will be checked with a confocal microscope. Moreover, quantitative real-time polymerase chain reaction (RT-qPCR) will be used on pooled larvae to check BBB tight junction proteins, neuroinflammatory markers, and dopamine synthesizing enzyme to see the fate of dopaminergic neurons.
Results: Expected results is higher leakage of the BBB in double-knockdown zebrafish larvae due to downregulation of tight junction protein expression that leads to the upregulation of inflammatory signals and increasing level of dopaminergic loss.
Conclusion: This study will indicate the impacts of ALCAM deficiency on the BBB, as well as lay the foundation for future studies by finding which ALCAM paralog is functionally more similar to the human ALCAM.
-It is supported by Tübitak 1001 Project (Project Number: 125Z347)

Keywords: Zebrafish, Blood-Brain Barrier, ALCAM, Neuroinflammation, Neurodegeneration

PP171

Unlocking Early-Life Pathological Imprinting of Alzheimer's Disease via Lifestyle Interventions

Ceren Aydoğan[1, 2], Ayça Arslan[2]

[1] UNAM - National Nanotechnology Research Center, Bilkent University, Ankara, Turkey
[2] Institute of Materials Science and Nanotechnology, Bilkent University, Ankara, Turkey

Background/Aim: Alzheimer's disease (AD) is the leading cause of dementia, and effective preventive strategies remain limited. Growing evidence suggests that early-life gut microbiota contributes to immune maturation and may influence susceptibility to neurodegenerative diseases later in life. Disruption of microbiota-dependent immune programming during the weaning period has been proposed to induce pathological imprinting, resulting in altered immune responses in adulthood. This study aimed to investigate whether the timing of microbiota colonization during early development affects susceptibility to AD-like pathology and to evaluate the potential impact of a ketogenic diet as a lifestyle intervention in an adult zebrafish AD model.
Materials and Methods: Gnotobiotic zebrafish were colonized with conventional microbiota at different developmental stages (exGF3, exGF5, and exGF7) to model distinct windows of microbial exposure. In adulthood, an amyloid-β42-induced zebrafish model of AD was established. A separate cohort received either a ketogenic or control diet before amyloid-β42 administration. Behavioral performance was evaluated using the Novel Tank Test, Swim Endurance Test, and Novel Object Recognition Test, while neuroinflammatory responses were examined by immunofluorescence and molecular analyses.
Results: Behavioral, molecular, and immunohistochemical analyses of the adult AD model are ongoing to determine whether early-life microbial exposure and ketogenic diet intervention modulate neuroinflammatory responses and AD-associated phenotypes.
Conclusion: This study provides a comprehensive framework to investigate the contribution of early-life microbiota-dependent immune programming to Alzheimer's disease susceptibility and to evaluate the therapeutic potential of ketogenic diet intervention. The findings are expected to improve our understanding of gut–immune–brain interactions and may support the development of novel preventive strategies targeting neurodegenerative diseases.

Keywords: Alzheimer's disease, zebrafish, gut microbiota, pathological imprinting, ketogenic diet

PP172
17-DMAG Improves Healthspan and Stress Resistance with Trade-offs in Lifespan and Reproductive Capacity in Caenorhabditis Elegans

Buse Özden[1, 2], Simge Emlik Gümüş[2], Enes Bal[2], Burcu Ayhan Şahin[2], Pelin Özfiliz Kılbaş[2], Çağatay Tarhan[1]

[1] Department of Molecular Biology and Genetics, Faculty of Science, Istanbul University, Istanbul, Turkiye
[2] Department of Molecular Biology and Genetics, Faculty of Science and Letters, Istanbul Kultur University, Istanbul, Turkiye

Background/Aim: Aging is characterized by a progressive decline in proteostasis, stress resistance, and physiological functions, ultimately compromising survival. HSP90 is a molecular chaperone essential for proteostasis and cellular stress responses. Its inhibition may activate compensatory stress mechanisms and thus holds potential to modulate lifespan and healthspan. 17-Dimethylaminoethylamino-17-demethoxygeldanamycin (17-DMAG) is an HSP90 inhibitor with reported geroprotective and senotherapeutic potential in cellular and animal models. This study investigated the dose-dependent effects of 17-DMAG on lifespan, physiological function, reproduction, and stress resistance in Caenorhabditis elegans (C. elegans).
Materials and Methods: Wild-type N2 C. elegans were treated with vehicle or 10, 15, 20, and 30 µM 17-DMAG from the first day of adulthood. Animals were maintained at 20 °C except during heat-stress exposure. Lifespan, pharyngeal pumping, locomotion, survival under acute heat and paraquat-induced oxidative stress, and total viable progeny production were evaluated.
Results: 17-DMAG reduced median lifespan at all concentrations. Nevertheless, 10 and 15 µM preserved pharyngeal pumping and locomotor activity during aging and increased survival under heat stress, with the most prominent effects at 15 µM. Only 15 µM improved paraquat-induced oxidative stress resistance. Higher concentrations were less beneficial, with 20 µM reducing survival under oxidative stress and 30 µM impairing physiological performance and heat-stress resistance. Total viable progeny production decreased following treatment with 15 and 30 µM 17-DMAG.
Conclusion: 17-DMAG did not extend lifespan but exerted dose-dependent effects on healthspan and stress resistance. Moderate concentrations, particularly 10-15 µM, preserved physiological function and enhanced stress resistance, whereas 30 µM was detrimental. The healthspan benefits observed at 15 µM coincided with reduced progeny production, suggesting a trade-off between reproductive investment and somatic maintenance. These findings indicate that HSP90 inhibition may improve specific healthspan parameters independently of lifespan extension, while highlighting the importance of dose and reproductive cost in evaluating the geroprotective potential of 17-DMAG.

