



# **TuPA International Proteomics Congress** 6<sup>th</sup> Turkish National Proteomics Congress

**11-12 October 2024** Kocaeli University, Kocaeli, Türkiye



**ABSTRACT BOOK**





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**October 11<sup>th</sup> 2024, Friday**

09:00-09:30

**Welcome Notes**

09:30-11:15

**Session 1**

Chair: Murat Kasap

09:30-10:15

**Analysis of Posttranslational Modifications in Cell Division**

*Nurhan Özlü*

Koc University, Molecular Biology and Genetics

10:15-10:45

**Application of Structural Proteomics in the Development of a Therapeutic Inhibitor to Target Ubiquitin-Dependent Degradation Mechanism in Breast Cancer**

*Gizem Dinler Doğanay*

Istanbul Technical University,  
Department of Molecular Biology and Genetics

10:45-11:15

**Proteomic Insight of Cellular Senescence**

*Servet Özcan*

Molecular Biology at Erciyes University

11:15-11:30

**Coffee Break** 

11:30-12:30

**Session 2**

Chair: Banu İskender İzgi

11:30-12:00

**A New Statistical Model for Open Search Localization**

*Daniel J. Geiszler*

Koç University, School of Medicine

12:00-12:30

**Cellular Energy Metabolism and Proteomics**

*Hüseyin Çimen*

Gebze Technical University Central Research Laboratory Application and Research Center

12:30-13:20

**Oral Presentations**

12:30-12:40

**S001**

**Proteomic analysis of cancer cell interactions**

*Nazan Saner, Ceren Uzun, Büşra Aytül Akarlar, Sena Nur Özkan, Daniel Jon Geiszler, Ece Öztürk, Nurcan Tunçbağ, Nurhan Özlü*

**October 11<sup>th</sup> 2024, Friday**

12:40-12:50	<b>S002</b> Thymoquinone-Induced Proteomic Changes Highlight Cellular Metabolism Alterations in MCF-7 Cells <i>Tuğcan Korak, Merve Gulsen Bal Albayrak, Murat Kasap, Gurler Akpınar</i>
12:50-13:00	<b>S003</b> Proteomic changes in osteoarthritis pre- and post-surgery <i>Duygu Sarı Ak, Nazlı Helvacı, Alev Kural, Hayriye Ecem Yelkenci, Cemal Kural, Mustafa Çağlar Beker</i>
13:00-13:10	<b>S004</b> The Relationship Between FoxO3a and Lipid Metabolism in Breast Cancer <i>Pelin Ozfiliz Kilbas</i>
13:10-13:20	<b>S005</b> Serum Proteomic Signatures of Elite Athletes: Insights into Cardiovascular Health <i>Hasan Basri Kılıç, Melis Şardan Ekiz, Ömür Çelikbıçak, Lale Tokgözoğlu, Yusuf Çetin Kocaefe</i>
13:20-14:15	<b>Lunch</b> 
14:15-14:45	<b>Satellite Symposium</b>  Chair: Mehmet Atakay
14:15-14:45	Serum Proteome Profiling by Deep Parallel Multiplexing NGS <i>Kamil Önder</i> CEO of Procomcure Biotech
14:45-15:45	<b>Session 3</b>
14:45-15:05	Discovery of Novel Proteins by Using Omics Technologies <i>Nevin Gül Karagüler</i> Istanbul Technical University
15:05-15:25	Absolute Quantification of Proteins in Complex Matrices via Targeted Proteomics: A Comparative Look at Peptide vs. Protein Calibration Strategies <i>Merve Öztuğ Kılınç</i> TÜBİTAK National Metrology Institute

