TuPA
Turkish Proteomics Association

TuPA International Proteomics Congress
7th Turkish National Proteomics Congress

18-19 September 2025, İstanbul

İstanbul Technical University,
Süleyman Demirel Cultural Center

İTÜ

ABSTRACT BOOK

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September 18th, Thursday

09:00-09:30	**Opening Ceremony** *Gizem Dinler Doğanay* İstanbul Technical University *Nurhan Özlü* Koç University
09:30-10:30	**Session 1 - Biomolecules and Protein Folding** **Chair:** Aysel Özpınar
09:30-10:30	**Keynote Speaker** Using proteomics to understand multiscale biology and evolution. *Eugene Shakhnovich* Harvard University
10:30-11:00	The Proteostasis Index: A Framework for Healthy Aging and Resilience *Eugenia Clerico* University of Massachusetts Amherst
11:00-11:15	**Coffee Break**
11:15-13:00	**Session 2 - Structural Proteomics: Advances in Covalent Labeling, Crosslinking, Native MS, Cryo-EM, Crystallography** **Chair:** Gizem Dinler Doğanay
11:15-11:45	Analysis of the conformation, dynamics and interactions of proteins in solution by hydrogen-deuterium exchange mass spectrometry (HDX-MS) *Kasper Dyrberg Rand* University of Copenhagen
11:45-12:10	Beyond the Canonical Motifs: Mapping the Novel Contact Sites in the Bcl-2–Beclin 1 Complex *Miray Türk* Istanbul Technical University
12:10-12:35	Beyond the Numbers: Exploring the Edge of Peptide-Centric Proteomics *Süreyya Özcan Kabasakal* Middle East Technical University
12:35-13:00	Ion Mobility-Mass Spectrometry for Conformational Analysis of Biopharmaceuticals *Mehmet Atakay* Hacettepe Üniversity
13:00-14:30	**Lunch / Poster Presentation**

September 18th, Thursday

14:30-15:00	**Satellite Symposium**  A New Era in Protein Analysis: Ultra-High Resolution and Ultra-Fast Scanning Combined.
15:00-16:45	**Session 3 - Omics Technologies** **Chair:** Süreyya Kabasakal
15:00-15:25	Membrane proteins and proteomics: love is possible, but so difficult *Murat Kasap* Kocaeli University
15:25-15:50	Role of Long Non-Coding RNA X-Inactive-Specific Transcript (XIST) in Neuroinflammation and Myelination: Insights from Cerebral Organoids and Implications for Multiple Sclerosis *Alaattin Şen* Pamukkale University
15:50-16:15	4D proteomics™ in the post-genomic area Single-cell sensitivity and deep sequencing, extreme versatility, and comprehensive characterization with the new timsULTRA AIP and timsOMNI *Daniel Vlacil* Country Sales Manager LSMS Czech Republic & Slovakia
16:15-16:45	**Oral Presentations** **S01** Verification of Protein Biomarkers for Endometrial Cancer Using Mass Spectrometry *Öykü Su Yıldırım, Berat Doğan, Esin Şahin, Senem Arda Düz, Yıldırım Karslıoğlu, Murat Öz, Gamze Nur Cimilli Şenocak, Abdullah Karaer, Süreyya Özcan Kabasakal* **S02** Alterations of Endothelial Secretomes in Hypoxic Cancer *Özel Çapık, Ömer Faruk Karataş*
16:45-17:00	**Coffee Break** 
17:00-17:50	**Session 4 - Food Safety and Chemistry** **Chair:** Mehmet Atakay
17:00-17:25	From Molecules to Meals: Proteomics in Food Science *Beraat Özçelik* Istanbul Technical University
17:25-17:50	Integrating Proteomic Modalities to Secure Bee Products' Quality and Biofunctionality *Emir Akyıldız* Altıparmak Food Industry and Trade Inc., Dr.

September 19th, Friday

09:00-10:30	**Session 5 - Clinical Proteomics** **Chair:** Özge Can
09:00-09:30	The Mitochondrial Relay with the Nucleus: Form and Function *Michelangelo Campanella* Queen Mary University of London
09:30-10:00	Investigating the role of gut microbiome in the pathogenesis and development of Alzheimer's disease with a multiomics approach *Ahmet Tarık Baykal* Acibadem University
10:00-10:30	Implementing Single-Cell Proteomics as a Core Facility Service: Challenges, Strategies, and Impact *Virginie Sjoelund* Northeastern University
10:30-10:45	**Coffee Break** 
10:45-12:00	**Session 6 - Mass Spectrometry in Pharmaceutical Applications** **Chair:** Ahmet Tarık Baykal
10:45-11:05	Decoding the Sugar Code: How Glycomics is Reshaping Personalized Medicine *Hacı Mehmet Kayılı* Karabük University
11:05-11:25	Critical Quality Attributes (CQAs) and Quality Target Product Profile of Biosimilars *Beyhan Çabukel* Atabay Pharmaceuticals
11:25-11:45	New generation therapeutic peptides *Özge Can* Acıbadem Mehmet Ali Aydınlar University

September 19th, Friday

11:45-12:00	**Oral Presentations** **S03** Oxygen-Defined Drug Responses in Brain Endothelial Cells via Quantitative Proteomics *Sarah Barakat, Fan Yang, Hayriye Ecem Yelkenci, Kıvanç Kök, Giovanni E. Mann, Emrah Eroğlu*
12:00-13:00	**Lunch / Poster Presentation**
13:00-13:45	**Keynote Speaker** **Chair:** Nurhan Özlü Enabling precision medicine using multi-omics, AI, and systems biology *Adil Mardinoğlu* King's College London
13:45-14:00	**Biotechnology Valley** *Ercan Varlıbaş* Chairman of the Board of BIOSAD
14:00-14:45	**Biotechnology Industrialists Association (Biyosad) Presentation and Industry–University–Startup Collaboration Panel** **Moderatör:** Levent Doğanay **Panelists:** - Ercan Varlıbaş - Adil Mardinoğlu - Berk Özdemir - Doğan Taşkent - Ali Demir Seze

September 19th, Friday

14:45-16:15	**Session 7 - Future Prospects** **Chair:** Hacı Mehmet Kayılı
14:45-15:15	Unlocking Protein-RNA Interactions: Time-Resolved Serial Crystallography of Ribosome-Complexes as a Paradigm *Hasan Demirci* Koç University
15:15-15:45	Structural studies of membrane protein complexes involved in translocation and quality controll *Burak Kabasakal* Turkish Accelerator and Radiation Laboratory (TARLA)
15:45-16:15	Hsp70 chaperone accelerates protein folding *Rahmi İmamoğlu* Protein Scientists and Team Leader at Proteros PhD at Max-Planck Institut of Biochemistry
16:15-16:30	**Coffee Break**
16:30-17:05	**Session 8 - Metabolomics & Proteomics** **Chair:** Hasan Demirci
	Metabolomics in Motion: Fluxomics to Decoding Cellular Dynamics *Emirhan Nemutlu* Hacettepe University
17:05-17:35	**Oral Presentations**
	S04 Establishing Palaeoproteomics in Türkiye: ZooMS on Faunal Remains from Antandros *Seher Elif Mekik, Nur Banu Bayram, Dilara Temizkan, Eren Erişçi, Kahraman Yağız, Gürcan Polat, Ercan Arıcan*
	S05 Regulatory Role of EML1 in Microtubule Organization and Cell Division *Dilaray Tüfekçi, Helin Bahar Çalı, Berfu Nur Yiğit, Büşra Aytül Kırım, Valeria Viola, Fiona Francis, Nurhan Özlü*

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ORAL PRESENTATIONS ABSTRACTS

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S001
Verification of Protein Biomarkers for Endometrial Cancer Using Mass Spectrometry

Öykü Su Yıldırım[1], Berat Doğan[2], Esin Şahin[1], Senem Arda Düz[3], Yıldırım Karslıoğlu[4], Murat Öz[5], Gamze Nur Cimilli Şenocak[6], Abdullah Karaer[3], Süreyya Özcan Kabasakal[1]

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This study aims to validate previously identified markers for endometrial cancer (EC) as a critical step in biomarker development pipeline, and to assess their utility as diagnostic and prognostic markers.
Multiple reaction monitoring (MRM) was developed to quantify nine proteins: transgelin (Q01995), peptidyl-prolyl-cis-trans isomerase (Q00688), cyclophilin-A (P62937), macrophage-capping-protein (P40121), pyruvate-kinase-M1/M2 (P14618), glutathione-S-transferase-P (P09211), manganese-superoxide-dismutase (P04179), cytosol-aminopeptidase (P28838), and complement-component-C4A (P0C0L4). Isotopically labeled standards were used to optimize LC–MS parameters, refine transition selection, and minimize interferences, with analyses performed on LC-QQQ-MS. Peptide separation achieved using C18 column. Tissue samples from 23 endometrioid EC (EEC) and 20 non-endometrioid EC (NEC) patients and 26 controls were extracted, denatured, reduced, alkylated, and digested with Trypsin/Lys-C. Statistical analysis was performed using MSstatsShiny and R, including normalization, group comparison, PCA, PLS-DA, and ROC evaluation.
MRM–MS method was successfully developed with robust, reproducible analytical performance across nine proteins, with R^2 (0.9796–0.9992), limits-of-detection (9.1×10^{12} to 3.0×10^{9} μmol/μL), and limit-of-quantification (2.0×10^{8} to 1.0×10^{7} μmol/μL). Instrument stability (CV% = 0.3–15.8%) met EMA validation standards. In EEC-healthy comparison, P40121, Q00688, P14618, P28838, and P09211 were upregulated in EEC, whereas Q01995 and P0C0L4 were downregulated ($p < 0.05$). ROC analysis demonstrated strong diagnostic performance (AUC > 0.80) for P14618, P28838, Q01995, and P0C0L4. In NEC-healthy comparison, P40121, P14618, and P28838 were upregulated, while Q01995 was downregulated in NEC. P14618 exhibited strong diagnostic performance. There were not significant differences for subtype comparison. PCA and PLS-DA results were consistent with these findings.
This study presents first MRM–MS method for verifying EC-associated proteins. Method achieved robust analytical performance and reproducibility. High diagnostic performance of four proteins demonstrated potential for clinical validation. These findings provide a rationale for integrating these markers into clinical workflows for EC detection and lay groundwork for larger-scale clinical validation studies.

Keywords: Endometrial cancer, Targeted proteomics, Multiple reaction monitoring, Biomarker, Mass spectrometry

S002
Exosome-Mediated Proteomic Alterations of Endothelial Secretomes in Hypoxic Cancer

Ozel Capik, Omer Faruk Karatas

Erzurum Technical University, Molecular Biology and Genetics Department, Erzurum, Turkey

Purpose: Tumor microenvironment (TME) is a complex and dynamic milieu that consists of diverse cell types such as stromal cell components of the micromilieu and cancer cells, as well as extracellular matrix structural proteins and various signaling molecules. Tumor cells and its micromilieu respond to hypoxic stress by secreting hypoxia induced tumor derived exosomes (hiTDExs) that carry complex biological communication tools with the ability to orchestrate the behavior of endothelial cells and the fate of the tumor. Therefore, continuous efforts are being made to elucidate the specific functions of hiTDExs on endothelial cells and their contributions to the remodeling of the TME, notably in head and neck squamous cell carcinoma (HNSCC). This study aimed to elucidate how hypoxia-induced HNSCC-derived exosomes modulate the protein profile of recipient endothelial cells' secretome within the tumor micromilieu and contribute to tumor progression in HNSCC.

Method: We examined whether hiTDExs reprogram endothelial cells within the TME, thereby altering their secretome composition. Human umbilical vein endothelial cells (HUVECs) were treated with hiTDExs derived from FaDu cells. Human cytokine profiling and functional assays such as cell viability and cell migration potential revealed a tumor-promoting role of secretome-derived CCL26 in HNSCC, validated by neutralization experiments.

Findings: In HUVECs's secretomes treated with hypoxic exosomes from FaDu cells, 52 proteins were significantly altered compared to normoxic controls. ELISA confirmed a marked increase in CCL26 levels. Functionally, CCL26-enriched secretomes enhanced HNSCC cell viability and cell migration potential promoting pro-tumorigenic phenotypes within the hypoxic TME, while CCL26 neutralization abrogated these effects. These alterations suggest a pivotal role in remodeling the HNSCC microenvironment.

Conclusion: hiTDExs reprogram endothelial secretomes via CCL26 induction, driving HNSCC progression. This reveals a novel exosomal mechanism shaping the hypoxic TME and highlights CCL26 as a potential therapeutic target to restrict metastasis and tumor advancement.

Keywords: Exosome, Hypoxia, Cytokine, Secretome, Head and neck cancer, Proteomic profiling

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S003
Oxygen-Defined Drug Responses in Brain Endothelial Cells via Quantitative Proteomics

Sarah Barakat[1], Fan Yang[2], Hayriye Ecem Yelkenci[1], Kivanc Kok[1,3], Giovanni E. Mann[2], Emrah Eroglu1[,4]

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Purpose: Traditional cell culture is performed under atmospheric oxygen levels (18 kPa), which do not accurately reflect the lower oxygen levels experienced by most cells in vivo. This is particularly important for brain endothelial cells exposed to oxygen levels no higher than 5 kPa. Hyperoxia (18 kPa O_2) imposes cellular oxidative stress, potentially skewing research and drug screening outcomes. In this study, we aimed to elucidate how oxygen tension affects the proteomic profile and modulates drug-induced responses in cerebral endothelial cells.

Method: Cerebral microvascular endothelial cells (hCMEC/D3) were adapted for 5 days to either physiological normoxia (5 kPa O_2) or standard hyperoxic conditions (18 kPa O_2). Mass spectrometry data were acquired using a SYNAPT G2-Si instrument and subsequently analyzed for quantitative proteomic profiling to compare global protein expression between the two oxygen conditions. To investigate how oxygen tension modulates drug responses, we further examined the proteomic impact of sulforaphane (SFN), a known inducer of NRF2-mediated antioxidant defense pathways, under both 5 kPa and 18 kPa O_2.