Keywords: 17-DMAG, HSP90, aging, healthspan, Caenorhabditis elegans

PP173
Temperature Shapes Zebrafish Embryonic Development and Molecular Responses

Selin Acar[1], Nuray Söğünmez Erdoğan[1]

[1] Kadir Has University, Istanbul, Turkey

Background/Aim: Extreme heat is a major public-health threat causing disproportionate mortality. Since early embryogenesis is highly sensitive to environmental perturbations, understanding temperature effect on molecular programs is essential for identifying heat-associated developmental risks. The ZSCAPE single-cell atlas indicated temperature-induced proteostasis disruption. However, fixed time points confound direct temperature effects with developmental shifts. Therefore, we aimed to identify temperature-responsive genes in stage-matched zebrafish embryos.
Materials and Methods: ZSCAPE data from 28°C and 32°C embryos were screened for temperature-responsive genes. Zebrafish embryos were maintained at 24°C, 28°C, or 32°C and collected at the protruding-mouth stage, corresponding to approximately 72 hours post-fertilization (hpf) at 28°C. Thus, groups were matched by developmental stage rather than chronologic age. Gene expression was quantified by qPCR with three biological replicates for hsp70, cct8, ptprfb, and cirbpb and two biological replicates for scrib and ybx1.
Results: Temperature significantly affected developmental timing: embryos reached the protruding-mouth stage at 98hpf, 72hpf, and 60hpf at 24°C, 28°C, and 32°C, respectively. Embryos raised at 24°C showed delayed growth and smaller body size ($p<0.05$), whereas those raised at 32°C developed more rapidly ($p<0.05$). Significant temperature-dependent expression differences were detected for hsp70 ($p<0.05$), cct8 ($p<0.001$), and ptprfb ($p<0.05$). Relative expression values at 24°C, 28°C, and 32°C were 2.21/1.00/7.23 for hsp70, 1.28/1.00/6.23 for cct8, and 1.39/1.00/0.46 for ptprfb. cirbpb did not differ significantly. In the two-replicate dataset, ybx١ tended to be lower at °٣٢C, while scrib was highest at 24°C and lowest at 32°C.
Conclusion: Temperature exposure during embryogenesis altered genes associated with two mechanistically distinct processes: the heat-shock and proteostasis response, represented by hsp70 and the TRiC/CCT chaperonin subunit cct8, and receptor protein tyrosine phosphatase-dependent sensory axon guidance, represented by ptprfb. These preliminary findings suggest that temperature may affect embryogenesis by modifying protein-homeostasis capacity and neural connectivity programs.

Keywords: Zebrafish, temperature, neurodevelopment , proteostasis

PP174

The Role of Brain-derived Neurotrophic Factor Signaling in the Molecular Remodeling of Inhibitory Neurotransmission During Feeding-Dependent Plasticity in the Ventromedial Hypothalamus

Zeynep Bilgetekin[1], Muhammed Deniz Oksal[2], Hanife Dilvin Yıldırım[2], Yasemin Önder[3]

[1] Computational Applied Sciences and Engineering M.Sc. Program, School of Graduate Studies, Kadir Has University, Istanbul, Turkey
[2] Computational Applied Sciences and Engineering Ph.D. Program, School of Graduate Studies, Kadir Has University, Istanbul, Turkey
[3] Department of Molecular Biology and Genetics, Faculty of Engineering and Natural Sciences, Kadir Has University, Istanbul, Turkey

Background/Aim: Brain-derived neurotrophic factor (BDNF), a key modulator of synaptic plasticity, is induced in the ventromedial hypothalamus (VMH), a critical satiety center, of fed mice. Although BDNF-dependent plasticity has been linked to energy balance in the VMH, the role of VMH inhibitory circuits remains unknown. We investigated the effect of acute BDNF signaling on γ-aminobutyric acid type A receptors (GABAARs) and GABA transporters (GATs) in VMH slices and primary hypothalamic neurons derived from mice.
Materials and Methods: For the slice pharmacology experiment, 300-µm VMH-containing brain slices were prepared from 10-week-old 24-h-fasted C57BL/6 mice (n=23) and incubated in oxygenated artificial cerebrospinal fluid (aCSF). K252a (200 ng/mL) was applied 30 min before BDNF (100 ng/mL) treatment, followed by VMH dissection. Primary hypothalamic neurons from P0 C57BL/6 pups were treated with the same protocol. RNA and protein were isolated from both models, and GABAAR subunit and GAT expression levels were quantified by qRT-PCR and Western blot.
Results: Following BDNF treatment in VMH slices, Slc6a11 mRNA levels trended toward downregulation (p=0.063) compared to control, while Gabra5 and Slc6a1 levels remained unchanged. Unexpectedly, Gabra2 was significantly increased in the BDNF+K252a group compared to control (p=0.026) ex vivo, and in vitro compared to control (p=0.003) and BDNF (p=0.028) groups. Gabra2 mRNA and protein expression showed consistent patterns across experimental models. BDNF-treated neurons showed a trend toward increased Bdnf mRNA expression compared to control (p=٠,٠٨٤) and BDNF+K٢٥٢a (p=٠,٠٥٨) groups, supporting treatment responsiveness. Protein levels did not significantly change under these conditions.
Conclusion: Our findings suggest that BDNF signaling differentially affects components of GABAergic neurotransmission, including a trend toward Slc6a11 downregulation and a consistent Gabra2 expression pattern across ex vivo and in vitro models, supporting the involvement of BDNF in hypothalamic inhibitory plasticity.

This project was supported by TUBITAK 2232 (121C128) and TUBITAK 1001 (124Z284) grants.

Keywords: BDNF, Inhibitory Neurotransmission, Plasticity, VMH

PP175

Cognitive Impairment in 179 Patients with Brain Tumors in Morocco: Severity and Distribution According to Tumor Type

Maria Legmouz[1], Fatimazahra Azzaoui[2], Abdessamad El Ouahabi[3]

[1] Faculty of Science, Ibn Tofail University, Kénitra, Morocco
[2] Faculty of Science, Ibn Tofail University, Kénitra, Morocco
[3] Faculty Of Medecine and Pharmacy, University Mohamed 5, Rabat, Morocco

Background/Aim: Brain tumors can be accompanied by significant cognitive impairment affecting memory, attention, executive functions and language. However, data regarding brain tumor in generally and cognitive functioning specifically of patients in Morocco remain limited. This study aimed to assess cognitive impairments in Moroccan patients with brain tumors and to examine its distribution according to tumors type.
Materials and Methods: This prospective study included 179 patients with brain tumors recruited from Specialty Hospital in Rabat, Morocco. Sociodemographic and clinical data, including sex and tumor type, were collected. Cognitive function was assessed using the Montreal Cognitive Assessment test (MoCA). The resulting scores were used to identify patients with pathological cognitive scores and to classify the severity of cognitive impairment as mild, moderate, or severe. The distribution and severity of cognitive impairment according to tumor type were analyzed.
Results: Among the 179 patients included in the study, 94 (52.5%) were men and 85 (47.5%) were women. Regarding tumor type, gliomas were the most common, followed by meningiomas and pituitary adenomas. Overall, 66.43% of patients had an abnormal cognitive score. Cognitive impairment was mild in 45% of patients, moderate in 39%, and severe in 16%. The distribution of cognitive impairment severity varied according to tumor type, with gliomas showing the highest proportions of mild, moderate, and severe cognitive impairment.
Conclusion: This study highlights a high prevalence of cognitive impairment among patients with brain tumors in Morocco, with varying degrees of severity according to tumor type. These findings underscore the importance of incorporating cognitive assessment into the clinical care of patients with brain tumors to enable early identification of cognitive deficits and facilitate appropriate management.