**October 11<sup>th</sup> 2024, Friday**

15:25-15:45

**Ubiquitin-Proteasome System: Its Role in Cancer Development, Resistance Mechanisms, and New Treatment Strategies**

*Azmi Yerli Kaya*

Kütahya Health Sciences University

15:45-16:45

**Poster Session & Coffee Break**



16:45-18:15

**Session 4**

Chair: Hüseyin Çimen

16:45-17:15

**G Protein-Coupled Receptors (GPCRs) as Novel Generation Pesticides**

*Necla Birgül*

Boğaziçi University, Dept. of Molecular Biology and Genetics

17:15-17:45

**Proteomic and Metabolomic Profiling to Elucidate the Impact of Epetraborole Antibiotic on Escherichia coli**

*Bekir Çöl*

Muğla Sıtkı Koçman University

17:45-18:15

**Opposing Roles for ADAMTS2 and ADAMTS14 in Myofibroblast Differentiation and Function**

*Kübra Karaosmanoğlu Yöneten*

Centre for Tumour Biology, Barts Cancer Institute, Queen Mary University of London

**October 12<sup>th</sup> 2023, Saturday**

09:00-10:30

**Session 5**

Chair: Tarrk Baykal

09:00-09:30

**Actinomycin D Mediated Cellular Stress Orchestrates the Cytosolic Localization of RNA Binding Proteins to Generate Phase Separated Condensates.**

*Sreeparna Banerjee*

Middle East Technical University ODTU

09:30-10:00

**IM-MS Unveils Global Protein Conformations in Response to Conditions that Promote and Reverse Liquid-Liquid Phase Separation**

*Rebecca Beveridge*

University of Strathclyde, Glasgow

10:00-10:30

**Teasing out Proteome and Function of the Mitochondrial Contact Sites with the Nucleus**

*Michelangelo Campanella*

P.S. My affiliation is Centre for Clinical Pharmacology and Precision Medicine, William Harvey Research Institute, Queen Mary University of London

10:30-10:45

**Coffee Break**



10:45-12:30

**Session 6**

Chair: Bekir Salih

10:45-11:15

**Proteomics and Phosphoproteomics for Uncovering Cancer Biology and Precision Oncology**

*Connie R. Jimenez*

Amsterdam UMC University Medical Centers

11:15-11:45

**Understanding the Role of Wnt/ $\beta$ -Catenin Signaling in Brain Demyelination and Remyelination using Proteomics in the Adult Zebrafish Model**

*Güneş Özhan*

İzmir Institute of Technology and a principal investigator at İzmir Biomedicine and Genome Center

11:45-12:30

**Credits, HPI/Kay Herschelmann**

*Bernhard Y. Renard*

Hasso-Plattner-Institut and the Digital Engineering Faculty of the University of Potsdam



**October 12<sup>th</sup> 2024, Saturday**

12:30-13:15

**Lunch** 

13:15-14:00

**Satellite Symposium**

Chair: Sreeparna Banerjee

13:15-14:00

**Making the Impossible Possible: New Horizons of 4-D Proteomics with TIMS Technology**

*Valeriia Kuzyk*

14:00-15:30

**Oral Presentations**

14:00-14:10

**S006**

**Conformational sampling for potential lead binding sites in TPMT enzyme**

*Ceyhan Ceran Serdar, Ece Bingül, Arzu Uyar*

14:10-14:20

**S007**

**An Omics View of Mitochondrial Damage in Megaconial CMD**

*Evrin Aksu Mengeş, Eray Taha Kumtepe, Cemil Can Eylem, Emirhan Nemutlu, Murat Kasap, Gürler Akpınar, Beril Talim, Burcu Balcı Hayta*

14:20-14:30

**S008**

**Targeting Sirt1: Novel Insights into Glioblastoma Therapy**

*İrem Öğütçü, Evren Önay-uçar*

14:30-14:40

**S009**

**Screening the Nanotoxicity of Single-walled Carbon Nanotubes Using nLC-MS/MS Analysis**

*Ayimgül Uzunyol, Nedim Hacıosmanoğlu, Fatih İnci*

14:40-14:50

**S010**

**Antibacterial peptides from Enterococcus hirae strain against vancomycin-resistant enterococci**

*Aurèle A. Assou Koffi, Baran Dingiloğlu, Gizem Dinler Doğanay, Nazmiye Özlem Şanlı, Nazlı Arda*