Findings: Functional annotation analysis showed that proteins differentially expressed between 18 kPa and 5 kPa O_2 were significantly enriched in pathways associated with energy metabolism and oxidative stress. Notably, SFN treatment elicited minimal proteomic changes at 5 kPa O_2, whereas under hyperoxia, it induced enrichment of oxidative stress-related pathways.

Conclusion: These findings highlight the critical importance of culturing cells under physiologically relevant oxygen levels to improve the clinical translation of in vitro models. Understanding how pericellular oxygen levels affect the phenotype of cells is essential for the design of drug discovery and high-throughput screening protocols.

Keywords: Brain endothelial cells, Physiological normoxia, Hyperoxia, Sulforaphane, Proteomic

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S004
Establishing Palaeoproteomics in Türkiye: ZooMS on Faunal Remains from Antandros

Seher Elif Mekik[1], Nur Banu Bayram[2], Dilara Temizkan[2], Eren Erişçi[2], Kahraman Yağız[3], Gürcan Polat[4], Ercan Arıcan[2]

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Aim: This study aimed to implement the first palaeoproteomic application at a university centre in Türkiye. To this end, Zooarchaeology by Mass Spectrometry (ZooMS) approach was applied to five archaeological bone finds dated to the Hellenistic period from the Antandros Ancient City excavations (Balıkesir, Türkiye).

Methods: Bone samples were collected under sterile conditions during the 2024 excavations at Antandros. In addition, bone grave gifts from earlier seasons were delivered to our laboratory. Exterior decontamination was performed with acetone and distilled water. The decontaminated samples were then powdered using a Dremel tool. Type I collagen was obtained by HCl demineralisation procedure followed by gelatinisation in ammonium bicarbonate buffer (AmBic). Following isolation, A205/A280 spectrophotometric readings and the BCA assay provided a preliminary assessment of collagen quantity. The five samples were then submitted for MALDI-TOF MS analysis at Acıbadem University following trypsin digestion. The resulting mass spectras were analysed with the semi-automated SpecieScan tool.

Results: MALDI spectra were obtained from all five samples; in 4/5 specimens, candidate higher-taxon/species groups passed the correlation threshold (mostly terrestrial mammals). In 1/5 specimens, peptide motifs indicating a marine mammal were observed despite being below the threshold. Although collagen preservation varied among samples, the workflow produced results quickly and showed consistent similarity values across technical replicates.

Conclusion: This pilot demonstrates that a ZooMS-based palaeoproteomic application and palaeoproteomics research environment can be established in Türkiye. The findings suggest potential raw-material diversity in Hellenistic bone objects; however, low-correlation signals will undergo additional validation. Peptide signals from a bone hairpin deposited as a grave good that indicate a marine mammal suggest that, as today, bones from particular species may also have been valued in that period. With a larger sample set curated by our ongoing project, the importance of palaeoproteomics for archaeology will be further highlighted.

Keywords: Palaeoproteomics, Peptide Mass Fingerprinting, Zooarchaeology by Mass Spectrometry

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S005
Regulatory Role of EML1 in Microtubule Organization and Cell Division

Dilaray Tüfekçi[1,2], Helin Bahar Çalı[2], Berfu Nur Yiğit[2], Büşra Aytül Kırım[2], Valeria Viola[3,4,5], Fiona Francis[3,4,5], Nurhan Özlü[2,6]

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Microtubules and microtubule-associated proteins (MAPs) are crucial for regulating intracellular transport, spindle formation, and cell division, all of which are critical for brain development and cortical organization. Among these, EML1 is a MAP associated with subcortical heterotopia, yet its molecular role in microtubule organization and the consequences of disease-associated mutations remain incompletely understood. To investigate the role of EML1, CRISPR-Cas9-mediated knockout in HeLa cells and Eml1 conditional knockout neural progenitors were used. Exogenous expression of GFP-tagged wild-type or disease-causing T243A mutant EML1 was employed to assess mutation effects on microtubule binding and cellular phenotypes. A combination of microtubule pelleting assays, confocal immunofluorescence imaging, label-free quantitative proteomics, and live-cell imaging was applied to evaluate microtubule stability, MAP recruitment, spindle organization, and mitotic progression. Loss of EML1 reduced taxol-stabilized microtubules, increased soluble tubulin, decreased α-tubulin acetylation, and enhanced vulnerability to nocodazole-induced depolymerization. These changes were accompanied by diminished recruitment of other MAPs such as EML4 and SEPT2 to microtubules, despite unchanged protein levels. EML1 was required for EML4's association with the network; its absence significantly reduced EML4 binding, contributing to impaired organization and mitotic fidelity. Mitotic abnormalities such as spindle disorganization and prolonged division were rescued by wild-type Eml1 but not by the T243A mutant, which showed impaired binding and aggregation. Cell cycle-specific proteomic analysis revealed that EML1 associates with distinct protein sets during interphase and mitosis, indicating dynamic regulation. NEK9, a mitotic kinase not previously reported as an EML1 interactor, was reproducibly identified in Eml1-GFP pulldowns. Importantly, EML1 regulates the dynamic localization of EML4 during cell division; knockout cells showed aberrant EML4 accumulation on spindle and midzone microtubules, which was restored by wild-type Eml1. Overall, EML1 acts as a scaffold-like regulator of microtubule stability, MAP interactions, and mitosis. Its loss or mutation disrupts cytoskeletal integrity, offering mechanistic insight into Eml1-related heterotopia.

Keywords: EML1, microtubule organization, microtubule-associated proteins, EML4, acetylation, mitotic defects

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POSTER PRESENTATIONS ABSTRACTS

P001
Protein Profiling of White and Brown Adipose Tissue Organoids

Ezgi Bulut-okumuş, Selinay Şenkal-turhan, Ayşegül Doğan

Yeditepe Üniversitesi, Genetik ve Biyomühendislik Bölümü

Purpose: Adipose tissue functions in energy balance, thermogenesis, and immune modulation. Its impairment contributes to metabolic diseases like obesity and type 2 diabetes, highlighting its therapeutic relevance. Recent studies highlight the importance of neuromesodermal progenitors (NMPs), a bipotent stem cell population, in the embryonic origin of adipose tissue and its differentiation. This study aims to investigate the potential of iPSC-derived NMPs to generate brown (BAT) and white (WAT) adipose tissue organoids and to elucidate their embryonic origin and underlying molecular mechanisms.

Methods:iPSCs were differentiated into NMPs, which were then further directed toward paraxial and lateral plate mesodermal lineages to generate BAT and WAT organoids, respectively. Protein profiling was performed using LC-MS/MS to analyze the total proteome composition of NMP, PM, LPM, and organoid groups. Heatmap visualization and principal component analysis assessed correlations and clustering among groups. Proteins upregulated in the organoid groups were further analyzed for their associated biological processes and molecular functions using the PANTHER classification system,while pathway enrichment was evaluated via KEGG analysis using ShinyGO. Additionally, protein-protein interaction networks were constructed and visualized through STRING analysis

Results: Proteomic analysis revealed dynamic expression of mesodermal markers (e.g., TBXT, FOXF1) and adipogenic regulators across differentiation stages. BAT organoids showed enrichment of mitochondrial proteins, while WAT organoids displayed increased expression of proteins associated with vacuole- and endosome-related pathways. Correlation and heatmap analyses indicated that mesoderm groups correlated closely with NMPs, and BAT organoids were more similar to their mesodermal origin than WAT organoids, suggesting a stronger lineage-specific differentiation at the proteomic level.

Conclusion: These findings demonstrate that iPSC-derived adipose organoids recapitulate key structural and molecular features of native adipose tissue, providing a physiologically relevant model for adipogenesis research.

Note: This study was supported by Scientific and Technological Research Council of Turkey (TUBITAK) under the Grant Number 222S560 (Project No: 222S560).

Keywords: adipose tissue, organoid, induced pluripotent stem cells

P002
Proteomics Analysis of BIA Biosynthesis in Turkish Opium Poppy Cultivars

İrem Taşdemir[1], Anne Frary[1], Sami Doğanlar[1], Bernhard Kuster[2], Fatih Leblebici[3]

[1] İzmir Institute of Technology
[2] Munich Technical University
[3] Turkish Grain Board

Purpose: Benzylisoquinoline alkaloids (BIAs), including morphine, thebaine, and noscapine, are key pharmaceutical compounds derived exclusively from the opium poppy (Papaver somniferum). This study aimed to investigate the proteomic basis of cultivar-specific BIA accumulation by analyzing differentially expressed proteins (DEPs) in five tissues of three Turkish cultivars bred for high morphine (Ofis 2), noscapine (Ofis NM), and thebaine (TMO T) content.

Method: Protein samples from mature/young capsules, latex-containing stems/latex-free stems, and roots were subjected to LC-MS/MS analysis. Differential expression was evaluated using MaxQuant and Perseus softwares. Alkaloid content was quantified by TLC-HPLC. GO and KEGG enrichment analyses were used for functional interpretation of DEPs (Figure1).

Results: A total of 8,820 proteins were identified, with mature capsules exhibiting the highest number of DEPs. Proteins involved in morphinan, noscapine, and papaverine biosynthesis showed cultivar-specific expression (Figure2). Key enzymes including T6ODM, SalAT, and COR varied in abundance across capsules and stems. Enrichment analyses revealed distinct biological processes associated with morphine and noscapine accumulation.

Conclusion: This study reveals clear proteome signatures across tissues and cultivars that correlate with BIA content. The identification of tissue-specific regulatory proteins and metabolic shifts provides targets for further functional validation. Furthermore, this work supports the strategic improvement of Turkish opium poppy cultivars for higher pharmaceutical yield. Such advancements will strengthen Türkiye's global competitiveness in legal opium production and contribute to the sustainable cultivation of this economically-vital crop.

Keywords: opium poppy, benzylisoquinoline alkaloids, BIA biosynthesis, proteomics, metabolomics

P003
Membrane Array-Based Protein Profile Analysis Of Induced Pluripotent Stem Cells

Hazar Eren Soydan, Ezgi Bulut-okumuş, Selinay Şenkal-turhan, Samed Özalay, Türkiz Sundu, Ayşegül Doğan

Yeditepe Üniversitesi

Aim: Induced pluripotent stem cells (iPSCs) offer great potential in disease modeling, drug discovery, and personalized and regenerative medicine due to their capacity to differentiate into various cell types while maintaining patient-specific genetic identity. Comprehensive characterization of their proteomic landscape is essential for advancing their translational applications. In this study, the proteome of iPSCs was investigated by using the RayBio® L-Series Human Antibody Membrane Array, which targets 1000 human proteins. Among these, 629 proteins were detected with clear signal intensity. From this pool, 165 highly expressed proteins were selected based on intensity and functional relevance for downstream analysis.

Method: Bioinformatics tools including STRING and PANTHER were used to characterize the protein interactions and associated biological pathways. The results revealed enrichment in key processes such as chemotaxis, cell adhesion, and intracellular signal transduction. Notably, several chemotaxis-related proteins showed strong expression, suggesting intrinsic capacities for microenvironmental sensing and immunomodulation. Additionally, the "binding" molecular function—covering protein-protein, receptor-ligand, and cytoskeletal interactions—was highly represented, indicating a proteome primed for dynamic cellular communication.

Results: These findings provide valuable insights into the molecular landscape of undifferentiated iPSCs and underscore their readiness to interact with external cues. The use of membrane-based antibody arrays enabled high-throughput and functional profiling of the iPSC proteome, supporting their utility in stem cell research, drug discovery, and tailored medical approaches.

Conclusion: these data reinforce the significance of proteomic profiling in iPSCs and highlight their promise in advancing personalized medicine through a deeper understanding of their inherent biological networks.

Keywords: iPSCs, Membrane Array, proteomic analysis

P004
Effects of Low-Fat Yogurt Consumption on Lipidomic Profiles in Prediabetes

Meşkure Pak[1], Ayşe Lamia Öztürk[1], Ozan Emre Eyupoğlu[2], Mazhar Müslüm Tuna[3], Esma Merve Arda Özkan[3], Neslihan Karaağaç[4]

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Aim: Lipidomics helps monitor nutrients' short-, medium- and long-term effects to make nutritional recommendations. Thus, it elucidates the interaction between disease, nutrition, and lipid metabolism. This study aims to investigate the impact of low-fat yogurt consumption on glycemic and lipid profiles in individuals with prediabetes.

Methods: Eight volunteers aged 25–60 years, diagnosed with prediabetes and without severe comorbidities, were recruited from the Endocrinology Polyclinic of Umraniye Training and Research Hospital (Istanbul, Türkiye) between May and August 2024.
Ethical approval was granted by the Ethical Committee of Istanbul Medipol University (Decision no. 2021-928), and all participants signed an informed consent form before intervention. Participants consumed 150 grams of low-fat yogurt daily for four weeks. Blood samples were collected before and after the intervention. Statistical analysis was performed using SPSS 21.0 with a significance level of $p<0.05$. Untargeted lipidomic profiling was conducted using LipidSearch 4.2 SP1 and Compound Discoverer 3.3 SP3 (Thermo Scientific).

Results: Decreases in fasting blood glucose (102.0±12.1; 98.0±10.3 mg/dL) and HOMA-IR (3.85±1.7; 3.71±1.7) were observed but were not statistically significant ($p>0.05$).
Fatty acids, glycerolipids, phospholipids, prenol lipids, sphingolipids, and sterol lipids were detected in the investigation. The most abundant lipid classes were triglycerides (TG, n=135), phosphatidylcholine (PC, n=112), ceramides (Cer, n=94), phosphatidylethanolamine (PE, n=63), diacylglycerols (DG, n=58), sphingomyelin (SM, n=48), phosphatidylserine (PS, n=48) and phosphatidylinositol (PI, n=44). At the end of the study, a significant increase was detected only in one lipid type from the phosphatidylcholine class (PC(14:1/22:2) ($p<0.01$) involved in insulin signaling.