Keywords: Brain tumors, Cognitive impairment, Morocco

PP176
Investigation of ALCAM/CD166 Transcript Variants in Microglia in Brain Aging and Alzheimer's Disease

Melis Karaca[2], Bora Deniz Yılmaz[2], Sena Dedeoğlu[3], Ayça Arslan[1]

[1] Bilkent University UNAM, Ankara
[2] İ.D. Bilkent University, Materials Science and Nanotechnology Program, Ankara
[3] İ.D. Bilkent University, Neuroscience Program, Ankara

Background/Aim: Activated leukocyte cell adhesion molecule (ALCAM/CD166) is an immunoglobulin superfamily protein that mediates cell–cell adhesion and immune signaling. Although ALCAM has been studied extensively in cancer and immunology, its function in the brain — particularly in microglia — and its contribution to aging and neurodegeneration remain poorly defined. ALCAM undergoes alternative splicing, generating several transcript variants in addition to the canonical protein-coding variant (V1). Among these is variant 4 (V4), a three-exon transcript containing one retained intron that is annotated as, and predicted to function as, a long non-coding RNA (lncRNA).
Materials and Methods: We have previously shown that ALCAM expression declines with age in the zebrafish brain and is reduced in the human Alzheimer's disease (AD) brain; that ALCAM overexpression induces senescence, accompanied by elevated p21, in glioblastoma cells; and that both ALCAM and the V4 transcript are more abundant in microglia than in epithelial cells.
Results: On this basis, we hypothesize that ALCAM is subject to post-transcriptional control in microglia and participates in aging- and neurodegeneration-associated processes, while V4 acts as a regulatory modulator of disease progression. To test this, we will (i) determine the effects of ALCAM V1 and V4 overexpression on cellular dynamics in HMC3 human microglial cells and on zebrafish embryonic development, and (ii) assess the consequences of ALCAM overexpression in the adult zebrafish brain during natural aging and in an Aβ-induced zebrafish model of AD.
Conclusion: Together, these experiments will clarify how ALCAM and its transcript variants are regulated and what they contribute to microglial biology.

Keywords: Alzheimer's disease, ALCAM, zebrafish

PP177

Functional Investigation of a Predicted GDAP1–IQSEC1 Interaction in Drosophila Melanogaster

Teeba Al-Fahadi [1], İnci Şardağ[2, 3, 4], Cansu Süloğlu[1], Eren Sicim[1], Aleena Iqbal[1], Arzu Çelik [2, 3, 4], Farzaneh Larti[1]

[1] Kadir Has University, Department of Molecular Biology and Genetics, Cibali Campus, Istanbul, Turkiye
[2] Bogazici University, Department of Molecular Biology and Genetics, 34342 Bebek, Istanbul, Turkiye
[3] Bogazici University, Center for Life Sciences and Technologies, 34342 Bebek, Istanbul, Turkiye
[4] Bogazici University, Center for Targeted Therapy Technologies, 34684 Kandilli, Istanbul, Turkiye

Background/Aim: Mutations in Ganglioside-induced differentiation-associated protein 1 (GDAP1) cause Charcot–Marie–Tooth disease (CMT), an inherited peripheral neuropathy affecting motor and sensory neurons. Although several GDAP1-interacting proteins have been reported, the molecular pathways through which GDAP1 regulates neuronal function remain poorly understood. IQ motif and SEC7 domain-containing protein 1 (IQSEC1), an ADP-ribosylation factor (ARF) guanine nucleotide exchange factor regulating membrane trafficking and synaptic organization, is a biologically relevant candidate. We combined structural modeling with Drosophila melanogaster genetics to identify and functionally investigate candidate GDAP1-interacting proteins in vivo.
Materials and Methods: AlphaFold3-based structural modeling was used to evaluate 37 reported GDAP1-interacting proteins curated in UniProt from two independent studies. Candidates were prioritized by interface confidence, structural quality, and evolutionary conservation in Drosophila. IQSEC1 isoform 1 emerged as the highest-confidence candidate. Functional analyses used ubiquitous Gdap1 RNAi-mediated knockdown and CRISPR-mediated integration cassette (CRIMIC) knockout alleles of Gdap1 and Iqsec1. Locomotor performance and neuromuscular junction (NMJ) morphology were assessed in third-instar larvae.
Results: IQSEC1 showed the highest-confidence predicted structural interaction with GDAP1. Several pathogenic GDAP1 variants mapped near the predicted protein-protein interface. Ubiquitous Gdap1 knockdown impaired larval locomotion and reduced NMJ branching. Homozygous Gdap1 CRIMIC mutants were viable to adulthood with similarly reduced larval NMJ branching. In contrast, homozygous Iqsec1 mutants were lethal, while heterozygotes showed increased satellite boutons. Together, these findings indicate that both genes contribute to normal larval NMJ development, although their genetic and physical interaction remains to be established.
Conclusion: Structural modeling identified IQSEC1 as a high-priority candidate of GDAP1-interacting protein. The distinct NMJ abnormalities following disruption of Gdap1 or Iqsec1 indicate that both genes contribute to larval synaptic development, but do not establish a direct interaction or shared pathway. Genetic interaction studies, cell-type-specific rescue experiments, and biochemical validation will help determine whether GDAP1 and IQSEC1 functionally or physically interact in neurons.