14:50-15:00

**S011**

**MetaproDB: A Collection of Biome-specific Protein Databases for Metaproteomics**

*Muzaffer Arıkan*

**October 12<sup>th</sup> 2024, Saturday**

15:00-15:10	<b>S012</b> REVEL Scores Demonstrate Superior Performance Compared to AlphaMissense Scores <i>Mustafa Tarik Alay</i>
15:10-15:20	<b>S013</b> Classification of RNA Sequence Data Using Machine Learning <i>Ferdi Güler, Melih Ağraz</i>
15:20-15:30	<b>S014</b> Investigating the Effects of Freeze-Thaw Cycles on Isolated Protein Stability <i>Sertan Çevik, Kübra Yıldız, Dilara Ulusal Sevimli, Eray Şimşek</i>
15:30-15:45	<b>Coffee Break</b> 
15:45-17:05	<b>Session 7</b> Chair: Bekir Çöl
15:45-16:05	Utilizing Enzymatic Biotinylation Approaches to Study Subcellular Proteomics <i>Mehmet Sarıhan</i> Kocaeli University, Institute of Medical Sciences, Medical Biology,
16:05-16:25	Spatial Alterations in 5xFAD Alzheimer's Disease Neonatal Mouse Model Detected by Mass Spectrometry Imaging <i>İrep Uras</i> Acibadem Mehmet Ali Aydınlar University
16:25-16:45	Human Corneal Epithelial Cells as a Model for SARS-CoV-2 infection of Ocular Compartments <i>Mohammad Haroon Qureshi</i> Bogaziçi University, Department of Molecular Biology and Genetics
16:45-17:05	Comprehensive Proteomic Analysis Reveals Molecular Insights into Lymph Node Metastasis in Distinct Breast Cancer Subtypes <i>Merve Gülsen Bal Albayrak</i> Kocaeli University, Faculty of Medicine, Basic Medical Sciences
17:05-17:25	<b>Award Ceremony and Closing</b>

# **ORAL PRESENTATIONS**

## **ABSTRACTS**

## S001 Proteomic analysis of cancer cell interactions

Nazan Saner<sup>1</sup>, Ceren Uzun<sup>2</sup>, Büşra Aytül Akarlar<sup>1</sup>, Sena Nur Özkan<sup>3</sup>, Daniel Jon Geiszler<sup>1</sup>, Ece Öztürk<sup>3,4</sup>, Nurcan Tunçbağ<sup>2</sup>, Nurhan Özlü<sup>1</sup>

<sup>1</sup> Department of Molecular Biology and Genetics, Koç University

<sup>2</sup> Department of Chemical and Biological Engineering, Koç University

<sup>3</sup> Koç University Research Center For Translational Medicine (kuttam), Koç University

<sup>4</sup> Department of Medical Biology, School of Medicine, Koç University

**Aim:** Cell-cell interactions are critical for the growth of organisms and maintaining homeostasis. In the tumor microenvironment, different types of cell interactions promote cancer progression. Given their importance in healthy and diseased conditions, we developed a method combining proximity labelling and quantitative proteomics to profile the cell-cell interactome using co-culture system.

**Methods:** This study employed a combinatorial approach of horseradish peroxidase (HRP)-dependent proximity biotinylation and stable isotope labeling by amino acids in cell culture (SILAC) to identify proteins involved in homotypic (same cell type) and heterotypic (different cell types) cancer cell interactions. Epithelial (MCF7) and mesenchymal (MDA-MB-231) breast cancer cells were co-cultured to model these interactions. HRP was targeted to the extracellular leaflet of the plasma membrane in 'bait' cells to label proximal proteins in 'prey' cells. Prey cells were metabolically labeled with heavy isotopes, enabling differentiation of self-biotinylated proteins of bait cells. Biotinylated proteins were affinity purified and analysed with mass spectrometry. To test the efficiency of proximity labelling in spheroids, cells were encapsulated in alginate hydrogel beads via CaCl<sub>2</sub>-based ionic crosslinking.