Conclusion: Daily low-fat yogurt consumption may improve insulin resistance in individuals with prediabetes by inducing favorable changes in the lipidomic profile, particularly through increasing phosphatidylcholine levels associated with insulin sensitivity.

Keywords: Lipidomics, Low-fat Yogurt, Prediabetes

P005
Structural Analysis of A.vinelandii NifW Using CD Spectroscopy and SAXS

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Background/Aim: Nitrogen can be utilized by organisms in the form of ammonia, produced through biological nitrogen fixation. This process is catalyzed by nitrogenase enzymes found in diazotrophic bacteria such as Azotobacter vinelandii and Klebsiella pneumoniae [1]. The NifW is one of the assembly factors involved in the maturation of the MoFe protein within the nitrogenase [2]. Previous studies suggest that NifW plays a conformational role in nitrogenase activity [3] and supports a protective role for nitrogenase against oxidative damage. In previous studies, NifW was expressed and purified recombinantly in E. coli [4]. Despite its functional significance, it hasn't yet been structurally characterized. This study aims to determine the solution stability of the recombinant NifW and investigate its structural properties by using Circular Dichroism (CD) Spectroscopy and Small Angle X-Ray Scattering (SAXS).

Method: NifW from Azotobacter vinelandii was overexpressed in E. coli BL21 (DE3) cells and purified with affinity chromatography and size exclusion chromatography. CD spectroscopy and SAXS measurements were performed under different pHs (4.0,6.0,7.0,8.0) and temperatures (10-90°C), and structural findings were compared with the theoretical models predicted by AlphaFold2.

Results: CD spectroscopy analysis revealed that the structure of NifW is disrupted at pH4.0. Both CD spectroscopy and SAXS analyses indicated that the protein maintains structural stability up to 40°C, while higher temperatures cause significant unfolding. Experimental CD spectroscopy data compared with the AlphaFold2 model via PDBMD2CD, providing experimental validation of the secondary structure of NifW. SAXS-based DAMMIF modeling further revealed distinct oligomeric states of NifW.

Conclusion: NifW protein has been shown to play a stabilizing/protective role for nitrogenase in the literature. Although it has been recombinantly produced and purified, its structural properties haven't yet been characterized. The data obtained in this study not only provide insights into the solution stability of the protein but also present the first structural information on NifW.

Keywords: NifW, SAXS, CD spectroscopy, structural analysis, recombinant protein, nitrogen fixation

P006
The Untargeted Serum HR-MS Analysis of Acute Lymphoblastic Leukemia Patients

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Aim: Acute lymphoblastic leukemia (ALL) is the most prevalent blood cancer in children, and its various subtypes reflect the unique genetic and environmental influences on each patient. Tackling this complexity, high-resolution mass spectrometry is commonly used as an effective tool in metabolomics. The innovative method reveals essential metabolic changes associated with cancer, paving the way for personalized treatment strategies that cater to individual needs.

Method: Untargeted serum metabolomic analysis was conducted on 15 pediatric ALL patients and 15 healthy controls using high-resolution mass spectrometry following standard methanol protein precipitation. Data preprocessing and statistical analyses were performed using TidyMass and MetaboAnalyst in R. Metabolite annotation was done by comparing spectra with public databases. To elucidate the dysregulated metabolic pathways, KEGG enrichment analysis was conducted.

Findings: A total of 11 distinct metabolites were identified as serum biomarker candidates for patients with ALL. Upregulated metabolites included diacetyl, 1-methyladenine, and N-acetylneuraminate (the most significant increase); downregulated metabolites included L-alanine, N-acetyl-L-tyrosine, O-hydroxy hippuric acid, N-methylnicotinamide, 4-methylcatechol sulfate, pyruvatoxime, 3,4-dihydroxy-L-phenylalanine, and nicotinamide-N-oxide. Pathway enrichment analysis revealed alterations in selenocompound metabolism; amino acid metabolism (alanine, aspartate, and glutamate; tyrosine); and amino sugar and nucleotide sugar metabolism.

Conclusion: The recent untargeted metabolomics study conducted on a small pediatric population with ALL revealed significant disturbances in energy, amino acid, and nucleotide metabolisms. The key differential molecules belong to the protein class, highlighting the crucial role of protein metabolism in the disease pathogenesis. While further validation through targeted multiomic workflows is essential, these findings could pave the way for identifying robust biomarkers, which can enhance the understanding and treatment strategies for pediatric ALL.

Keywords: Acute lymphoblastic leukemia, metabolomics, serum profiling, mass spectrometryx

P007
Absolute Quantification of Peptide Biomarker by Isotope Dilution-Mass Spectrometry

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Aim: Meat adulteration raises ethical, religious, and economic concerns while endangering the safety and trust of consumers. Conventional DNA-based polymerase chain reaction (PCR) and protein-based enzyme-linked immunosorbent assay (ELISA) techniques frequently give unreliable results for processed meat products due to denaturation and degradation risks. This research aims to establish a LC-MS/MS-based approach for sensitive detection of pork-specific peptide biomarkers, with a particular focus on processed meat products.

Method: The candidate reference materials (RMs), containing 1%, 5%, and 10% pork meat in beef matrix, as well as 100% pork meat were prepared. The conditions of protein extraction and digestion from 100% pork candidate RM were optimized. The resulting peptides were subjected to proteomic analyses by bioinformatic tools to identify pork-specific biomarkers, and the most abundant peptide was selected as quantitative target. The selected biomarker peptide was obtained synthetically and value assigned by Peptide Impurity Corrected Amino Acid Analysis (PICAA). Quantification of the biomarker peptide in protein digests of the candidate RMs was achieved by isotope dilution mass spectrometry (ID-MS), using a stable isotope-labeled (SIL) analogue of the peptide to construct calibration curves.

Results: The generated calibration curves exhibited high linearity for target peptide, with R2 values consistently above 0.999 across different days of analysis. The robustness of the method was confirmed across all selected adulteration levels by averaging the biomarker concentrations obtained from independent analyses. A single calibration curve was constructed for target biomarker, demonstrating that it could still be reliably detected and quantified even at 1% level.

Conclusion: The candidate RMs enabled reproducible quantification with low variation between sample replicates. The developed approach demonstrated high sensitivity and reliability, allowing accurate detection of pork meat adulteration even at low levels. These results supported the applicability of peptide biomarkers as effective tools for proteomic studies addressing meat authentication issues.

Keywords: Meat adulteration, Isotope dilution mass spectrometry ID-MS, Liquid chromatography-tandem mass spectrometry LC-MSMS, MS-based targeted proteomics, Quantitative proteomics

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P008
Lipidomic Characterization of Plasma Lipids in Hereditary Hemolytic Anemia Patients

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Aim: Hereditary hemolytic anemias (HHAs) are a group of rare genetic disorders characterized by premature red blood cell (RBC) destruction, leading to chronic anemia and related complications. RBC membrane lipids are crucial for cellular stability and function. Although alterations in lipid metabolism have been implicated in HHA pathophysiology, comprehensive lipidomic studies in these conditions remain limited. Here we present our untargeted plasma lipidomic findings in HHA patients.

Method: A total of 31 HHA patients including β-thalassemia (N=7), hereditary spherocytosis (N=7), sickle cell anemia (N=5), congenital dyserythropoietic anemia (CDA) (N=7), and hereditary xerocytosis (N=5) were involved in the study. The control group involved healthy individuals (N=20). Plasma lipids were extracted using the MTBE method and analyzed via LC-qTOF-MS.

Results: Three hundred seventy-five lipid species were identified by untargeted lipidomic profiling combined with multivariate statistical analysis via MetaboAnalyst. While PCA showed limited separation, PLS-DA revealed relatively clearer group discrimination. In comparison between all patients and controls, the model showed strong performance (R^2 = 0.9729, Q^2 = 0.4969). Similar trends were observed in subgroup analyses: β-thalassemia and sickle cell anemia (BT+SC) vs controls (R^2 = 0.9707, Q^2 = 0.5235), and hereditary spherocytosis and xerocytosis (HS+HX) vs controls (R^2 = 0.9906, Q^2 = 0.4532). Key discriminatory lipids with high VIP scores (>1.5) included sphingolipids (e.g., Cer 59:7;4O, SM 43:1;2O), glycerolipids (e.g., TG 57:3, DG 39:2), and phospholipids (e.g., PC 46:1, PC O-38:7).

Conclusion: Due to the non-normal distribution of the data, a non-parametric Kruskal-Wallis test was performed to compare the six groups. Among 375 lipids identified through MS analysis, 33 showed statistically significant differences with a p-value < 0.05.
This study provides the first comprehensive lipidomic characterization of plasma lipids in the plasma samples from various HHA patients.

The study has been granted by Hacettepe University Scientific Projects Coordination Unit (THD-2024-20908).

Keywords: Lipidomics, Untargeted Lipidomics, Hereditary Anemia, Plasma, RBC, Erythrocytes

P009
LC-MS/MS-Based Comparative Profiling of Post-Translational Modifications in mAbdi2 and Innovator

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Background/Aim: This study aimed to evaluate and compare the physicochemical properties of mAbdi2, a biosimilar candidate, with its reference innovator product. Post-translational modifications (PTMs) are critical quality attributes (CQAs) that can influence monoclonal antibody structure and function. Therefore, the extent and specific sites of PTMs in mAbdi2 were analyzed and assessed in relation to functional assay outcomes.

Method: An LC-MS/MS-based analytical workflow was employed for structural characterization. LC-MS top-down analysis was performed under both glycosylated and de-glycosylated conditions to confirm the primary structure. LC-MS/MS bottom-up peptide mapping was conducted for sequence verification, PTM profiling, and disulfide bond mapping to assess higher-order structural integrity. Additional targeted analyses included oxidation profiling in complementarity-determining regions (CDRs), glycosylation site confirmation, and evaluation of terminal modifications. Comparative studies involved PBS incubation, glutaminyl–peptide cyclotransferase (QPCT) enzyme treatment for pyroglutamate formation, and Carboxypeptidase B (Cp-B) enzyme treatment for C-terminal lysine removal. Charge variant profiling and relative potency assays were performed before and after enzymatic treatments.

Results: Peptide mapping confirmed sequence similarity between mAbdi2 and the innovator product, comparable oxidation profiles in CDRs, matching glycosylation sites, and consistent disulfide bond patterns indicative of proper folding and stability. The heavy chain of mAbdi2 showed a higher conversion of Gln to pyroglutamate compared to the innovator product. PBS incubation and QPCT treatment supported these findings. A relatively lower percentage of C-terminal lysine truncation was observed in mAbdi2, correlating with charge heterogeneity. Cp-B treatment eliminated charge heterogeneity, and potency assay results showed no significant difference between samples.

Conclusions: The LC-MS/MS workflow demonstrated a high degree of structural and functional comparability between mAbdi2 and the innovator product across all evaluated PTMs. Terminal modifications, including pyroglutamate formation and lysine truncation, did not significantly impact the biological activity of mAbdi2.

Keywords: Biosimilar Monoclonal Antibodies, Mass Spectrometry Characterization, Post-Translational Modifications, Critical Quality Attributes, Top-Down, Bottom-Up, Structural Analysis, Complementarity Determining Regions CDRs

P010
Profiling of IgG Glycopeptides in Non-Small Cell Lung Cancer

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Aim: The aim of this study was to quantitatively analyze IgG glycopeptides in serum samples from patients with non-small cell lung cancer (NSCLC) and healthy controls using liquid chromatography–tandem mass spectrometry (LC–MS/MS). The primary objective was to investigate whether specific glycosylation changes could serve as potential biomarkers for distinguishing NSCLC from non-cancer states.

Methods: Serum samples were collected from oncologically confirmed NSCLC patients and healthy controls. IgG was enzymatically digested with trypsin to obtain glycopeptides. IgG glycopeptides were characterized using nano-liquid chromatography–tandem mass spectrometry (nLC–MS/MS). Transitions were optimized for major glycoforms, and data processing was performed using glycopeptide-specific software. The relative abundance of each glycoform was calculated, and statistical analyses were conducted to compare cancer and control groups.

Results: Quantitative profiling revealed distinct differences in glycosylation patterns between patients with non-small cell lung cancer (NSCLC) and the control group. Changes in glycoform levels were determined using MSFragger and Skyline software. Only the statistically significant changes were considered. According to the results of multivariate analysis, specific combinations of glycoforms were able to distinguish NSCLC patients from the control group with high accuracy.

Conclusion: The LC–MS/MS method provided sensitivity and reproducibility for measuring IgG glycopeptides in serum. The distinct glycosylation changes observed between NSCLC patients and healthy controls indicate that IgG glycopeptide profiling could be used as a minimally invasive biomarker strategy for NSCLC detection. However, further studies with larger participant cohorts are needed to evaluate and validate the clinical applicability of these findings.

Keywords: Glycosylation, Glycopeptide, Mass Spectrometry, Biomarker

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P011
Iron Release from Transferrin Complexed with Meningitidis Transferrin-Binding-Proteins: QM/MM Calculations

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Aim: *Neisseria meningitidis* is a bacterium responsible for meningitis and meningococcemia. This pathogen requires ferric ions for survival and virulence, acquires Fe^3 directly from the C-lobe of human transferrin (hTf) via its transferrin-binding-proteins TbpA and TbpB. Understanding the molecular mechanism of Fe^3 release from hTf triggered by TbpA–TbpB binding is critical for the design and development of new therapeutics. Therefore, the purpose of this study is to provide computational insight into the Fe3+ release by meningitidis, focusing on the role of Lys359 in TbpA.