Keywords: GDAP1, IQSEC1, Charcot–Marie–Tooth disease, Drosophila melanogaster, neuromuscular junction

PP178
Tissue-Specific Functions of Septin 4 at the Drosophila melanogaster Neuromuscular Junction

Pınar Aydan Kekeç[1, 2, 3], Dr. Farzaneh Larti[4], Prof. Dr. Arzu Çelik Fuss[1, 2, 3]

[1] Bogazici University, Department of Molecular Biology and Genetics, 34342 Bebek, Istanbul, Turkiye
[2] Bogazici University, Center for Life Sciences and Technologies, 34342 Bebek, Istanbul, Turkiye
[3] Bogazici University, Center for Targeted Therapy Technologies, 34684 Kandilli, Istanbul, Turkiye
[4] Kadir Has University, Department of Molecular Biology and Genetics, Cibali Campus, Istanbul, Turkiye

Background/Aim: Septins are cytoskeletal regulators that assemble into hetero-oligomeric complexes and filaments, coordinating actin filaments, microtubules, and the plasma membrane. Such coordination is important at synapses, where remodeling regulates synaptic vesicle trafficking and neurotransmission. Previously, we showed that combined loss of Septin 2 and Septin 5 alters synaptic structure, supporting a role for septin complexes in synaptic organization. Septin 4 (Sep4) is highly conserved and expressed in neuronal and muscular tissues and has been implicated in synaptic function; however, its tissue-specific contribution to neuromuscular junction (NMJ) development remains uncharacterized. The Drosophila melanogaster larval NMJ provides a model for investigating synaptic plasticity and recapitulates key organizational features of vertebrate synapses. Here, we investigate whether Sep4 has distinct functions in presynaptic and postsynaptic compartments of the Drosophila NMJ.
Materials and Methods: Sep4 expression was knocked down ubiquitously, pan-neuronally, and muscle-specifically using the GAL4/UAS system. Third-instar larval NMJs (segment A4, muscles 6/7) were analyzed using immunofluorescence microscopy, and synaptic morphology was quantified. Sep4 knockdown efficiency and potential transcriptional compensation by Septin 1 (Sep1) were evaluated via RT-qPCR.
Results: Sep4 depletion produced cell-type dependent alterations in NMJ morphology. Pan-neuronal Sep4 knockdown triggered synaptic overgrowth, increasing axonal branching and bouton number. In contrast, muscle-specific depletion reduced bouton number without affecting branching. Ubiquitous knockdown promoted branching without altering bouton number or NMJ area, reflecting a composite morphological response. RT-qPCR revealed reciprocal changes in Sep1 and Sep4 transcript levels, consistent with a potential transcriptional compensatory response.
Conclusion: The distinct morphological responses of neuronal and muscle-specific Sep4 depletion suggest that Sep4 differentially regulates the pre- and postsynaptic mechanisms shaping NMJ development. Reciprocal changes in Sep1 and Sep4 transcript levels suggest potential compensatory regulation between these septins. Functional analysis of combined Sep1 and Sep4 depletion will be required to determine whether they act redundantly or cooperatively during synaptic development.

Keywords: Cytoskeleton, Septins, Tissue-Spesific Knockdown, Synaptic Remodeling

PP179
Functional Characterization of a CMT-Associated NARS1 Variant in Drosophila Melanogaster

Tuana Keskin[1, 2, 3], Farzaneh Larti[4], Arzu Çelik Fuss[1, 2, 3]

[1] Bogazici University, Department of Molecular Biology and Genetics, 34342 Bebek, Istanbul, Turkiye
[2] Bogazici University, Center for Life Sciences and Technologies, 34342 Bebek, Istanbul, Turkiye
[3] Bogazici University, Center for Targeted Therapy Technologies, 34684 Kandilli, Istanbul, Turkiye
[4] Kadir Has University, Department of Molecular Biology and Genetics, Cibali Campus, Istanbul, Turkiye

Background/Aim: Charcot-Marie-Tooth disease (CMT), a hereditary motor and sensory neuropathy, is one of the most common inherited neuromuscular disorders in humans affecting peripheral nerves. Six aminoacyl tRNA synthetases (aaRS) have been linked to different axonal forms of CMT that share similar clinical features, including progressive distal muscle atrophy and weakness, sensory dysfunction, gait disturbances, and skeletal abnormalities. This makes aaRSs the largest protein family associated with CMT, suggesting that additional aaRS genes may also contribute to the disease. In particular, asparaginyl-tRNA synthetase 1 (*NARS1*) variants have been associated with neuropathies. To study the functional and CMT-related effects of *NARS1*, we used a *Drosophila melanogaster* knock-in model carrying a homozygous *Y289C*-equivalent mutation in the endogenous orthologous locus. This variant is located within the catalytic domain and may impair enzymatic activity by disrupting protein structure. We aimed to characterize the effects of this variant in flies and assess its potential association with CMT-related phenotypes.
Materials and Methods: CRISPR-generated homozygous NARS1 Y289C-equivalent knock-in flies and their corresponding controls were analyzed. Neuromuscular junction (NMJ) morphology was examined in 3rd instar larvae by immunohistochemistry using Horseradish Peroxidase (HRP) and Discs Large (DLG) antibodies, followed by confocal microscopy. Adult eye morphology was examined using stereomicroscopy. Immunohistochemistry images were analyzed using Fiji (ImageJ), and graphs were generated using GraphPad Prism.
Results: Analysis of NMJ morphology revealed an increased number of synaptic branches in the NARS1 Y289C-equivalent mutant, as assessed by both HRP and DLG staining. Adult eye imaging revealed no rough-eye phenotype or abnormal ommatidial organization in the mutant flies.
Conclusion: These findings indicate that the introduced NARS1 Y289-equivalent mutation affects NMJ morphology without causing an overt adult eye phenotype. Additional behavioral and synaptic analyses that are under way will help to determine whether this structural alteration is associated with impaired neuromuscular function and a CMT-relevant phenotype.