**Results:** We mapped both the homotypic and heterotypic interactomes of epithelial and mesenchymal breast cancer cells. The enrichment of cell surface and extracellular proteins confirms the specificity of our methodology. This method revealed prominent signaling pathways orchestrating homotypic and heterotypic interactions of epithelial and mesenchymal cells. It also highlights the importance of exosomes in these interactions. The efficient proximity biotinylation in spheroids suggested that the methodology can be extended to 3D co-culture models.

**Conclusion:** This study presents a new pipeline of experimental protocols and strategies, along with bioinformatic analyses, that can be used to comprehensively examine the proteome of any type of cell-cell interaction.

Anahtar Kelimeler : Biomarkers · Inflammation · Osteoarthritis · Proteomics · Surgical intervention

Bildiri No : 6485

Ana Konu : Cell Biology and Functional Proteomics

Bildiri Türü : Oral Presentation

Sunucu : Duygu Sarı Ak

Eposta : duygusariak@gmail.com



## S002

### Thymoquinone-Induced Proteomic Changes Highlight Cellular Metabolism Alterations in MCF-7 Cells

Pelin Ozfiliz Kilbas, İlke Sarak, Yiğit Efe Aydın, Mustafa Atakan, Buket Özcan

Istanbul Kultur University

**Objective:** The rising incidence of breast cancer (BC) underscores the urgent need for new therapeutic targets and agents to combat resistance and toxic side effects associated with current treatments. Thymoquinone (TQ) has been shown to play a role in apoptosis, cell cycle regulation, cell proliferation, epithelial-to-mesenchymal transition, invasion, angiogenesis, and metastasis in various types of cancer. The anticancer mechanisms of TQ have been explored in various studies, yet a comprehensive analysis in BC remains lacking. This study aims to elucidate the proteomic changes induced by TQ treatment in MCF-7 BC cells and uncover the underlying molecular mechanisms.

**Methods:** MCF-7 cells were treated with 15  $\mu$ M TQ, the inhibitory concentration (IC<sub>50</sub>) determined in our previous study, for 48 hours. Protein extracts from treated and untreated control cells were analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Label-free quantitation (LFQ) identified differentially regulated proteins, with validation through Western blot analysis. Functional annotations were performed using the STRING database. Annotations with a false discovery rate less than 0.05 were considered significant. Subsequently, functional annotations were visualized using the SRplot.

**Results:** The LFQ analysis identified 629 regulated proteins, with 104 upregulated and 477 downregulated in TQ-treated cells, considering a two-fold change between groups. Western blot analysis confirmed a significant decrease in ATP synthase subunit alpha of Complex V (ATP5A) expression ( $p < 0.0001$ ). Functional annotations revealed significant enrichment in metabolic and biosynthetic pathways, including carbon metabolism, ribosome, and amino acid biosynthesis.

**Conclusion:** Thymoquinone induces significant proteomic changes in MCF-7 cells, predominantly affecting pathways related to cellular metabolism. These findings suggest that TQ's anticancer effects may be mediated through metabolic reprogramming, providing insights into its potential as a therapeutic agent for BC. In vivo validation and investigation of TQ's long-term effects are necessary to fully explore its therapeutic potential in metabolic reprogramming and BC treatment.

Anahtar Kelimeler : Breast cancer, Thymoquinone, Proteomics

Bildiri No : 6298

Ana Konu : Cell Biology and Functional Proteomics

Bildiri Türü : Oral Presentation

Sunucu : Tuğcan Korak

Eposta : tugcan.korak@kocaeli.edu.tr

### S003

#### Proteomic changes in osteoarthritis pre- and post-surgery

Duygu Sarı Ak<sup>1</sup>, Nazlı Helvacı<sup>2</sup>, Alev Kural<sup>3</sup>, Hayriye Ecem Yelkenci<sup>6</sup>, Cemal Kural<sup>4</sup>, Mustafa Çağlar Beker<sup>5</sup>

<sup>1</sup> Department of Medical Biology, Hamidiye International School of Medicine, University of Health Sciences, Istanbul 34668, Turkey

<sup>2</sup> Department of Medical Biochemistry, Hamidiye School of Medicine, University of Health Sciences, Istanbul 34668, Turkey