Method: Point mutations of Lys359 to alanine, arginine, and aspartic acid were constructed using TbpA-TbpB-hTf complex [1]. A large cluster of this complex was truncated including 12 Å surrounding of Fe. ONIOM QM/MM optimizations were performed using Gaussian 09, employing M06/6-31G(d,p) method for the QM region and AMBER force field for the MM region. Binding energies of Fe^3 to the TbpA–TbpB–hTf mutant complexes were calculated.

Results: Comparison of binding energies of mutant proteins to the wild-type (Lys359) verified that introducing a positively charged residue (Arg) destabilizes the holo protein, thus disfavoring Fe binding while negatively charged (Asp) mutation leads to the opposite effect. Results also supported our hypothesis that the Lys359 provides the acidic proton required for the disruption of Fe coordination as if mimicking the pH decrease in the endosome during iron release into the cell.

Conclusion: This work provides molecular insight into Fe^3 release in Neisseria meningitidis and highlights Lys359 as a potential target for therapeutic intervention in meningococcal infections.

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Keywords: Binding Energy, Neisseria Meningitidis, Quantum MechanicsMolecular Mechanics

P012
Salivary IgG Glycopeptide Profiling as Non-Invasive Biomarker for Biological Age

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Aim: This study evaluates the potential of the salivary IgG glycopeptide profile as a biomarker for biological age by comparing it with the serum IgG profile.By identifying age-associated glycopeptide structures in both biofluids, it aims to assess the feasibility of saliva as a non-invasive alternative to blood-based glycan analysis. Additionally, it examines variations in the serum IgG N-glycome by sex and age and determines the optimal storage temperature for saliva samples.

Methods: IgG stability tests were conducted to identify optimal saliva storage conditions. Samples were stored at -80, -20, +4, and +25 °C for 1, 5, and 15 days. Glycopeptide profiles were then generated and compared. IgG was isolated from dried blood spot (DBS) cards and saliva using protein G Sepharose beads. Glycopeptides from both sources were characterized by nano-liquid chromatography–tandem mass spectrometry (nLC–MS/MS).Salivary and serum IgG glycosylation patterns were statistically compared to evaluate correlations between glycan compositions. Serum IgG N-glycans were analyzed at the glycan level using hydrophilic interaction liquid chromatography with fluorescence detection (HILIC–HPLC–FLD).

Results: Stability tests showed -80 °C as the optimal saliva storage condition. Specific salivary IgG glycopeptides were identified that corresponded to serum structures. Several age-associated glycopeptide traits were conserved between biofluids. Statistical analysis revealed significant similarities in glycopeptide patterns, with some showing strong correlations between saliva and serum IgG. Serum IgG N-glycan profiles varied with age and sex.

Conclusion: Salivary IgG glycopeptide profiling shows promise as a non-invasive biomarker for biological age. Correlations between salivary and serum IgG glycopeptides support saliva-based analysis for aging research and clinical use. Further studies are required to develop predictive models and confirm the robustness of salivary IgG glycopeptides as aging biomarkers, as well as to clarify the influence of sex and age on serum IgG N-glycan profiles.

Keywords: Saliva, Immunoglobulin G, Biological Age, Serum

P013
Effect of Recombinase&Checkpoint Kinase Inhibitors On Radiosensitivity in Prostate Cancer

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Aim: Castration-resistant prostate cancer (CRPC) has a poor prognosis and recently, DNA repair mechanisms have been targeted in new treatment regimens. RAD51 recombinase is the key protein of recombinational repair process. Wee1 kinase is a negative regulator of mitosis. The purpose of the study was to determine the effectiveness of Rad51 and Wee1 inhibition on radiosensitivity in CRPC cells in vitro.

Methods: Cultured metastatic prostate cancer cells (PC-3) were exposed to ionizing radiation with pre-treatment of Rad51 inhibitor (B02) and/or Wee1 inhibitor (AZD1775). Cell survival was determined by MTT assay and clonogenic assay. The apoptotic rate of the cells was measured by flow cytometry following Annexin V-FITC/PI staining. One-way ANOVA with post-hoc Tukey's test was used for intergroup comparison.

Results: AZD1775 and B02 increased the radiosensitivity of PC-3 cells as single treatments; so did combined treatment ($p<0.001$). AZD1775 strongly decreased PC-3 cells' viability, while B02 had a moderate effect ($p<0.001$, $p<0.01$). The combined treatment reduced PC-3 cell viability more than either single treatment ($p<0.001$). AZD1775 strongly decreased colony formation in PC-3 cells, while B02 did less ($p<0.001$, $p<0.05$). The combined treatment reduced colony formation more than both single treatments ($p<0.001$). AZD1775 strongly increased the entry into early apoptosis of PC-3 cells, while B02 did so less ($p<0.001$, $p<0.05$). The combined treatment also significantly increased apoptosis ($p<0.001$).

Conclusion: The evidence of this study showed that AZD1775 and B02 are promising radiosensitizing agents for prostate cancer cells. Therefore, single or combined therapy may be a novel strategy to improve the clinical outcome in patient with CRPC.

Keywords: dna damage, dna repair proteins, radiosensitivity, castration-resistant prostate cancer

P014
OCB-Defined MS IgG Glycosylation and Prospective Biochemistry Integration

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Background: Multiple sclerosis (MS) is a heterogeneous neuroinflammatory disease in which oligoclonal band (OCB) patterns provide valuable diagnostic information. Fc-linked IgG glycosylation modulates immune effector functions and has been associated with disease activity in autoimmune conditions. Translating glycan signatures into clinically useful biomarkers requires integration with routine clinical biochemistry parameters obtained from serum.

Objective: This study aims to establish the groundwork for developing glyco-biomarker panels capable of predicting MS disease course. We profiled serum IgG glycosylation from MS patients stratified by five distinct OCB profiles and will integrate these glycan signatures with selected patient-specific clinical biochemistry parameters to identify potential predictive markers.

Methods: IgG was purified from serum using Protein G affinity chromatography, followed by enzymatic release of N-linked glycans and MALDI-TOF MS analysis. Glycan structures were classified according to fucosylation, galactosylation, and sialylation features. In the planned next phase, these glycan datasets will be correlated with available biochemical parameters such as C-reactive protein (CRP), total cholesterol, HDL cholesterol, triglycerides, fasting glucose, creatinine, ALT, AST, albumin, vitamin D, ferritin, and homocysteine, depending on test availability for each patient.

Preliminary Results: OCB-defined patient groups exhibited distinct IgG glycosylation profiles, with significant differences observed in galactosylation, sialylation, and the abundance of bisected N-glycans. These preliminary findings suggest that specific glycan alterations may reflect underlying disease mechanisms and could serve as candidates for future biomarker development.

Future Directions: We will combine glycan profiles with selected biochemical markers to explore their predictive value in MS subtype classification and progression risk. This integrated approach may enable the development of clinically applicable biomarker panels to support patient stratification and personalized therapeutic strategies.

Keywords: MALDI-TOF, Immunoglobulin G, Glycosylation, Multiple Sclerosis, Biomarker

P015
NK-92-Induced Secretome Remodeling in Luminal A and TNBC Cells

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Objective: Variation in immune microenvironment across breast cancer subtypes can shape their response to immune-driven cytotoxic mechanisms. This study aimed to evaluate natural killer (NK)-92 cell–mediated cytotoxicity and secretome proteomic alterations in Luminal A (MCF-7) and triple-negative (MDA-MB-231) breast cancer cells.

Methods: MCF-7 and MDA-MB-231 cells were exposed to NK-92 cells at effector-to-target (E:T) ratios that achieved roughly 50% loss of viability, as determined by WST-1 assays (5:1 for MCF-7; 10:1 for MDA-MB-231). After 4 hours of co-culture, conditioned media were harvested for LC-MS/MS analysis to detect secreted proteins with fold changes ≥1.5 relative to untreated controls. Functional enrichment was performed using KEGG pathway analysis via Enrichr platform.

Results: WST-1 assays revealed significant, dose-dependent reductions in viability for both subtypes at the optimized E:T ratios ($p<0.05$), with MCF-7 cells showing greater sensitivity. Secretome profiling identified 23 significantly altered proteins in MCF-7+NK-92 and 12 in MDA-MB-231+NK-92 co-cultures compared with controls. In MCF-7, enriched pathways were mainly related to ferroptosis and complement/coagulation cascades. In MDA-MB-231, pathway enrichment was predominantly associated with lipid metabolism and PPAR signaling. Ferroptosis was the only pathway found to be enriched in both models.

Conclusion: Interaction with NK-92 cells triggers distinct patterns of cell killing and secretome remodeling in Luminal A and triple-negative breast cancer models. Luminal A cells predominantly activate oxidative stress– and complement-associated pathways, whereas triple-negative cells undergo metabolic reprogramming linked to lipid processing. These subtype-dependent responses point to immune-metabolic mechanisms that could be exploited to improve the effectiveness of NK cell–based therapeutic strategies in breast cancer.

Keywords: NK-92 cells, breast cancer subtypes, Luminal A, triple-negative breast cancer, secretome proteomics, lipid metabolism, ferroptosis, NK cellbased immunotherapy

P016
Proteomics and Molecular Docking of Lacticaseibacillus rhamnosus-Syringic Acid Interaction

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Aim: Probiotics are "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host". Furthermore, certain phenolic compounds in foods and beverages can exert beneficial effects on human health. The interaction between these two health-promoting components can result in more advantageous combinations, termed synbiotics, thereby positively influencing the gut microbiota. Thus, this study aimed to investigate the effects of the phenolic compound syringic acid on the probiotic bacterium Lacticaseibacillus rhamnosus (LGG) using proteomics and bioinformatics approaches.

Methods: Effects of syringic acid on physiological characteristics of LGG, including microbial growth, aggregation, antioxidant activity, and adhesion, were determined. Then, potential effects on probiotic metabolism were elucidated through proteomic analyses. Finally, proteins identified in the proteomic analysis were subjected to molecular docking to investigate the conformations of interactions between syringic acid and probiotic proteins. All results were evaluated using appropriate statistical methods.

Results: Physiological effects: Syringic acid did not inhibit microbial growth, and the highest effects on aggregation and adhesion were observed at a concentration of 1000 μg/L. In the synbiotic interaction, the antioxidant activity of LGG was enhanced. Proteomics: Two predicted proteins, putative HNH nuclease YajD and WelJ, were identified based on proteomic data, although their functions have not yet been experimentally confirmed. Docking: Molecular docking suggested stable interactions between syringic acid and these proteins, indicating potential binding orientations.

Conclusion: In conclusion, syringic acid positively influenced key probiotic traits of LGG, particularly aggregation, adhesion, and antioxidant activity without inhibiting growth. Proteomic and molecular docking analyses revealed potential targets that may mediate these effects. These findings suggest syringic acid could serve as a functional synbiotic component, requiring further studies to clarify its molecular mechanisms and health-promoting potential.

Acknowledgment: This study was supported by Health Institutes of TÜRKİYE (TÜSEB) (37713).

Keywords: Lacticaseibacillus rhamnosus, Molecular Docking, Proteomics, Syringic Acid, Exoproteome

P017
Resveratrol in Parkinson's Disease: A Transcriptomics-Guided Approach

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Aim: Parkinson's disease (PD), characterized by the loss of dopaminergic neurons, oxidative stress, and mitochondrial dysfunction, requires the development of accessible in vitro systems for mechanistic studies and preclinical examinations. We identified resveratrol as a candidate therapeutic modulator guided by integrated transcriptomic analyses of SH-SY5Y and LUHMES cells treated with 6-hydroxydopamine (6 OHDA) and 1-methyl-4-phenylpyridinium (MPP+). This study primarily aims to evaluate resveratrol's capacity to mitigate neurotoxicity using a retinoic acid (RA)-differentiated SH-SY5Y model for PD.

Methods: Transcriptomic datasets (GSE196190, GSE229460, GSE203522, and GSE198009) from SH-SY5Y and LUHMES cells treated with 6-OHDA and MPP+ were analyzed using R to identify differentially expressed genes and network-level interactions. DrugBank was utilized to identify drug-target correlations and create a list of potential medications. For in vitro validation, SH-SY5Y cells differentiated with RA (10 μM) were exposed to MPP+ (1 mM, 24 h) or 6-OHDA (100 μM, 12 h). The viability of these cells will be assessed upon the application of resveratrol.

Results: Phase-contrast imaging demonstrated that RA (10μM) caused SH-SY5Y cells to generate neurites, an indication of efficient neuronal development. After 12 to 24 hours of treatment with MPP+ (1mM) or 6-OHDA (100μM), cell viability decreased significantly over time compared to untreated controls. Based on DrugBank mapping and public transcriptome analysis, resveratrol emerged as a candidate with the potential to enhance survival in cultures exposed to toxins, offering a promising direction for future validation and therapeutic exploration.

Conclusion: Cross-dataset transcriptome analysis identified resveratrol as a potential modulator, which improved viability in cells treated with MPP+ and 6-OHDA. Future work will validate pathways and explore advanced delivery methods, such as resveratrol-loaded exosomes, to enhance and sustain therapeutic effects.

Keywords: Transcriptomic analysis, Parkinsons disease, neuroprotection, resveratrol

P018
N-Glycan Profiling of Cell Lines by HPLC-HILIC-FLD

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Aim: This study aimed to develop an HPLC-HILIC-FLD–based method for profiling N-glycans in different cell lines. The goal was to establish a reproducible workflow that allows detailed evaluation of cell line–specific glycosylation patterns.

Method: N-glycans were enzymatically released from cell pellets, fluorescently labeled, and purified by solid-phase extraction prior to chromatographic analysis. A dextran ladder was employed for GU calibration, providing precise peak identification and consistent alignment of glycan signals across samples.