Keywords: CMT, Drosophila melanogaster, Animal Model, Aminoacyl tRNA synthetases

PP180
Unraveling the Effect of Long-term Hypothermia Induced by GABAergic DMH Neurons on Cognitive Functions

Hilal Badoglu[1], Aysenur Ozpinar[2, 3, 4], Buse Balaban[2, 5, 6], Pelin Dilsiz[2, 7]

[1] Department of Molecular Biology-Genetics and Biotechnology, Istanbul Technical University, Istanbul, Türkiye
[2] Research Institute for Health Sciences and Technologies (SABITA), Istanbul Medipol University, Istanbul, Türkiye
[3] Department of Physiology, School of Medicine, Istanbul Medipol University, Istanbul, Türkiye
[4] Graduate School of Health Sciences, Istanbul Medipol University, Istanbul, Türkiye
[5] Department of Medical Biology, School of Medicine, Istanbul Medeniyet University, Istanbul, Türkiye
[6] Department of Medical Biology, Institute of Health Sciences, University of Health Sciences, Istanbul, Türkiye
[7] Department of Molecular Biology and Genetics, Faculty of Science and Letters, Istanbul Technical University, Istanbul, Türkiye

Background/Aim: Thermoregulation is a feedback mechanism that maintains the core body temperature within a physiological range. Body temperature below the optimal range affects wide range of physiological and behavioral processes. Low body temperature has long been known to provide neuroprotection, albeit hypothermia induced by exogenous cooling may cause multiple side effects, including heart rhythm disturbances, hypokalemia, coagulopathies, and infections. Learning and memory rely on synaptic plasticity, which is sensitive to temperature changes in the brain, however, the impact of hypothermia on cognitive functions remains unclear. Here, we investigated the role of hypothermia induced by chemogenetic inhibition of GABAergic neurons located in the dorsomedial hypothalamus (DMH) on cognitive functions.
Materials and Methods: To this end, we intracranially administered rAAV2/1-EF1a-DIO-hM4D(Gi)-mCherry (n=8) or rAAV2/1-CAG-DIO-GFP (n=8) to the DMH of Vgat-Cre mice. Hypothermia was induced by intraperitoneal administration of clozapine, the chemogenetic ligand. Chemogenetic inhibition of GABAergic neurons in the DMH decreased core body temperature. Behavioral tests were performed to assess the effect of hypothermia on learning and memory.
Results: Hypothermia induced by silencing the GABAergic neurons in the DMH improved cognitive functions. Hypothermic mice demonstrated enhanced recognition memory compared to normothermic mice detected by novel object recognition test. Similarly, low body temperature improved spatial working memory evaluated by spontaneous alternation performance in the Y-maze test. The anxiety-like behavior of hypothermic and normothermic mice were comparable.
Conclusion: We demonstrated that a prolonged hypothermic state induced by chemogenetic inhibition of GABAergic neurons in the DMH modulated cognitive performance. Our findings suggest that endogenously induced hypothermia improved cognitive functions and enhanced memory consolidation bypassing the side effects of exogenous cooling.

Keywords: Hypothermia, dorsomedial hypothalamus, cognition, learning and memory

PP181
nrg3b Perturbation Alters Neuronal Markers and CNS Morphogenesis and is Linked to PI3K/AKT Signaling in Zebrafish

Elham Tarahomi[1], Nuray Söğünmez Erdoğan[1], Sepand Rastegar[2]

[1] Kadir Has University, Istanbul
[2] Institute of Biological and Chemical Systems - Biological Information Processing (IBCS-BIP), Karlsruhe Institute of Technology, Karlsruhe, Germany.

Background/Aim: Neuregulin (NRG)–ERBB signaling contributes to nervous system development, yet the function of nrg3b during early zebrafish CNS development remains unclear. We characterized spatiotemporal expression of nrg3b and determined whether its perturbation affects glutamatergic and GABAergic neuronal molecular programs and their relationship with PI3K/AKT signaling.
Materials and Methods: nrg3b expression was examined by whole-mount in situ hybridization and quantified by RT-qPCR at 1, 3, and 5 days post-fertilization (dpf). Gain-of-function was induced by injection of in-vitro-transcribed nrg3b mRNA, whereas loss-of-function used a splice-blocking morpholino. Expression of nrg3b, gad1b (GABAergic marker), and slc17a6a (glutamatergic marker) was quantified by RT-qPCR. PI3K/AKT pathway involvement was assessed by exposing 2 dpf embryos to 8 or 16 µM LY294002.
Results: nrg3b showed brain-enriched expression that increased from 1–5 dpf, while overexpression altered slc17a6a levels (0.51-fold at 24 hpf, $p = 0.0008$; 2.26-fold at 48 hpf, $p = 0.0047$), while gad1b remained unchanged. Injection of 0.40 pmol/embryo nrg3b morpholino significantly reduced both gad1b and slc17a6a expression (both $p<0.001$) and induced morphological abnormalities, including reduced brain size and eye diameter, compared with embryos injected with the same dose of control morpholino. In contrast, injection of 0.3 pmol/embryo did not significantly alter either neuronal marker and morphology. LY294002 significantly affected nrg3b expression, with a 1.24-fold increase at 16 µM compared to WT ($p = 0.0168$). gad1b decreased dose-dependently to approximately 0.69- and 0.54-fold of WT at 8 and 16 µM, respectively ($p=0.0191$ and $p=0.0015$). slc17a6a showed a downward trend but did not reach significance (ANOVA $p=0.1921$).
Conclusion: These findings identify nrg3b as a developmentally regulated, CNS-enriched gene whose dosage influences glutamatergic and GABAergic marker expression and early CNS morphogenesis. The stage-dependent effects of nrg3b overexpression, dose-sensitive molecular and morphological phenotypes following knockdown, and reciprocal nrg3b/gad1b response to PI3K inhibition further suggest that nrg3b-associated neuronal development is linked to PI3K/AKT signaling.

Keywords: nrg3b, zebrafish, central nervous system, neuronal differentiation, PI3K/AKT

PP182
Antagonistic Activity of Aureobasidium Pullulans Against Fusarium Culmorum

Berkay Erdur[1], Mehmet Arslan[1], Gülruh Albayrak[2], Figen Mert[3]

[1] Istanbul University, Institute of Sciences, Programme of Molecular Biology and Genetics, Istanbul, Turkiye
[2] Istanbul University, Faculty of Sciences, Department of Molecular Biology and Genetics, Istanbul, Turkiye
[3] Çanakkale Onsekiz Mart University, Faculty of Agriculture, Department of Plant Protection, Çanakkale, Turkiye