<sup>3</sup> Clinic of Medical Biochemistry, Bakırkoy Dr. Sadi Konuk Training and Research Hospital, University of Health Sciences, Istanbul 34668, Turkey

<sup>4</sup> Orthopaedics and Traumatology Clinic, Bakırkoy Dr. Sadi Konuk Training and Research Hospital, University of Health Sciences, Istanbul 34668, Turkey

<sup>5</sup> Department of Physiology, School of Medicine, Istanbul Medeniyet University, Istanbul, Turkey

<sup>6</sup> Regenerative and Restorative Medical Research Center (remer), Research Institute For Health Sciences and Technologies (sabita), Istanbul Medipol University Istanbul, Turkey

Osteoarthritis (OA) is a degenerative joint disease with a significant burden on public health. Understanding the molecular alterations associated with OA, particularly before and after surgical interventions, can improve diagnostic and therapeutic strategies. This research aims to investigate the proteomic changes in OA patients pre- and post-surgery to identify key proteins involved in disease progression and recovery.

A cohort of OA patients undergoing surgical treatment was selected for this study. Blood samples were collected from these patients both pre-operatively and six weeks post-operatively. The control group consisted of healthy individuals. Proteomic profiling was conducted using mass spectrometry, and the resulting data were analyzed using bioinformatics tools, including DAVID for pathway analysis and STRING for protein-protein interaction networks. Statistical analyses were performed to identify significantly altered proteins between the groups.

Proteomic analysis revealed significant molecular disparities between pre-operative OA patients and healthy controls, with a marked upregulation of proteins related to inflammation, extracellular matrix remodeling, and metabolic processes. Post-surgery, several of these proteins returned to baseline levels, suggesting partial normalization of the molecular pathways affected by OA. However, some proteins remained altered, indicating incomplete recovery and potential targets for adjunctive therapies.

This study highlights key proteomic changes in OA patients before and after surgery. The findings suggest that surgical intervention helps mitigate some molecular disruptions associated with OA, but certain pathways remain dysregulated. These proteins could serve as potential biomarkers for monitoring surgical outcomes and guiding post-operative care in OA patients. Further research is needed to explore therapeutic interventions targeting these persistent molecular alterations.

Anahtar Kelimeler : Biomarkers · Inflammation · Osteoarthritis · Proteomics · Surgical intervention

Bildiri No : 6485

Ana Konu : Cell Biology and Functional Proteomics

Bildiri Türü : Oral Presentation

Sunucu : Duygu Sarı Ak

Eposta : duygusariak@gmail.com

## S004

### The Relationship Between FoxO3a and Lipid Metabolism in Breast Cancer

Pelin Ozfiliz Kilbas, İlke Sarak, Yiğit Efe Aydın, Mustafa Atakan, Buket Özcan

Istanbul Kultur University

The tumor suppressor FoxO3a is involved in several important physiological functions, such as metabolism, apoptosis, and cell cycle regulation. This transcription factor has a particularly significant role in breast cancer. Different metabolic traits, such as differences in lipid metabolism, are present in breast cancer subtypes and can impact tumor development. Due to this heterogeneity, we aim to investigate more about the connection between lipid metabolism and FoxO3a. Gaining knowledge about how FoxO3a affects lipid metabolic pathways in various subtypes of breast cancer may be very helpful in developing novel therapeutic approaches and individualized treatment plans.

LC-MS/MS studies in breast cancer cells analyzed changes in total protein expression levels in response to FoxO3a overexpression. Fatty acid synthase enzyme inhibitor Orlistat treatment was performed to determine the effects on cell survival, growth, and proliferation of breast cancer cells through MTT Assay, Trypan blue dye exclusion assay, and fluorescence staining. The role of FoxO3a overexpression and orlistat treatment on lipid metabolism-mediated apoptosis was investigated by immunoblotting techniques. Identification of proteins potentially interacting with FoxO3a among various candidates involved in lipid metabolism was detected by *in silico* computational approaches. The selected potential protein target was validated by immunoprecipitation techniques.