Results: The established HILIC-FLD workflow generated reproducible and distinct N-glycan profiles for each cell line. Dextran ladder calibration enabled accurate peak assignment and facilitated structural interpretation of glycan signals.

Conclusion: A robust and practical HPLC-HILIC-FLD–based approach for N-glycan profiling of cell lines was developed. By incorporating dextran ladder calibration, the method ensures reliable peak identification and comparability across analyses, providing a strong foundation for future studies on glycosylation dynamics and biomarker exploration.

Keywords: N-Glycan Profiling, HILIC, Fluorescence Detection, Cell Lines

P019
Comparative Evaluation of HILIC-Based Sorbents for N- and O-Glycan Analysis

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Aim: N- and O-glycan purification is crucial for accurate glycomic analyses. This study aimed to compare the efficiency of different HILIC-based sorbents—BioMagHILIC, glycan SPE, cellulose, and sepharose—in purifying N-glycans from standard serum samples.

Method: N-glycans were enzymatically released from standard human serum and subjected to purification using glycan SPE, cellulose, and sepharose-based HILIC sorbents. The performance of each sorbent was assessed through UHPLC-HILIC-FLD analysis, and structural profiles were validated by LC-MS.

Results: The three HILIC sorbents were comparatively evaluated regarding recovery, selectivity, and reproducibility. Differences in purification efficiency were observed among the sorbents, and the most suitable HILIC material for N-glycan analysis was determined. Conclusion: This study provides a comparative overview of glycan SPE, cellulose, and sepharose sorbents for N-glycan purification from serum. The results underline the importance of sorbent selection in achieving reliable and efficient glycan analysis.

Keywords: Glycomics, N-glycan, HILIC-based sorbents, Glycan SPE, Cellulose, Sepharose

P020
Proteomic Profiling of MSC Neurogenesis in a 3D Bioprinted Model

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Aim: The objective of this study is to investigate whether paracrine signals originating from human primary astrocytes promote the neuronal differentiation of bone marrow-derived mesenchymal stem cells (BM-MSCs) within a three-dimensional (3D) microenvironment generated using 3D bioprinting technologies, and to elucidate the associated proteomic alterations.

Methods: A 3D bioprinted platform was created by embedding bone marrow-derived MSCs in GelMA hydrogels. Cells were cultured for seven days in either neuronal differentiation medium (DIFF) or DIFF mixed 1:1 with astrocyte-conditioned medium (DCM). Neuronal markers (TUJ1, MAP2, GFAP) were assessed at days 4 and 7 by immunostaining. To further analyze neuronal fate, TMT-based LC–MS/MS proteomics was performed. Data were filtered, batch-corrected, variance-stabilized, and analyzed with limma. Differentially expressed proteins were defined at FDR <0.2 and fold change >1.5, followed by functional enrichment analysis.

Results: Immunostaining of DCM constructs showed a transient increase in neuronal and glial markers at day 4 that declined by day 7, indicating an early but unstable lineage activation. Complementary proteomics on day 7 revealed astrocyte conditioning enriched neurotrophic and ECM-remodeling proteins (NGF, galectin-1, decorin), while DIFF was dominated by plasma and structural proteins. Although temporal data were limited to DCM, integrating both datasets suggests astrocyte-derived cues drive an early mixed neuronal–glial phenotype and reprogram the proteome to support neurogenic processes at later stages.

Conclusion: This study establishes a new differentiation protocol for differentiation of human neurons from MSCs within a 3D microenvironment that mimics physiological conditions, using astrocyte-conditioned cues. The protocol successfully yields neuron-like cell populations characterized by increased expression of neuronal and glial markers and induces a reorganization of the MSC proteome toward neurotrophic and extracellular matrix–associated programs. This 3D bioprinted, human cell–based neuronal culture system holds significant potential for applications ranging from drug screening to the development of neuroregenerative strategies.

Keywords: mesenchymal stem cells, neuronal differentiation, astrocyte-conditioned medium, 3D bioprinting, proteomics

P021
Pan-Cancer Adenocarcinoma Analysis through Weighted Reaction Co-Expression Networks

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Adenocarcinomas, including lung, prostate, colon, stomach, ovarian, and pancreatic types, are major causes of cancer mortality, marked by cellular reprogramming that promotes tumor survival. Altered energy metabolism, lipid turnover, and amino acid use contribute to both tumor growth and therapy resistance. Identifying shared molecular traits among these cancers could aid precision oncology. This study addresses the need to integrate transcriptomic data with metabolic modeling to test the hypothesis that common reaction networks underlie adenocarcinoma progression, potentially uncovering novel biomarkers and therapeutic vulnerabilities that are not apparent in single-cancer analyses. RNA-Seq data from The Cancer Genome Atlas (TCGA) were obtained via the Genomic Data Commons (GDC) Cohort Builder for LUAD, PRAD, COAD, STAD, OV, and PAAD. After FPKM normalization, data was exported and processed with a Python script to create unified datasets. Human-GEM, a genome-scale metabolic model with 13,069 reactions, 3,067 genes, and 8,366 metabolites, served as the reference. Gene expression data were mapped to reactions using the parsedGPR method. Downstream analysis in R included Weighted Reaction Co-expression Network Analysis (WRCNA) and Differential Expression (DEG) analysis. Reaction Set Enrichment Analysis (RSEA) with Bonferroni correction revealed statistically significant enrichment of key metabolic pathways. This integrative approach links TCGA transcriptomic data with genome-scale modeling to uncover conserved and cancer-specific metabolic reprogramming in adenocarcinomas by analyzing TCGA RNA-seq from adenocarcinoma types using DEG, WRCNA, and RSEA, mapping expressions to reactions for network validation and therapy targets. Results identified shared upregulated pathways such as steroid hormone biosynthesis (enriched in PRAD, STAD, OV, and LUAD) and purine metabolism (prominent in PAAD, OV, and COAD), alongside tryptophan metabolism and arachidonic acid metabolism as common features, highlighting potential targets for pan-adenocarcinoma therapies.

Keywords: Bioinformatics, Adenocarcinoma, Transcriptomics

P022
Potential Serum Biomarkers in Bladder Cancer by UHPLC-MS

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Background: Bladder cancer(BC) ranks among the ten most common cancers, with 573,278 new cases reported worldwide. Novel early-stage diagnostic markers are needed to the detection of recurrence in patients. Serum biomarkers represent a promising approach for early-stage cancer diagnosis due to their non-invasive nature, high accuracy, and low cost. This study aimed to investigate the serum metabolomic profiles of NMIBC patients and healthy controls to identify potential candidate biomarkers.

Method: 90 serum samples (n=54 cancer, n=36 control) were analyzed using a Orbitrap Exploris 240 High Resolution LC/MS(Thermo Scientific). Statistical analysis of the metabolites were performed using Compound Discoverer 3.3 SP1(Thermo Scientific) and MetaboAnalyst 6.0. Volcano plot analysis, PCA(principal component analysis) analysis and orthogonal partial least squares discriminant analysis(OPLS-DA) model were employed to distinguish between NMIBC patients and controls. For pathway analysis, KEGG metabolic pathway database was used.

Findings: To investigate potential changes in serum metabolic profile associated with cancer, NMIBC patients were compared to controls. The significantly upregulated metabolites in the bladder cancer group ($log_2FC>1.5$, $p<0.05$) include Phosphatidylcholine, 1-Acyl-sn-glycero-3-phosphocholine, Choline, Acetylcholine, Sphinganine, Sphingomyelin, Pantothenate, L-Valine, Uracil, Spermidine, 4-Guanidinobutanoate, Hydroxyproline, L-Proline, Uridine, 5-Oxoproline, and Spermidine. The identified metabolites were associated with key metabolic pathways, including arachidonic acid, linoleic acid, alpha-linolenic acid, glycerophospholipid, sphingolipid metabolisms, pantothenate and CoA biosynthesis, arginine and proline metabolism, pyrimidine metabolism, and glutathione metabolism.

Conclusion: This study is one of the few to use untargeted UHPLC-MS specifically in early-stage BC patients to investigate serum metabolic alterations. Our findings are consistent with Zhao et al., who identified specific metabolites for distinguishing NMIBC, and with Guo et al., who reported altered pyrimidine metabolism in NMIBC. Our study suggest that specific metabolites are differentially expressed in NMIBC patients compared to healthy controls, indicating their potential as early-stage biomarkers. However, further validation studies are required to confirm their usability as potential biomarkers.

Keywords: NMIBC, UHPLC-MS, serum, metabolomics, biomarkers

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P023
Potential Biomarkers in Bladder Cancer

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Aim: Bladder cancer is one of the most common malignancies of the urinary tract, and there is a need for non-invasive and reliable biomarkers to support early diagnosis and patient management. The aim of this study was to quantitatively evaluate the serum levels of trimethylamine-N-oxide (TMAO), choline, carnitine, and betaine metabolites that have been increasingly recognized in the literature as potential biomarkers using LC-MS/MS in bladder cancer patients compared with healthy individuals. By assessing the diagnostic performance of these metabolites, the study seeks to provide novel insights into their potential use as non-invasive diagnostic tools.

Methods: Serum samples were collected from 52 healthy controls and 47 bladder cancer patients, including 22 Ta and 25 T1 cases. Quantitative analysis of TMAO, choline, carnitine, and betaine was performed using a validated LC-MS/MS method. Descriptive statistics were generated, and comparative analyses between groups were conducted using the Mann–Whitney U test. In addition, Spearman's rank correlation analysis was applied to investigate the associations between metabolite levels and clinical parameters such as age and body weight.

Results: The correlation analysis revealed that TMAO displayed the strongest positive association with tumor stage, followed by carnitine and choline, while no significant association was observed for betaine. Neither age nor body weight had a significant influence on metabolite levels. Group comparisons indicated that all metabolites, except betaine, showed significant differences between patients and controls, suggesting their potential role in distinguishing bladder cancer cases from healthy individuals.

Conclusion: Our findings suggest that TMAO, carnitine, and choline may serve as promising serum biomarkers for the non-invasive detection of bladder cancer, while betaine demonstrated limited diagnostic utility. These results provide supportive evidence that serum metabolite profiling using LC-MS/MS could contribute to the development of more reliable diagnostic strategies and may ultimately improve early detection and clinical decision-making in bladder cancer.

Keywords: bladder, cancer, tmao, choline, carnitine, betaine, LC-MSMS, biomarker

P024
ω9 Fatty Acids May Support Newborn Health in Hypothyroid Stages

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Aim: The effects of ω9 fatty acids (FAs) on human health have been less studied than those of ω3 and ω6 FAs. Until recently, colostrum ω9 FA studies were limited to Asian countries, which consistently demonstrated that ω9 FAs are most abundant in colostrum and decrease as lactation progresses. Recent European studies confirmed that ω9 erucic, gondoic, and nervonic acids are most abundant in colostrum, decreasing later, and are even higher in the colostrum of mothers with premature deliveries. ω9 FAs stimulate the brown adipose tissues, rapidly generating heat; thus, a relationship with birth weight was presumed. Furthermore, the colostrum and transitional milk production interval aligns with the period when neurocognitive development is vulnerable to a deficiency of thyroid hormones (THs), which regulate fetal adipogenesis. Based on these backgrounds, we analyzed associations between colostrum FAs, birth weight, and THs.

Methods: 22 FAs in the colostrum of 78 healthy mothers were measured with LC-MS/MS. FT3, FT4, and TSH levels were analyzed in the mothers' serum, and newborns' TSH in heel-pricked specimens. Correlations were defined in subsets, separated according to birth weight, THs, and mothers' body mass index, with Phyton Software.

Results: In the entire cohort, five of 22 FAs correlated with birth weight, all positively, including ω9 gondoic, erucic, and nervonic acids, and also saturated behenic and lignoceric acids, produced by the same elongases. These correlations sustained for gondoic, nervonic, behenic, and lignoceric acids among mothers with low FT4, and for erucic acid among mothers with high TSH.

Conclusions: The breast epithelial cells are prepared prenatally to adjust colostrum quality by shared mechanisms with fetal fat accrual. Thus, ω9 monounsaturated and saturated FAs over 18 carbon length may be released more into the colostrum to support thermoregulation against the harm of TH deficiencies.

Keywords: 9 Fatty Acids, Hypothyroid Stages, Newborn Health

P025
Palmitoylation Profiling of ZDHHC5 Knockout Cells

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Post-translational modifications (PTMs) are one of the main regulatory factors of cell division. Cellular membrane palmitoylation, a reversible post-translational modification, plays a critical role in membrane association and signaling processes. The cell membrane is a structure undergoes marked remodeling particularly during cell division. As palmitoylation is a reversible post-translational modification that enables proteins to associate with the membrane, the identification and profiling of palmitoylated proteins depending on cell phases is of particular interest in this regard. The palmitoyl acyltransferase ZDHHC5 has been linked to controlling dynamic protein localization at the plasma membrane, but little is known about how it affects cytokinesis. n this study, we aimed to investigate the palmitoylation profile of cytokinesis-synchronised ZDHHC5 knockout HeLa Kyoto cells compared with non-targeting (NT) control counterparts. Palmitoylated proteins were enriched and profiled by metabolic feeding of 17-octadecynoic acid (17-ODIA) or palmitic acid coupled by click chemistry-based biotinylation and the samples analyzed by LC-MS/MS. We identified differentially expressed proteins in ZDHHC5 KO compared to NT cells. As an ongoing project, the future experiments will focus on validating these proteins to clarify their functional relevance in cytokinesis.