Background/Aim: Fusarium culmorum is a devastating fungal pathogen of cereals. Naturally occurring isolates of the yeast-like fungus Aureobasidium pullulans represent a promising source of biological control agents against plant-pathogenic fungi. This study evaluated the antagonistic potential of A. pullulans isolates recovered from olive and quince leaves against F. culmorum and investigated their selected biocontrol-related traits.
Materials and Methods: Eleven A. pullulans isolates recovered from leaves were identified by translation elongation factor 1-alpha (TEF1-α) sequencing. Antagonistic activity was assessed against four field isolates and one reference isolate of F. culmorum using dual-culture assays. Protease production was screened on skim milk agar and quantified using the Folin–Ciocalteu method. Chitinase activity was determined using the 3,5-dinitrosalicylic acid assay. Biofilm formation was examined by crystal violet staining. Expression of the protease gene ALP5 and a glycoside hydrolase family 18 (GH18) chitinase gene was quantified by qPCR in selected A. pullulans isolates from dual cultures. Associations between growth inhibition and biocontrol-related traits were evaluated by correlation analysis.
Results: All A. pullulans isolates significantly inhibited F. culmorum mycelial growth. Maximum inhibition was 62.67 ± 0.76% against TY21 and 62.33 ± 1.80% against the reference isolate FcUK99. EpO-2, EpO-4, EpO-5, and EpO-6 showed consistently strong inhibitory activity. In the presence of F. culmorum, EpO-2 showed higher GH18 expression and EpO-4 higher ALP5 expression than the weak antagonist EpO-1. Growth inhibition was strongly correlated with protease production on solid medium and protease activity in liquid culture ($r = 0.85$ and $r = 0.87$, respectively; $p < 0.001$), whereas chitinase activity showed no significant correlation.
Conclusion: Proteolytic activity was strongly associated with A. pullulans-mediated suppression of F. culmorum, suggesting a potential role for extracellular proteases in antagonism. The most effective isolates warrant further evaluation under plant and field conditions.

Keywords: Aureobasidium pullulans, Fusarium culmorum, biological control, protease, fungal antagonism.

P183
Wheatgrass Protein–Aronia melanocarpa Juice Complexes: Structural Characterization, Molecular Docking, and Enhanced Bioaccessibility

Rumeysa Bayhan[1], Büşra Yusufoğlu*[2], Rukiye Gündoğan[3]

[1] Department of Chemistry, Faculty of Science and Letters, Istanbul Technical University, Ayazağa Campus 34469, Istanbul, Turkey
[2] Department of Chemistry, Faculty of Science and Letters, Istanbul Technical University, Ayazağa Campus 34469, Istanbul, Turkey
[3] Department of Food Engineering, Faculty of Chemical and Metallurgical Engineering, Istanbul Technical University, Ayazağa Campus 34469, Istanbul, Turkey

Background/Aim: Anthocyanins from Aronia melanocarpa juice (AMJ) exhibit strong antioxidant activity linked to reduced cancer, cardiovascular, and diabetes risk, but degrade extensively during digestion, limiting intestinal stability and bioaccessibility. Wheatgrass protein (WGP) contains functional groups capable of interacting with anthocyanins through hydrogen bonding and hydrophobic interactions, potentially forming protective protein–polyphenol complexes. This study aimed to develop a plant-based delivery system based on WGP-AMJ complexation to improve phenolic retention and digestive stability.
Materials and Methods: WGP-AMJ complexes were prepared at weight ratios of 100:25, 100:50, and 100:100 and freeze-dried. Conformational and intermolecular changes were evaluated by Fourier-transform infrared (FTIR) spectroscopy, focusing on the O-H stretching region and amide I/II bands. Complexes underwent standardized static INFOGEST in vitro digestion, and the resulting intestinal-phase fractions were analyzed for phenolic content and antioxidant activity (DPPH, CUPRAC; one-way ANOVA, Scheffé's post-hoc test). Wheat germ agglutinin (WGA; PDB ID: 1WGC) was prepared in ChimeraX, and four aronia anthocyanins were docked into its carbohydrate-binding pocket using AutoDock Vina under acidic gastric-phase conditions.
Results: FTIR spectra showed concentration-dependent broadening of the O-H band and shifts in amide I/II regions, suggesting hydrogen-bond-mediated interactions and conformational rearrangement of WGP upon binding. Docking predicted favourable binding of all four anthocyanins to WGA, ranked cyanidin-3-O-galactoside (−7.821 kcal/mol) > cyanidin-3-O-glucoside (−7.614) > cyanidin-3-O-rutinoside (−7.607) > cyanidin-3-O-arabinoside (−7.505), while experimental findings showed significantly greater intestinal-phase phenolic retention in WGP-AMJ complexes than in uncomplexed AMJ (83.81 vs. 23.82 mg GAE/g), with sixfold higher DPPH and threefold higher CUPRAC activity.
Conclusion: The combined findings demonstrate the potential of WGP-AMJ complexation as a plant-based delivery system for improving the intestinal stability of aronia-derived phenolics. FTIR analysis indicated protein-polyphenol interactions with conformational rearrangement of WGP, while docking suggested favorable interactions between the anthocyanins and a wheat-derived protein binding site. By preserving anthocyanin bioactivity, this strategy may support delivery of health-promoting phenolics.

Keywords: wheatgrass protein, Aronia melanocarpa, protein–polyphenol interaction, in vitro digestion, molecular docking

PP184

Investigation of the Molecular and Phenotypic Effects of Mixed Infections Induced by PVY and CMV in Nicotiana Benthamiana and Solanum Tuberosum Plants.

Serap Sakarya[1], Stuart James Lucas[1, 2]

[1] Faculty of Engineering and Natural Sciences, Sabanci University, Istanbul, Turkiye.
[2] Nanotechnology Research and Application Center (SUNUM), Sabanci University, Istanbul, Turkiye.

Background/Aim: Plant viruses are important molecular pathogens that can infect wild and cultivated plants and cause significant agricultural losses. Mutation and recombination can generate new viral variants and infect new hosts. Mixed viral infections are important because viral interactions can alter disease development, viral accumulation, plant defense, and yield. Depending on the timing and order of infection, mixed infections can occur as co-infections or sequential infections. Potato virus Y (PVY) and cucumber mosaic virus (CMV) are economically important plant viruses that can cause severe symptoms and yield losses. However, only a limited number of studies have investigated the interactions between PVY and CMV during mixed infections. This project aims to systematically investigate the phenotypic and molecular effects of single, co-, and sequential infections with PVY and CMV.
Materials and Methods: The model plant *Nicotiana benthamiana* and the crop plant *Solanum tuberosum* will be infected with PVY and CMV individually or in different mixed infection combinations. Viral presence and accumulation will be evaluated using molecular detection and real-time PCR. Gene expression, transcriptomic, and small RNA analyses will be used to investigate changes in plant defense responses and molecular mechanisms associated with mixed infections.
Results: At the current stage of the project, qPCR experiments using *Nicotiana benthamiana* have been completed. The obtained data will be further evaluated through bioinformatics analyses to compare gene expression patterns among the infection groups. In future, experiments using *Solanum tuberosum* will be performed, followed by molecular and bioinformatics analyses.
Conclusion: This study will provide insights into how infection timing and order influence PVY–CMV interactions, viral population dynamics, and plant defense responses. The findings are expected to contribute to understanding mixed viral infections and provide basic information for future studies on RNAi-based strategies for controlling plant viral diseases.