Due to FoxO3a overexpression and orlistat treatment, cell viability and survival of breast cancer cells were reduced depending on the hormone receptor level difference. It was observed that the expression levels of proteins such as FASN, ACC, ACL and PPAR- $\gamma$  were altered following Orlistat treatment. *In silico* computational approaches showed potential binding partners of FoxO3a. The potential interaction between lipid metabolism pathway members and FoxO3a could influence biological processes such as the regulation of cellular metabolism, oxidative stress response, or fatty acid metabolism. Further experimental studies could elucidate the functional significance of this interaction.

Anahtar Kelimeler : FoxO3a, lipid metabolism, orlistat, breast cancer

Bildiri No : 6500

Ana Konu : Cell Biology and Functional Proteomics

Bildiri Türü : Oral Presentation

Sunucu : Pelin Ozfiliz Kilbas

Eposta : p.ozfiliz@iku.edu.tr



## S005

### Serum Proteomic Signatures of Elite Athletes: Insights into Cardiovascular Health

Hasan Basri Kılıç<sup>1</sup>, Melis Şardan Ekiz<sup>2</sup>, Ömür Çeliklebiçak<sup>2</sup>, Lale Tokgözoğlu<sup>3</sup>, Yusuf Çetin Kocaefe<sup>1</sup>

<sup>1</sup> Hacettepe University, School of Medicine, Department of Medical Biology, Ankara/turkey

<sup>2</sup> Hacettepe University, Advance Technologies Research and Application Center, Hunitek , Ankara/turkey

<sup>3</sup> Hacettepe University, School of Medicine, Department of Cardiology, Ankara/turkey

This study investigates serum proteomic profiles of elite athletes (marathoners and weightlifters), controls with age and sex matched, and cancer patients who received doxorubicin, a known inducer of cardiac fibrosis. We aimed to identify potential serum biomarkers for cardiovascular health and explore the impact of contrasting exercise modalities on serum protein composition.

Serum samples were processed using the iST kit and analyzed via mass spectrometry using nLC-QTOF-maxis II ETD. Raw data was analyzed with MaxQuant (2.6.3.0). Statistics and visualization were performed with Perseus (2.1.1.0). Across all groups, we identified 300 unique proteins. A comparative analysis with the stringency of 1% FDR and t-test p-value cut-off was 0.05 revealed significantly abundant peptides between groups.

In particular, fibronectin (FN1) levels decreased significantly in weightlifters' serum compared to marathoners and sedentary controls. Conversely, sex hormone-binding globulin (SHBG) was significantly elevated in weightlifters compared to marathoners. Cancer patients exhibited elevated levels of acute-phase reactants compared to all other groups. These findings suggest that FN1 may serve as a potential detectable serum biomarker for vascular changes, and decreased FN1 levels could indicate vascular degeneration. Interestingly, while exercise upregulates FN1 in slow-twitch muscle fibers, it is conversely suppressed in fast-twitch fibers, suggesting FN1's potential utility in discriminating muscle fiber types and fiber switches.

The observed differences in SHBG levels between athlete groups are particularly intriguing given recent studies that indicate that weightlifters tend to have shorter life expectancies than marathoners. Lower SHBG levels have been associated with increased all-cause mortality, particularly with cardiovascular causes.

This study provides novel insights into the serum proteomic signatures associated with opposing modes of athletic (elite) training as well as cancer therapy. Our findings may have implications for understanding cardiovascular health in these populations and could guide future research on exercise-related health outcomes and potential biomarkers for cardiovascular risk assessment.