Keywords: palmitoylation, cytokinesis, ZDHHC5

P026
Spatial Control of Keratin8 Phosphorylation by Aurora B Enables Cytokinesis

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Keratins assemble into mechanically resilient polymers that physically stabilize epithelial cells. When epithelial cells divide, keratin polymers must be severed to allow cell separation during cytokinesis. Phosphorylation has been implicated in this process, but how keratins are regulated during cell division is not understood. Aurora-B kinase, which is part of the chromosome passenger complex (CPC) accumulates at the cell center during cytokinesis and has been implicated in regulating intermediate filaments. We mapped six Aurora B kinase sites in Keratin 8. Phosphorylation of Keratin 8 at S34 occurred specifically at the cleavage furrow and persisted at the midzone until the completion of cytokinesis. Inhibition of Aurora B or expression of a non-phosphorylatable Keratin 8 mutant impaired keratin disassembly at the cleavage furrow. We propose that Aurora B–mediated phosphorylation promotes localized keratin filament disassembly at the cleavage furrow, allowing spatially regulated disassembly during cytokinesis. Aurora B binds to keratin filaments, and its localization to midzones was reduced in Keratin 8 knockout cells, showing that Keratin 8 facilitates Aurora B targeting during cytokinesis. This suggests a positive feedback cycle whereby Keratin 8 promotes midzone-localization of Aurora B and in turn, is locally disassembled by its kinase activity. This cycle is required for successful furrow ingression and completion of cell division in cancer cells of epithelial origin and might provide a target for solid tumor treatment.

Keywords: keratin, cytokinesis, Aurora B kinase, phosphorylation, Keratin solubility, cleavage furrow, epithelial origin

P027
Structural and Functional Characterization of Bag-1S:p97/VCP Binding Site

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Background & Aim: Proteostasis involves all cellular processes responsible for maintaining protein balance, including protein quality control (PQC) systems such as molecular chaperones, endoplasmic reticulum-associated degradation (ERAD), and the ubiquitin-proteasome system (UPS). Bag-1, a protein found to be upregulated in breast cancer cells, contains two key domains: a BAG domain and a ubiquitin-like (UbL) domain. Previous studies conducted in our laboratory identified the interaction interface of the Bag-1S:p97 complex using Hydrogen Deuterium Exchange Mass Spectrometry (HDX-MS) and Cross-Linking Mass Spectrometry (XL-MS). Based on these findings, we designed six different peptides that target p97/VCP. Binding affinities of these peptides were studied using experimental methods. The aim of this study is to design inhibitory peptides targeting the Bag-1S:p97/VCP complex to interfere with protein degradation mechanisms in cancer cells.

Methods: Purity analysis and characterization of peptides were investigated using HPLC. Also, intact mass analysis performed using Mass Spectrometry to determine the molecular weight of peptides. To evaluate the ability of candidate peptides to modulate the ERAD mechanism, ATPase activity of p97/VCP was measured.

Results: As a result, the peptides were characterized and molecular weights of the peptides were determined. Our study identified two candidate peptides that effectively block the interaction between Bag-1S and p97/VCP, as evidenced by decreased ATPase activity.

Conclusion: The findings of this study demonstrate that several candidate peptides, designed based on the Bag-1S sequence, are capable of inhibiting the Bag-1S:p97/VCP interaction, offering a promising strategy for therapeutic intervention in cancer. Additionally, novel peptides derived from the p97/VCP sequence have been designed and are currently being evaluated in the ongoing phases of the study.

Keywords: ERAD, peptide, structure, protein

P028
Investigating Novel Interaction Interfaces Between Hsp70/Hsc70 and Bag-1 Using HDX-MS

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Aim: The interaction between the molecular chaperone Hsp70/Hsc70 and its nucleotide exchange factor Bag-1 is essential for maintaining cellular proteostasis through regulation of the ATP-dependent chaperone cycle. While the C-terminal BAG domain of Bag-1 is known to interact with the nucleotide-binding domain (NBD) of Hsp70/Hsc70 to enhance ADP release and substrate turnover, the contribution of additional or transient contact sites remains unclear. This study aims to investigate possible alternative or extended interaction interfaces between Hsp70/Hsc70 and Bag-1 using hydrogen-deuterium exchange mass spectrometry (HDX-MS).

Methods: HDX-MS was performed using previously optimized protocols from our laboratory, which had successfully identified novel interfaces in Bag-1:c-Raf and Bag-1:VCP/p97 complexes. Herein, the proteins were recombinantly produced, purified, and characterized by immunoblotting and circular dichroism (CD) spectroscopy to confirm proper folding. In vitro binding assays were used to verify interaction prior to HDX-MS.

Results: Preliminary data confirmed proper folding of both proteins and their direct interaction in vitro. Initial HDX-MS kinetics have been analyzed and revealed novel noncanonical binding regions.

Conclusion: This study is expected to uncover new structural details of the interaction of Hsp70/Hsc70 and Bag-1, potentially uncovering regulatory regions beyond the canonical BAG–NBD interface. Such insights may contribute to understanding chaperone–co-chaperone regulation in processes like protein folding, degradation, and stress response, with implications for diseases linked to proteostasis imbalance.

Keywords: HDX-MS, protein-protein interactions, chaperone

P029
Functional Characterization of the MSH2 A733T Variant of Uncertain Significance

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Aim: Lynch Syndrome is a genetic disease that increases the risk of cancers, including colon cancer, due to loss-of-function mutations in DNA mismatch repair genes: MLH1, MSH2, PMS2, MSH6. MSH2 and MSH6 form the MutSα complex, which recognizes and repairs DNA mismatches through binding MutLα. When this repair process is disrupted, mutations accumulate by increasing the cancer risk. Our previous study that screened 370 colorectal cancer patients and 914 healthy individuals in the Turkish population identified multiple variants of uncertain significance (VUS) in MSH2/6 genes, including an Ala733Thr missense mutation in MSH2 (Previously published in: Akcay IM et al. (2021) Int J Cancer 148, 285–295). Here, we aim to characterize MSH2A733T and its functional effects at the protein level.

Method: We initiated our experiments by performing circular dichroism analysis and then a pull-down assay to assess effect of mutation on protein stability, structure and binding, respectively. Following that,
MSH6
transcript levels were analyzed using qPCR. After transient transfection to MSH2-deficient LoVo cells, protein levels were also analyzed following proteasome inhibitor treatment, to assess the effect of the mutation at the translational level. Since MSH2 mediates DNA damage-induced apoptosis, we examined the functional role of mutant MSH2 protein in apoptosis by treating cells with cisplatin to induce excessive DNA damage, and further analyzed key signaling proteins.

Results: We found that mutant MSH2 overexpression resulted in a higher stability, while decreasing MSH6-binding capacity and increasing proteasomal degradation of MSH6. We also report that mutant fails to promote apoptosis, as it reduced levels of cell death proteins compared to wild-type.

Conclusion: These findings contribute to the potential reclassification of MSH2A733T as pathogenic rather than a VUS by providing evidence that it compromises the apoptotic role of the MMR pathway. We proposed a molecular mechanism underlying the pathogenicity of MSH2A733T

Keywords: DNA Mismatch Repair, MSH2, MSH6, Lynch Syndrome, Variant of Uncertain Significance, Cisplatin

P030
Bag1:Bcl-2 Interaction Surface: Structural and Functional Insights in Apoptosis

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Aim: Apoptosis is a tightly regulated cellular process, which is frequently suppressed in cancer cells to enable uncontrolled proliferation and resistance to conventional therapies. Modulating apoptotic pathways represents a promising therapeutic approach. In this context, the interaction between the anti-apoptotic Bcl-2 and its assistant Bag1, which is known to potentiate Bcl-2 activity, plays a pivotal role in the suppression of cell death signals. The aim of this study is to probe the binding interface of the Bag1–Bcl-2 complex to gain a comprehensive understanding of its structural basis and dynamics, which could guide the design of targeted inhibitors aimed at modulating apoptosis resistance in cancer.

Methods: Recombinant full-length Bag-1S and Bcl-2 proteins were expressed, purified and characterized. Direct interaction of Bcl-2 and Bag-1S was assessed by in vitro binding assay under both ATP-present and -absent conditions. Afterwards, interaction surface(s) of Bag1:Bcl-2 complex was probed by using HDX-MS approach through comparison of the H/D exchange rates in the complex and non-complex conditions. Samples collected at different deuteration times were used to identify solvent-protected regions in the complex condition and interpreted as interaction interfaces.
Results: Our data revealed that Bag1 and Bcl-2 was functional, interacted directly, and thus, this interaction is dependent on the presence of ATP. HDX-MS analysis identified Bag1-interacting regions of Bcl-2 depending on the reduced H/D exchange rates upon partner binding. Our data indicate that several regions on Bcl-2 contribute to Bag1 interaction.

Conclusion: Our study provided a comprehensive analysis on Bag1:Bcl-2 interaction due to use of full-length proteins. These findings contribute to the understanding of this interaction and may pave the way for the development of novel cancer therapeutics.

Keywords: Yapısal Biyoloji, Apoptoz, Kanser

P031
RSM-Based Optimization of Protein Extraction from Pancreatic Cancer Cell Lines

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Protein extraction is essential in proteomics, as it affects protein yield and profile. Several cell-based extraction methods exist, each with multiple steps, buffers, and chemicals, making parameter optimization particularly challenging in complex matrices. Statistical tools enable faster, more effective optimization. Response-Surface-Methodology (RSM) provides a systematic approach by evaluating multiple factors and their interactions. This study aims to develop optimized protocol using RSM to maximize protein extraction efficiency from BxPC-3 cells, a human pancreatic cancer cell line.

Extraction buffer was optimized for detergent, salt, and pH based on literature-reported concentration ranges, considering their roles in membrane disruption, stability, and solubility. RSM was utilized with total protein concentration (TPC) as the key response factor. The design included 30 experiments from 13 buffer compositions with replicates. For each experiment, five hundred thousand BxPC-3 cells were extracted, and TPC was measured using BCA assay on a spectrophotometer. Minitab 19 was used to identify significant parameters and generate response surface graphs. Finally, SDS-PAGE was performed to assess protein distribution, providing complementary information.

Three RSM surface graphs were generated to investigate how pairs of parameters affect protein concentration after extraction. RSM showed a distinct TPC peak at moderate detergent–salt levels. High salt with neutral to alkaline pH increased yield. Low detergent with slightly alkaline pH gave the highest yield, highlighting detergent as a major factor influenced by pH. RSM analysis revealed that buffer composition strongly influences protein extraction from BxPC-3. Optimal TPC was achieved at low detergent, high salt, and high pH. RSM results showed that concentration ranges reported in the literature were insufficient to achieve optimal protein yield. To better understand how buffer conditions affect protein and peptide profiles, the range of buffer components will be expanded by testing additional parameters and integrating mass spectrometry analysis.

Keywords: Response-Surface-Methodology, Protein Extraction, Cell Lysis, Cancer Cell Lines, Proteomics

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P032
Screening Inhibitory Peptide Disrupting Bag-1S/C-Raf Interaction in Cancer Cell Lines

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Background: Acquired drug resistance often results in cancer treatment failure. To cope with ADR, it is important to identify new drug targets that play a key role in the signaling pathways that provide an advantage to cancer cells to prevent tumor progression. After determining the interface of the Bag-1S/C-Raf complex, an inhibitor peptide was developed and the peptide binding to Bag-1S was detected through the Bag-1S/C-Raf interaction. Thus, targeting the Bag-1/C-Raf interaction has the potential to provide an inhibition. Therefore, an inhibitor molecule has been discovered that may enable the modulation of multiple interactions that play a critical role in the long-term survival of cancer cells.

Aim: The aim is to investigate the therapeutic potential of inhibitory peptide to overcome acquired drug resistance and improve cancer treatment efficacy.

Materials and methods: The study involves synthesizing the inhibitory peptide, verifying it via mass spectrometry, and identifying the most responsive cancer types and effective dose of the peptide via MTT assay. The affected signaling pathways and changes in target protein levels are investigated using immunoblotting analyses.

Results: Designed inhibitory peptide was verified via mass spectrometry. Its effective dose in cancer lines such as MCF-7 was determined. Moreover, affected signaling pathways and changes in target protein levels were investigated. It was seen that target protein levels in signaling pathways and cell survival were affected.

Conclusion: This study will provide data for the targeted use of the developed peptide inhibitor in cancer.

Acknowledgements: TÜSEB- 39352

Keywords: Bag-1S, c-Raf, anticancer agents

P033
Development of PETase Enzyme for Efficient Microplastic Biodegradation

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Background: PETase (polyethylene terephthalate hydrolase) was first identified in the bacterium Ideonella sakaiensis and plays a crucial role in the biological degradation of polyethylene terephthalate (PET) polymers. The enzyme hydrolyzes ester bonds, producing bis(2-hydroxyethyl) terephthalate (BHET), mono(2-hydroxyethyl) terephthalate (MHET), and terephthalic acid (TPA) as the main products. Due to this property, PETase is considered an important tool for mitigating the environmental impact of microplastics.

Aim of the study: For improving the efficiency of industrial recycling and bioremediation applications, the identification of PETase, along with the detailed characterization of its structural properties and catalytic mechanism, is of great importance. This study aims to optimize the production, purification and characterization of the enzyme and to investigate its activity on PET polymers.

Materials and methods: The PETase plasmid (with a C-terminal 6xHis tag) was transformed into a Rosetta strain. Following the transformations, the production conditions of the enzyme were optimized, and subsequently, the purification protocols were refined. After obtaining pure PETase, characterization studies were conducted using SDS-PAGE, Circular Dichroism (CD), and SEC-HPLC. Enzyme activity was determined via HPLC using a C18 column.

Results: PETase from Ideonella sakaiensis was successfully expressed, purified, and structurally confirmed with α-helix content. Activity tests via HPLC verified its function.

Conclusion: These results support PETase's potential for improved applications in recycling and bioremediation in industry.Following these results, the stability of the active PETase enzyme will be investigated to assess its suitability for industrial applications.

Keywords: PETase, MHET, BHET, TPA, purification, characterization.