Keywords: Sequential infection,, mixed viral infections,, PVY,, CMV,, plant-virus interactions,

PP185
Transfer Learning with Multiomics Integration and Deep Neural Networks Reveals Drug Resistance Mechanisms In Cancer

Semih Alpsoy[1], Osman Ugur Sezerman[2]

[1] Turkish-German University
[2] Acibadem Mehmet Ali Aydinlar University

Background/Aim: Drug resistance remains one of the major challenges limiting the effectiveness of cancer therapy. Identifying resistance mechanisms and improving predictive performance for treatment response are critical for precision medicine approaches. This study aimed to develop a transfer learning (TL)-based deep neural network (DNN) framework integrating multiomics data to predict drug responses and identify molecular mechanisms associated with resistance in cancer.
Materials and Methods: Gene expression, mutation, and copy number alteration (CNA) datasets were integrated with drug response profiles using a multiomics approach. Drug sensitivity data obtained from the Genomics of Drug Sensitivity in Cancer (GDSC) database were used for model training. Transfer learning models were constructed using drugs with related mechanisms and evaluated on independent Patient-Derived Xenograft (PDX) and The Cancer Genome Atlas (TCGA) datasets. Pathway enrichment analyses were performed to identify common and drug-specific resistance mechanisms.
Results: The proposed framework successfully predicted drug responses across independent datasets and improved prediction performance through multiomics integration. Gene expression combined with mutation or CNA profiles frequently produced robust predictive outcomes. Pathway enrichment analyses identified both shared and unique resistance-associated pathways and highlighted candidate genes potentially involved in therapeutic resistance.
Conclusion: The integration of transfer learning with multiomics data improved predictive performance and enabled the identification of biologically meaningful resistance mechanisms. This approach may contribute to precision oncology by supporting personalized treatment strategies and improving understanding of cancer drug resistance.

Keywords: Multiomics, Transfer Learning, Deep Neural Networks, Drug Resistance, Precision Oncology

PP186
Cell Cycle and Stress Activate PI(3,5)P2 Synthesis Through Distinct Pathways

Ahmed Alsaftawi[1], Nurhan Ersoy[1], Ayşe Koca Çaydaşı[1]

[1] Koç University, Department of Molecular Biology and Genetics, Istanbul, Türkiye

Background/Aim: Phosphatidylinositol 3,5-bisphosphate (PI(3,5)P2) is a conserved signaling lipid of the endolysosomal-system that is essential for membrane trafficking, stress responses, and lysosome and endosome homeostasis. Although present at very low levels under basal conditions, PI(3,5)P2 can be rapidly synthesized in response to specific stimuli such as hyperosmotic stress. Our previous work showed that PI(3,5)P2 levels also increase during mitosis in budding yeast, specifically on vacuolar membranes inherited by the daughter cell. This asymmetric enrichment of PI(3,5)P2 promotes daughter-cell rejuvenation and mother-cell aging by regulating vacuolar acidity.
Materials and Methods: Despite the importance of this mitotic increase, the mechanisms that trigger PI(3,5)P2 synthesis during cell division have remained unknown. Here, I will present our unpublished findings showing that PI(3,5)P2 production is directly regulated by the cell cycle machinery.
Results: We found that Vac14, the scaffold protein at the core of the PI(3,5)P2-synthesizing complex, becomes hyperphosphorylated during mitosis in a manner dependent on the polo-like kinase Cdc5. Preventing Vac14 phosphorylation, either by depleting Cdc5 or by mutating key phosphorylation sites in Vac14, abolishes the mitotic increase in PI(3,5)P2. Remarkably, these same mutants retain the ability to induce PI(3,5)P2 synthesis in response to hyperosmotic stress. This finding demonstrates that PI(3,5)P2 production can be activated through distinct upstream pathways depending on cellular context: cell cycle-dependent regulation during mitosis and environmental signaling during osmotic stress.
Conclusion: Together, our results reveal how cells use different regulatory inputs to control the synthesis of a key signaling lipid, enabling PI(3,5)P2 to support diverse functions ranging from stress adaptation to organelle inheritance during cell division.

Keywords: Cell cycle, lipid signaling, P(3,5)P2, organelle inheritance, Aging, Polo kinase

PP187
Computational Analysis of Human NARS1 Variants

Inci Sardag[1], Farzaneh Larti[2], Sehime Gulsun Temel[3], Arzu Celik Fuss[0]

[1] Bogazici University
[2] Kadir Has University
[3] Bursa Uludag University

Background/Aim: Asparaginyl-tRNA synthetase 1, Nars1, plays a key role in translation by catalyzing the attachment of asparagine to its corresponding tRNA. Mutations in the human Nars1 gene have been associated with several neurological and developmental disorders. Elucidating the molecular basis of these conditions paves the way for the development of targeted therapies. In this study, we conducted dynamical analyses of missense variants classified as variants of uncertain significance (VUS) and associated with neurodevelopmental delay in various genomic databases, as well as a novel c.866A>G (Y289C) variant identified through whole-exome sequencing and verified by Sanger sequencing in a Turkish patient.
Materials and Methods: All-atom molecular dynamics (MD) simulations were used to monitor changes in local environments at the mutant sites, uncovering their effects on the overall structure–function relationship.
Results: Our results indicate that the Y289C and L350P variants exhibit altered structural motifs, with Y289C further inducing distortion of the global protein structure and dimerization. In this mutant, the C289–C546 disulfide bond was found to be feasible, which resulted in a rearrangement of the C-terminal loop orientation compared to wild type, displacing it from the interface consequently distorting the dimer configuration. In the L350P mutant, a shift in orientation of the F347 side chain was observed, which appears to stabilize the N-terminal loop of the opposing chain, thereby affecting the dimerization. The G132C mutant showed weakened structural coordination between the tRNA-binding domain and the Bira bifunctional domain, while the N221S mutant may compromise tRNA binding due to potential backbone clashes. Analysis of allosteric communication pathways corroborated the findings from neighborhood interaction analysis, revealing the absence of common links at the dimer interface in the mutant proteins.
Conclusion: Overall, this study advances the investigation of a set of Nars1 VUS variants, along with a novel variant, by shedding light on their molecular consequences and potential relevance to diseases.