Anahtar Kelimeler : Serum Proteomics, Cardiovascular Health, Elite Athletes, Myokines, Cachexia, Slow-Twitch and Fast Twitch Muscle Fibers

Bildiri No : 6447

Ana Konu : Clinical Applications

Bildiri Türü : Oral Presentation

Sunucu : Hasan Basri Kılıç

Eposta : kilichb7@gmail.com



## S006

### Conformational sampling for potential lead binding sites in TPMT enzyme

Ceyhan Ceran Serdar<sup>1</sup>, Ece Bingöl<sup>3</sup>, Arzu Uyar<sup>2</sup>

<sup>1</sup> Ankara Medipol University, School of Medicine, Department of Medical Biology and Genetics, Ankara, Turkey

<sup>2</sup> İzmir Institute of Technology, Faculty of Engineering, Department of Bioengineering, İzmir, Turkey

<sup>3</sup> Ankara Medipol University, School of Medicine, Ankara, Turkey

**Aim:** Metal toxicity, which can lead to detrimental outcomes ranging from lipid peroxidation to genomic instability and even cancer development, was previously shown to inhibit various metabolic enzymes such as creatine kinase, delta-aminolevulinic acid dehydratase, hexokinase, catalase, etc. Thiopurine S-methyltransferase (TPMT), an enzyme that catalyzes the S-methylation of thiopurine drugs such as 6-mercaptopurine (6-MP), is of immense clinical importance as its enzymatic activity significantly influences the therapeutic dosage of thiopurine drugs required for each individual patient. To investigate the potential for lead-induced deactivation of thiopurine S-methyltransferase in-silico, we compared the enzyme's putative lead-binding domains with its prospective pockets that are expected to interact with 6-mercaptopurine (6-MP; the substrate), S-Adenosyl L-methionine (SAM; the methyl-donor), and S-adenosyl-L-homocysteine (SAH; the by-product).

**Methods:** 300 different conformations of the enzyme were initially generated through ClustENMD, an efficient sampling algorithm. Then, 13 distinct conformations with significant deviations from the original X-ray structure of TPMT were chosen and subsequently used for lead-docking through Metal ion-binding site prediction and modeling server (MIB2), and for docking of 6-MP, SAM or SAH through CB-dock server.

**Results:** Seven new putative lead-binding pockets, distinct from the six pockets originally pinpointed through the analysis of the static X-ray structure, were identified through the analysis of the dynamic structures. The overall results indicate the putative pockets where lead-docking coincides with substrate-binding, suggesting a potential for lead-induced allosteric inhibition.

**Conclusion:** Further in vitro and in-vivo analyses are necessary to elucidate the mechanism of a potential lead-induced TPMT-deactivation.

Anahtar Kelimeler : TPMT, lead-docking, in-silico

Bildiri No : 6536

Ana Konu : Bioinformatics

Bildiri Türü : Oral Presentation

Sunucu : Ceyhan Ceran Serdar

Eposta : ceyhan.ceran@ankaramedipol.edu.tr

## S007

### An Omics View of Mitochondrial Damage in Megaconial CMD

Evrin Aksu Mengeş<sup>1</sup>, Eray Taha Kumtepe<sup>1</sup>, Cemil Can Eylem<sup>2</sup>, Emirhan Nemutlu<sup>2</sup>, Murat Kasap<sup>3</sup>, Gürler Akpınar<sup>3</sup>, Beril Talim<sup>4</sup>, Burcu Balcı Hayta<sup>1</sup>

<sup>1</sup> Hacettepe University, Faculty of Medicine, Department of Medical Biology, 06100 Sıhhiye, Ankara, Turkey.

<sup>2</sup> Hacettepe University, Faculty of Pharmacy, Department of Analytical Chemistry, 06100 Sıhhiye, Ankara, Turkey

<sup>3</sup> Kocaeli University, Faculty of Medicine, Department of Medical Biology, 41001 İzmit, Turkey

<sup>4</sup> Hacettepe University, Faculty of Medicine, Department of Pediatrics, Pathology Unit, 06100 Sıhhiye, Ankara, Turkey

**Aim:** Megaconial Congenital Muscular Dystrophy-CMD is the first type of CMD characterized by a lipid biosynthesis defect. The gene responsible for this disease has been identified as choline kinase beta (CHKB)

in patients, the majority of whom were from Turkey. CHK Bencodes the enzyme that catalyzes the first step of the biosynthesis pathway of phosphatidylcholine (PC), the most abundant phospholipid in eukaryotic cell membranes. The most characteristic histopathological finding of the disease is the presence of enlarged(megaconial) mitochondria in skeletal muscle, concentrated close to the sarcolemma. This study aims to shed light on the causes and/or consequences of mitochondrial dysmorphology and dysfunction seen in the pathogenesis of Megaconial CMD with -omics approaches.