P034
Computational Analysis of NARS1 Missense Variants Associated with Pathological Conditions

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Asparaginyl-tRNA synthetase 1, NARS1, plays a key role in translation by catalyzing the attachment of asparagine to its 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 dynamic analyses of six 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. All-atom molecular dynamics (MD) simulations were used to monitor changes in the local environments at the mutant sites, uncovering their effects on the overall structure–function relationship. Simulations of the wild-type protein were performed in parallel as controls. Our results indicate that the Y289C and L350P variants induce alterations in key structural motifs. Specifically, Y289C promotes distortion of the global protein structure and dimerization. In this mutant, the formation of a C289–C546 disulfide bond appears feasible, leading to a rearrangement of the C-terminal loop orientation, which displaces the loop from the dimer interface, consequently distorting the dimer configuration. In the L350P mutant, a shift in the F347 side chain orientation was observed, which appears to stabilize the N-terminal loop of the opposing chain, thereby affecting dimerization. The G132C mutant showed weakened structural coordination between two domains, while the N221S mutant may compromise tRNA binding due to potential backbone clashes. Analysis of allosteric communication pathways corroborated the neighborhood interaction analysis, revealing the absence of common links at the dimer interface in the mutants. Overall, this study advances our understanding of a set of Nars1 VUS variants, along with a novel variant, by shedding light on their molecular consequences and potential relevance to disease mechanisms.

Keywords: Nars1, variant analysis, molecular dynamics simulations, novel variant, dimerization, allostericity, tRNA binding

P035
Sunflower Proteins: Linking In Vitro/Silico Bioactivity by LC-MS/MS

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Sunflower seeds are one of the most widely grown crops for the manufacture of vegetable oil worldwide. As it becomes increasingly popular to utilize food industry by-product streams as new, high-protein sources for human and animal use, de-oiled sunflower cake can be considered as a profitable and high quantity by-product. This study examined bioactive protein hydrolysates from cold-press sunflower (Helianthus annuus L.) cake. Alkali extraction-isoelectric precipitation (AE-IP) was used to manufacture sunflower protein isolates (89% protein). The acquired isolates were subjected to enzymatic hydrolysis using microbial proteases (Alcalase and Flavorzyme), followed by ultrafiltration (UF) with 3 and 10 kDa molecular weight cut-off (MWCO) membranes. DH (degree of hydrolysis %), ACE (angiotensin-converting enzyme) inhibitory action, DPP-IV (dipeptidyl peptidase-IV) inhibitory activity, DPPH (2,2-diphenyl-1-picrylhydrazil) radical scavenging effect, and TPC (total phenolic content) were conducted. Proximate and amino acid compositions were also investigated for the seeds, cold-press cake, protein isolate, proteolytic hydrolysates, and their corresponding fractions. The ACE-inhibition of the hydrolysate and its fractions were found to be in a range of 75.5-96.3%. DPP-IV inhibition values were between 33.3 and 86.1%. The total phenolic content of samples varied between 1.5 -10.5 GAE g/ 100 g. Antioxidant activities of fractions were found in the range of 4.1 -10.9 Trolox Equivalent g/100 g based on and DPPH assay. Peptides profiles of the UF fractions were investigated as a function of UF duration using a liquid chromatography triple quadrupole mass spectrometry method, which further facilitated the in-silico prediction of bioactivity for each characterized peptide sequence. Molecular docking was used to mimic the interactions of the sample peptides with ACE and DPP-IV.

Keywords: Sunflower derived peptides, plant proteins, bioactive peptides, molecular docking, valorization, in silico

P036
Graph Neural Networks Reveal Differentially Expressed Genes in Synthetic RNA-Seq

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Purpose: This study aims to explore the use of Graph Neural Networks (GNNs) to detect differentially expressed genes (DEGs) in synthetically generated RNA-Seq datasets. GNNs are employed to learn gene relationships via graph structures, providing a novel perspective for gene-level classification and downstream biomarker discovery.

Method: Synthetic RNA-Seq data was generated using a negative binomial distribution with varying sample sizes (n = 50, 200, 500), differential expression percentages (5% and 30%), and dispersion parameters (ϕ = 1 and 100). Each dataset consisted of exactly 1000 genes, allowing controlled benchmarking under high-dimensional transcriptomic conditions. Graphs were constructed from correlated gene expression profiles. For differential gene expression analysis, a graph neural network model was trained to classify each gene as differentially expressed or not. Model performance was evaluated using the True Detection Rate (TDR), which measures the proportion of correctly identified DEGs.

Findings: The GNN model demonstrated robust performance in high-signal and large-sample scenarios. For instance, with 500 samples and 30% DEGs, the model achieved a TDR of 0.9296. These results highlight the importance of both sufficient sample size and a meaningful DEG signal for effective learning. On the contrary, all high-dispersion (ϕ = 100) conditions led to poor performance, with TDR dropping to zero in most cases. This demonstrates the critical impact of class imbalance, graph sparsity, and expression variance on model success.

Conclusion: This study confirms that GNN-based frameworks can effectively detect differentially expressed genes in synthetic RNA-Seq data composed of 1000 genes, particularly under optimal conditions of low dispersion and large sample size. The results also indicate that DEG ratio and variance significantly influence detection performance. DEGs identified through this approach may provide candidates for downstream pathway enrichment analysis or biomarker discovery, thereby contributing to biological insight and translational relevance.

Keywords: Graph Neural Networks, Differentially Expressed Genes, Synthetic RNA-Seq Data

P037
Molecular Signatures of Coagulation in Diabetic Nephropathy

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Objective: Diabetic nephropathy is one of the most severe microvascular complications of diabetes, leading to progressive renal damage. In addition to inflammation and metabolic dysfunction, disturbances in vascular integrity and coagulation mechanisms play a key role in its pathogenesis. This study aimed to identify molecular alterations associated with coagulation and vascular dysfunction in patients with diabetic nephropathy.

Methods: This descriptive and comparative study included 16 patients diagnosed with diabetic nephropathy and 16 age- and sex-matched healthy controls. Plasma samples were collected from all participants. Molecular profiling was performed using high-sensitivity analytical techniques. Bioinformatic and statistical analyses were conducted to evaluate differences between groups, with a significance level set at $p<0.05$. Enrichment analyses were used to identify the biological pathways most affected.

Results: Significant alterations were detected in biological pathways related to platelet activation, hemostasis, complement cascades, and vascular inflammation in the diabetic nephropathy group. Additionally, disruptions in lipid metabolism, oxidative stress response, structural components, and immune regulation were observed. These findings reflect a pro-thrombotic and inflammatory molecular environment and suggest the activation of multiple pathological mechanisms in the disease process.

Conclusion: This study reveals that diabetic nephropathy is associated with pronounced molecular changes involving coagulation and vascular homeostasis. These alterations may contribute to disease progression and offer potential biomarkers for early diagnosis. Understanding these mechanisms can support the development of targeted therapeutic strategies to improve clinical outcomes.

Keywords: diabetic nephropathy, coagulation pathways, vascular dysfunction, molecular alterations

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P038
Mitochondrial proteomics highlight energy and aminoacid defects in Ullrich CMD

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Purpose: Collagen VI–related myopathies, including Ullrich congenital muscular dystrophy (UCMD), are caused by mutations in COL6A1, COL6A2, or COL6A3, leading to extracellular matrix defects and secondary mitochondrial dysfunction. Mitochondrial abnormalities in morphology, cristae structure, and function have been reported in UCMD; however, the global proteomic landscape of patient-derived mitochondria remains largely unexplored. This study aimed to characterize mitochondrial proteomic alterations in primary myoblasts of UCMD patients using an optimized isolation method.

Methods: Mitochondria were isolated from primary skeletal muscle myoblasts of genetically confirmed UCMD patients using the developed hypotonic swelling method. Mitochondrial proteins were analyzed by liquid chromatography–tandem mass spectrometry (LC–MS/MS). Differentially expressed proteins were identified and subjected to Gene Ontology (GO) gene enrichment analysis.

Findings: A total of 52 mitochondrial proteins were significantly altered in UCMD myoblasts compared to controls. Key affected pathways included the citrate (TCA) cycle, oxidative phosphorylation, glyoxylate and dicarboxylate metabolism, glycine/serine/threonine metabolism, and tryptophan metabolism. Upregulated proteins —such as CYCS, ATP5PO, NDUFB1/B6, UQCRFS1, COX15, DLST, DLD, TOMM22, and VDAC2— indicated compensatory activation of certain respiratory chain components and mitochondrial biogenesis factors. Conversely, COX4I1, NDUFS1, ATP5PD, MDH2, VDAC1, SHMT2, SLC25A3/4/6, and GRPEL1 were downregulated, suggesting impaired electron transport, substrate utilization, and protein import.

Conclusion: This study provides the first comprehensive mitochondrial proteomic map of UCMD patient-derived myoblasts, revealing combined defects in energy metabolism, amino acid pathways, and mitochondrial protein homeostasis. The coexistence of compensatory upregulation and functional loss within oxidative phosphorylation components suggests an adaptive yet insufficient mitochondrial stress response. These findings advance the understanding of UCMD pathophysiology and identify potential biomarkers and therapeutic targets for restoring mitochondrial function. Beyond UCMD, the identified proteomic alterations may have broader relevance to other neuromuscular disorders with secondary mitochondrial dysfunction.

This study was supported by Hacettepe University, Scientific Research Projects Coordination Unit(Project no:TSA-2021-19199).

Keywords: primary myoblast, mitochondrial proteomics, Ullrich congenital muscular dystrophy

P039
Impact Of Hyperglycemia-Induced Glucose Toxicity On Inflammatory Response Markers

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Background/Objectives: The aim is to evaluate the effects of hyperglycemia on the cellular senescence process and to investigate the regulatory role of resveratrol on inflammatory responses associated with hyperglycemia. In this context, the effects of resveratrol administration under high glucose conditions on cellular senescence markers and inflammation-related molecular pathways will be examined.

Methods: HUVEC cells were treated with 45 mM high glucose (HG) for 48 h and subsequently with 35μM resveratrol (Res) for 48 h, both at MTT-determined doses. Supernatants were analyzed for Interleukin (IL)-6 by ELISA, and RT-PCR assessed gene expression. Results: Concurrently, there was a marked increase in the HG group, upregulation in IL-6 (~3-fold) gene expression, relative to control groups. There was a significant difference between the HG group and both the control and HG+Res groups in IL-6 gene expression and protein levels ($p<0,05$). Concurrently, there was a marked increase in the HG group, upregulation in IL-6 (~3-fold) gene expression, relative to control groups. However, no significant difference was found between the control and HG+Res groups in IL-6 gene expression and protein levels ($p>0,05$).

Conclusion: Our findings show that resveratrol protects endothelial cells from toxicity caused by high glucose by suppressing IL-6 levels, demonstrating its potential as a therapeutic agent against hyperglycemia-induced inflammation.

Grant: This work was supported by Kahramanmaras Sutcu Imam University Department of Scientific Research Projects (grant number: 2024/9-27M).

Keywords: Huvec, IL-6, High Glucose

P040
Regulatory Impact of STN1 on EMT-Related Proteins in Pancreatic Tumors

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Objective: Pancreatic cancer is a highly aggressive malignancy that progresses through epithelial-mesenchymal transition (EMT) and metastasis. The role of STN1 in these processes remains unclear. This study aims to investigate how STN1 overexpression affects EMT and metastasis-associated protein levels and their interaction networks in pancreatic cancer cells.

Methods: Genes upregulated upon STN1 overexpression were identified, and KEGG pathway analysis was performed to determine the affected biological processes. Key gene-pathway relationships were visualized using Cytoscape to construct a protein-protein interaction (PPI) network, highlighting central hub genes. Additionally, TCGA datasets were analyzed to evaluate correlations between STN1 expression and selected EMT/metastasis-related genes, providing clinical relevance.

Results: Our analyses revealed that STN1 overexpression primarily influences the Pathways of Neurodegeneration and modulates hub genes critical to EMT/metastasis, including GNAQ, LDHA, VDAC1, TUBB, and TUBA1B. The Cytoscape PPI network demonstrated strong interactions among these genes, suggesting that STN1 may act as a central molecular regulator. Correlation analyses with TCGA data confirmed significant positive associations between STN1 and these target genes, reinforcing their potential biological and clinical relevance.

Conclusion: STN1 overexpression in pancreatic cancer cells enhances the expression of EMT and metastasis-associated proteins and organizes them into a functional interaction network. These findings suggest that STN1 could serve as a potential biomarker and therapeutic target, playing a key role in the molecular mechanisms underlying pancreatic cancer progression. Further studies are warranted to explore the mechanistic pathways through which STN1 modulates tumor aggressiveness and metastatic potential.

Keywords: STN1, Pancreatic cancer, EMT Epithelial-Mesenchymal Transition, Protein-protein interaction network PPI

P041
ONX-0914 Improves Diabetic Wound Healing via Immunoproteasome Inhibition

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Purpose: Wound healing is markedly impaired in Diabetes Mellitus (DM), primarily due to sustained inflammation and increased oxidative stress. Immunoproteasomes, highly expressed in immune cells, contribute to the maintenance of this pro-inflammatory state. Their inhibition represents a promising therapeutic strategy. This study aimed to evaluate the effects of the immunoproteasome inhibitor ONX 0914 on wound healing in a streptozotocin (STZ)-induced diabetic rat model, representing the first investigation of ONX 0914 specifically in the context of diabetic wound healing.

Method: Twenty-eight Wistar rats (250–300 g, 10–12 weeks old) were used. The control group (n=4) received intraperitoneal PBS. Diabetic rats (T2D) was induced in experimental animals (n=12) by intraperitoneal STZ (45 mg/kg). After a 7-day chronicity period, the T2D group (n=12) received PBS, while the treatment group (n=12) was administered ONX 0914 (10 mg/kg, i.v., three times weekly) for 28 days. Wound healing was evaluated macroscopically and histologically; oxidative stress markers and cytokine levels were analyzed biochemically. Healing rates were compared among groups, and wound tissues were collected for histology. Proteasome inhibition was confirmed by fluorometric activity determination. Data were analyzed statistically; $p<0.05$ was considered significant.