Keywords: MD simulations, NARS1, VUS, neurodegenerative diseases

PP188
Breast Milk Microbiota–Derived Extracellular Vesicles as Next-Generation Nanotherapy for Breast Cancer

Seval Taliboglu[1], Fadime Kiran[2]

[1] Ankara University, Graduate School of Natural and Applied Sciences, Department of Biology, Ankara, Türkiye
[2] Ankara University, Faculty of Science, Department of Biology, Ankara, Türkiye

Background/Aim: Bacterial extracellular vesicles (EVs) have gained attention in cancer therapy due to their bioactive cargo and potential as non-invasive therapeutic agents. This study aims to investigate the anticancer potential of EVs derived from the breast milk microbiota member Pediococcus pentosaceus EIR/HM1-strain against breast cancer.
Materials and Methods: P. pentosaceus-derived EVs (PpEVs) were isolated from bacterial culture medium using an ultracentrifugation-based approach. Morphology was assessed by electron microscopy and atomic force microscopy, while biochemical characterization was performed using Qubit nucleic acid quantification and bicinchoninic acid protein assay. The cytotoxic potential of PpEVs was evaluated in breast cancer MCF-7 cells following 24 hours of treatment, using a cell viability assay. The antiproliferative effects of PpEVs on MCF-7 cells were investigated through migration and clonogenic assays. Intracellular reactive oxygen species (ROS) accumulation and apoptosis were analyzed by flow cytometry, while the effects of PpEVs on gene expression were assessed by quantitative PCR analysis.
Results: The obtained PpEVs exhibited a spherical bilayer lipid membrane structure and contained 1.5 µg/mL DNA, 6.86 µg/mL RNA, and a protein content of 480 µg/mL. The PpEVs exhibited cytotoxicity against MCF-7 cells, with an IC50 of 82 ± 2 µg/mL. Furthermore, the PpEVs showed strong antiproliferative effects on MCF-7 cells by reducing migration and clonogenic activity. The PpEVs induced early apoptosis of the MCF-7 cells, with significant regulation of apoptosis-related genes. Additionally, intracellular ROS levels increased, accompanied by changes in oxidative stress–related gene expression.
Conclusion: The results indicate that microbiota-derived EVs could serve as potential agents for the development of next-generation nanotherapeutic approaches in cancer treatment.

This work was supported by Ankara University Scientific Research Projects Coordination Unit (BAP) under Project No: FBA-2025-3985.

Keywords: Extracellular vesicles, Microbiota, Breast cancer, Nanotherapy,

PP189
Preliminary Findings on PBMC Mitochondrial Respiration and Metabolic Adaptation in Night-Shift Workers

Duygu Güçlü[1], Ceyda Ergül[2], Asuman Gedikbaşı[3, 4], Şükrü Anıl Dogan[5], Duygu Aydemir[4]

[1] Department of Nutrition and Dietetics, Faculty of Health Science, Bezmialem Vakif University, Istanbul 34065, Türkiye
[2] Department of Biotechnology, Institute of Health Sciences, Bezmialem Vakif University, Istanbul 34820, Istanbul, Türkiye
[3] Faculty of Medicine, Pediatric Nutrition and Metabolism Division, Istanbul University, Istanbul, Türkiye
[4] Department of Basic Sciences, Child Health Institute, Division of Genetics, Istanbul University, Istanbul, Türkiye
[5] Department of Molecular Biology, Beykoz Institute of Life Sciences and Biotechnology, Bezmialem Vakif University, Istanbul 34820, Türkiye

Background/Aim: Night-shift work disrupts the alignment between circadian rhythms and metabolic processes and may induce adaptive changes in cellular energy metabolism. This pilot study aimed to evaluate mitochondrial respiratory capacity in peripheral blood mononuclear cells (PBMCs) and serum biochemical, hematological, and anthropometric characteristics in night- and day-shift workers.
Materials and Methods: Three night-shift and three day-shift workers were included in the study. PBMCs were isolated and analyzed using high-resolution respirometry with the Oroboros O2k system. Routine respiration (R), leak respiration (L), and maximal electron transfer system (ETS) capacity (M) were assessed, together with the R/E, L/E, and (R−L)/E ratios. Serum biochemical and hematological parameters, anthropometric measures, including body mass index (BMI) and waist circumference, and bioelectrical impedance-derived body fat percentage and basal metabolic rate were also assessed.
Results: PBMC routine respiration (~8.5 vs. ~5.2 pmol $O_2 \cdot s^{-1} \cdot 10^6$ cells^{-1}) and maximal ETS capacity (~10.7 vs. ~7.5 pmol $O_2 \cdot s^{-1} \cdot 10^6$ cells^{-1}) were higher in night-shift workers. Although leak respiration showed an increasing trend, the L/E ratio remained comparable between groups, suggesting that the increased respiratory activity was not primarily attributable to increased proton leak. The higher R/E ratio (~0.80 vs. ~0.74) further suggested greater utilization of the available mitochondrial respiratory capacity by PBMCs. BMI, waist circumference, body fat percentage, basal metabolic rate, and serum glucose, total cholesterol, triglyceride, and LDL-cholesterol levels tended to be higher in night-shift workers.
Conclusion: These preliminary findings suggest that night-shift work may induce an adaptive bioenergetic response in PBMCs rather than suppressing mitochondrial oxidative metabolism. The increases in routine and maximal respiratory capacity, along with differences in metabolic parameters, may indicate cellular energy adaptation associated with circadian disruption. Integration of mitochondrial respiration data with BMAL1, CLOCK, PER, and PPAR gene expression profiles may provide mechanistic insight into the relationship between circadian misalignment, metabolic regulation, and cellular energy adaptation.

Keywords: Night-shift work, circadian rhythm, PBMC, mitochondrial respiration, bioenergetic adaptation, energy metabolism