Findings: ONX 0914 accelerated wound healing in diabetic rats, confirmed by morphometric measurements on days 7, 14, and 21. STZ increased oxidative stress. In diabetic rats, GSH, GPx, and SOD levels decreased, while MDA increased; CAT activity was unchanged. ONX 0914 reduced oxidative stress and lowered pro-inflammatory cytokines, with decreased IL-1β and TNF-α levels.

Conclusion: ONX 0914 improved wound healing in diabetic rats by reducing oxidative stress and inflammatory cytokines. Immunoproteasome inhibition with ONX 0914 may represent a novel immunotherapeutic approach for diabetic wound treatment.

Keywords: ONX0914, Wound Healing, Immunoproteasome, Diabetes Mellitus

P042
Phycocyanin effects on senescence and responses in wheat leaves

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Aim: Arthrospira platensis is a cyanobacteria species rich in nutritional content and biologically active phycocyanin. This study aimed to investigate the effects of phycocyanin solutions on senescence, growth, and physiological responses of wheat (Triticum aestivum L.) leaf segments.

Method: Extracts containing 35% phycocyanin were used to prepare solutions at concentrations of 0.05 g/L and 0.1 g/L. These solutions were applied to wheat leaf segments, and their effects on growth parameters, pigment profile (chlorophyll, carotenoids, and anthocyanins), protein content, and antioxidant enzyme activity [peroxidase (POD) and catalase (CAT)] were evaluated after four days.

Results: Application of 0.05 g/L phycocyanin solution increased carotenoid and anthocyanin contents, whereas 0.1 g/L solution enhanced fresh weight, chlorophyll, and protein content while decreasing POD activity. No significant change was observed in CAT activity.

Conclusion: The findings suggest that phycocyanin delays senescence, promotes plant growth, and enhances physiological defense systems. Therefore, phycocyanin may serve as a potential biostimulant for sustainable agricultural practices.

Keywords: Arthrospira platensis, phycocyanin pigment, leaf segment, chlorophyll content, biostimulant

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P043
Anticancer Effects of Curcumin and ATRA Investigated by LC-MS/MS Method

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Aim: This study investigated the anticancer effects of curcumin and all-trans retinoic acid (ATRA) using LC-MS/MS. Both compounds possess antitumor properties, and their combination exerts synergistic effects on glioblastoma. We evaluated their impact on signaling pathways regulating proliferation, apoptosis, and survival in glioblastoma cells, aiming to reveal potential synergistic effects and contribute to novel therapeutic strategies.

Method: U87 cells were treated with ATRA, curcumin, and their combination for 48 hours. Cell pellets were stored at −80 °C and processed for proteomic analysis. Proteins were extracted, digested by in-solution trypsinization, and analyzed with nano-LC-MS/MS (Q Exactive Orbitrap). Data were processed using MaxQuant and UniProt database search, followed by statistical evaluation with Perseus. Differentially expressed proteins were examined by PANTHER to assess pathways and molecular functions.

Results: Proteomic profiling of U87 cells treated with ATRA, curcumin, and their combination identified 1,457 proteins, of which 71 were significantly differentially expressed. The combination group showed the most distinct changes (44 proteins: 26 downregulated, 18 upregulated), clearly separating from single-agent treatments. These alterations mainly involved ribosomal proteins, cell cycle regulators, and oxidative stress-related proteins. Pathway analysis indicated enrichment of amino acid and vitamin metabolism in the ATRA group, and energy/redox-related pathways in the curcumin group. The combination treatment modulated a broader range of biological processes, including apoptosis, cell cycle, ubiquitin-proteasome, and Wnt signaling pathways, highlighting its stronger regulatory impact at the proteome level.

Conclusion: This study highlights the distinct and overlapping effects of ATRA and curcumin on glioblastoma cells at the proteomic level. While ATRA primarily reprogrammed amino acid metabolism and suppressed oncogenic signaling pathways, curcumin induced both cytotoxic and adaptive responses. Importantly, their combination produced an anti-cancer effect, disrupting tumor-supportive networks more extensively than single treatments. These findings suggest that ATRA and curcumin may serve as a complementary therapeutic strategy for glioblastoma.

Keywords: ATRA, curcumin, proteomics, LC-MSMS, anti-cancer

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P044
New Insights into Inhibition of the "Undruggable" K-Ras G12D

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The Ras protein family, including the N-Ras, H-Ras, and K-Ras, regulates essential cellular processes such as signaling, division, and differentiation. These proteins act as molecular switches, cycling between an active GTP-bound state and an inactive GDP-bound state. The first 80 amino acids of the conserved G-domain contain the Switch I and Switch II regions, which are critical for effector binding. Mutations in Ras can lock the protein in its active state, leading to persistent downstream signaling and uncontrolled cell growth. Ras mutations occur in approximately 30% of human cancers, with KRAS mutations accounting for about 85% of these cases. The discovery of a pocket between the Switch I/II regions has accelerated the development of K-Ras inhibitors. Recent advances have identified small molecules such as MRTX-1133, TH-Z835, and TH-Z827, which bind near the frequently mutated 12th amino acid (Aspartic Acid) in the Switch II region, stabilizing K-Ras in its inactive conformation. However, the K-Ras G12D variant remains particularly challenging to target, and resistance to available therapies often arises. In this study, we identify alternative drug candidates through virtual screening of a ZINC20-derived molecular library (11,854,078 compounds). A KNIME workflow was used to filter molecules (8,719,045) according to Lipinski's Rule of Five, and molecular fingerprints were compared with positive control inhibitors using 3D similarity analysis. Compounds exceeding the similarity threshold (122) were docked to K-Ras G12D. The top-performing candidate was further evaluated through molecular dynamics simulations of both K-Ras G12D and the wild-type protein, followed by cross-correlation analysis and free energy calculations with umbrella sampling. Importantly, simulations revealed that the ligand interacts with an allosteric site located between the α3 and α4 helices. This binding was associated with detectable allosteric effects on K-Ras dynamics, suggesting a potential mechanism for therapeutic inhibition beyond the classical Switch I/II pocket.

Keywords: cancer, allosteric, virtual screening, kras, molecular dynamics simulations

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P045
TurboID-Based Mapping of Nek2 Interactions Uncovers Uniquationation and Repair Links

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Aim: Nek2A is a centrosome-associated mitotic kinase frequently overexpressed in cancers, linked to chromosomal instability and drug resistance. While its role in centrosome separation is established, its cancer-specific functions remain underexplored. Our lab has previously demonstrated that Nek2 overexpression promotes multipolar mitosis by disrupting centrosome clustering, highlighting its oncogenic potential. Here, we aimed to comprehensively characterize the cell cycle-dependent interactome of Nek2 using proteomics-driven approaches.

Methods: We developed a doxycycline-inducible TurboID-Nek2 proximity labeling system in U2OS cells and confirmed centrosomal localization. Following double-thymidine block synchronization, cells were enriched in G1/S, late S, and G2/M phases. Biotinylated proteins were isolated by streptavidin pulldown and profiled by LC–MS/MS. Interactome dynamics were compared between Nek2 wild-type and kinase-dead variants, and selected candidates were validated by immunoblotting and CRISPR/Cas9-mediated Nek2 knockout proteomics.

Results: Our analysis identified more than 150 high-confidence Nek2 interactors across cell cycle phases. Known partners such as APC/C components, casein kinase, and KIF24 were consistently recovered, while novel interactors including Nusap1, KIF2C, MAPRE3, and MPG emerged. Functional enrichment analyses revealed significant associations with centrosome clustering, microtubule dynamics, and ubiquitin-mediated proteolysis. Importantly, DNA damage response proteins (MPG, PARP2, RAD9A, SIRT7) were enriched across multiple phases, suggesting unanticipated roles of Nek2 in genome maintenance. We examined the Nek2–Nusap1 relationship in U2OS dox-inducible Nek2 cells and observed a time-dependent decrease of Nusap1 upon Nek2 overexpression. Treatment with the proteasome inhibitor MG132 rescued Nusap1 levels, indicating proteasomal regulation. Based on this link to ubiquitination, we considered the involvement of an E3 ligase and tested the novel Nek2 interactor SMURF2 as a candidate regulator.

Conclusion: This work delivers the first cell cycle-resolved Nek2 interactome mapped by proximity labeling proteomics. In addition to uncovering Nek2's regulation of Nusap1 through proteasomal degradation, with recovery by MG132 and a potential link to E3 ligase activity, our dataset also reveals novel connections between Nek2 and DNA repair pathways. Together, these findings provide a valuable proteomic resource for future studies and highlight Nek2 as a potential regulator of DNA repair–related vulnerabilities in cancer.

Keywords: Nek2, Repair, Mapping

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P046
Mapping ABCB1 Transcription Factors in Taxane-Resistant Prostate Cancer by Genomic Locus Proteomics

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Aim: Prostate cancer (PCa) often progresses into castration-resistant prostate cancer (CRPC), where treatment options such as taxanes lose efficacy due to taxane resistance. A major contributor to this resistance is the overexpression of the multidrug resistance gene ABCB1 (MDR1), which limits drug accumulation in cancer cells. While chemical inhibition of ABCB1 can reverse resistance, it has immunological side effects. This study aims to identify novel transcription factors and regulatory proteins controlling ABCB1 expression, with the goal of uncovering therapeutic targets to restore taxane sensitivity in CRPC.

Methods: In contrast to mass spectrometry that profiles global proteomes, our genomic locus proteomics approach uses a fusion plasmid encoding dCas9 tethered to the peroxidase APEX2 (CASPEX), enabling locus-specific biotinylation and providing us with the regulatory landscape surrounding the ABCB1 gene. gRNAs were designed to target the ABCB1 promoter, validated by ChIP assays, and used to biotinylate proximal proteins. Biotinylated proteins were identified through mass spectrometry, analyzed with bioinformatics pipelines (MaxQuant, DEP), and filtered using multiple criteria including enrichment scores, literature evidence and correlation with ABCB1 expression across cancers. Candidate transcription factors were further prioritized for functional validation via shRNA/CRISPR-Cas9 knockout assays to assess their role in taxane resistance.

Results: Among 2873 biotinylated proteins, we identified 127 enriched transcription factors, of which 10 novel candidates with potential regulatory impact on ABCB1 were selected. Importantly, known ABCB1 regulators, as well as proteins involved in DNA repair, chromatin remodeling, and histone modification, were also detected, validating the robustness of our approach. The in-silico and proteomic analyses highlighted new regulatory layers contributing to ABCB1 overexpression in resistant PCa cells.

Conclusion: This unbiased proteogenomic approach revealed previously unrecognized regulators of ABCB1 expression. By identifying transcription factors and chromatin-associated proteins enriched at the ABCB1 promoter, our study provides novel insights into the mechanisms underlying taxane resistance in CRPC. These findings open the way for developing targeted strategies to modulate ABCB1 activity and improve therapeutic outcomes in advanced prostate cancer.

Keywords: Mapping, ABCB1, Taxane-Resistant Prostate Cancer

P047
Identification of Regulatory Factors at the ATP7B Promoter Using CASPEX Proximity-Labeling

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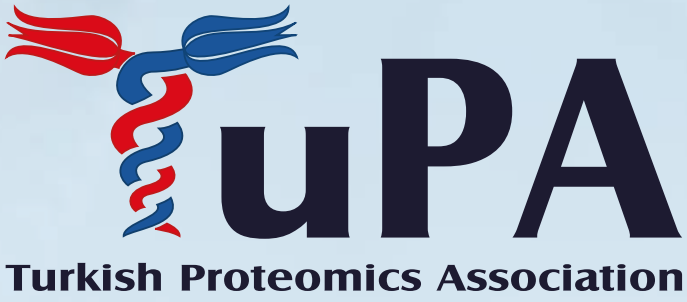
Aim: ATP7B encodes a copper-transporting ATPase essential for copper balance. Dysregulation of ATP7B contributes to Wilson's disease and influences cisplatin resistance in cancer. This study aimed to interrogate transcriptional regulation at the ATP7B promoter, focusing on the transcription start site (TSS) –3000 region, and to identify regulatory factors that control ATP7B expression.
Methods: For genomic locus proteomics analysis, stable CASPEX (CRISPR-dCas9-APEX2) expressing cell lines targeting the ATP7B TSS –3000bp in 7 loci were generated. Successful localization of CASPEX to the target regions were verified by ChIP-qPCR. Following validation, biotinylation reactions were performed with Biotin-Phenol in living cells, and enriched proteins were purified using streptavidin pulldown. Samples were subsequently analyzed by LC-MS/MS. Candidate factors were filtered using stringent identification thresholds for significancy, publicly available ChIP-Seq and TF-binding databases.

Results: Mass spectrometry identified a reproducible set of candidate regulatory proteins enriched at the ATP7B promoter. Analysis highlighted transcription factors and chromatin-associated proteins with roles in DNA repair and oxidative stress response. Following this analysis, we focused on MEN1, HOXB6, GATA6, and TOX4. Using TOX4 overexpression cells, ChIP-qPCR demonstrated that TOX4 directly localized to the ATP7B promoter.

Conclusion: This work establishes CASPEX as a powerful strategy to map regulatory complexes at the ATP7B promoter. Verification by ChIP-qPCR confirmed locus-specific targeting, and downstream proteomic analyses identified novel factors potentially modulating ATP7B transcription. The focused validation of MEN1, HOXB6, GATA6, and TOX4—particularly the confirmed promoter binding of TOX4—provides strong evidence for their regulatory role. These findings offer new leads to elucidate ATP7B regulation in copper homeostasis and chemoresistance.

Keywords: ATP7B, CASPEX, Regulatory Factors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