**Method:** After examining the expression levels of proteins involved in mitochondrial dynamics by Western Blot and immunofluorescence staining, untargeted (GC-MS)/targeted (LC-MS/MS) metabolomic and GC-MS based fluxomic analyses were performed to investigate mitochondrial energy production in primary skeletal muscle cells. Then, following the isolation of pure mitochondria from primary cells, proteomic analyses were performed by LC-MS/MS, and proteins differentially expressed in the patient's mitochondrial fraction compared to the control group were confirmed by qRT-PCR and Western Blot.

**Results:** A statistically significant decrease was detected in the expression levels of mitochondrial fission proteins in patient cells, while no difference was found in the expression levels of fusion proteins between the control and the patient. Metabolomic and fluxomic analyses revealed decreased levels of phosphonucleotides, Krebs cycle intermediates, ATP, and energy metabolism-related metabolites. Also, proteomic analysis revealed an ATP/ADP transport defect in Megaconial CMD skeletal muscle cells.

**Conclusion:** Our results indicate that altered mitochondrial dynamics and energy metabolism, as well as defective ATP/ADP transport contribute to mitochondrial dysmorphology and dysfunction in the pathogenesis of Megaconial CMD.

This study was supported by Hacettepe University, Scientific Research Projects Coordination Unit (Project no: THD-2015-7717, TSA-2021-19199).

Anahtar Kelimeler : megaconial congenital muscular dystrophy, mitochondrial dysfunction and dysmorphology, omic analysis, skeletal muscle

Bildiri No : 6419

Ana Konu : Cell Biology and Functional Proteomics

Bildiri Türü : Oral Presentation

Sunucu : Evrim Aksu Mengeş

Eposta : evrim.aksu@hacettepe.edu.tr

## S008 Targeting Sirt1: Novel Insights into Glioblastoma Therapy

İrem Öğütçü<sup>1</sup>, Evren Önay-uçar<sup>2</sup>

<sup>1</sup> Department of Molecular Biotechnology and Genetics, Institute of Graduate Studies In Sciences, Istanbul University, Istanbul, Turkey

<sup>2</sup> Department of Molecular Biology and Genetics, Faculty of Science, Istanbul University, Istanbul, Turkey.

**Purpose:** Glioblastoma (GB) is one of the most malignant primary brain tumors, associated with a poor prognosis. Sirtuin 1 (SIRT1) is a NAD<sup>+</sup>-dependent deacetylase, plays a crucial role in tumor growth, metastasis and the response to cancer therapies. This study aimed to investigate the effects and underlying mechanism of SIRT1 on glioblastoma.

**Methods:** U-87MG 3D-tumor spheroids were formed via the hanging-drop/agarose method. SIRT1 expression was blocked using siRNA transfection (knockdown efficiency for 2D and 3D cell culture as 79.7% and 57.6%, respectively). The effects of siRNA-mediated SIRT1 silencing in were assessed through cell viability, migration, angiogenesis, EMT, apoptosis, autophagy, ROS analysis, fluorescence imaging, and spheroid growth. The underlying mechanism of the anticancer effects of SIRT1 silencing was investigated by western blot and immunofluorescence analysis.

**Results:** Silencing SIRT1 decreased glioma cell viability, colony formation, angiogenesis, heat shock response, and growth in 2D and 3D cultures. It increased ROS levels and ER stress, downregulated the SIRT1/FOXO1/NF- $\kappa$ B axis, and shifted protein localization towards apoptosis. Additionally, SIRT1 silencing reduced p-ERK1/2 and p-AKT levels, potentially inducing autophagy and slowing GB spheroid growth.

**Conclusion:** In summary, siRNA-mediated SIRT1 silencing in U-87MG glioma cells leads to the suppression of EMT, decreased migration and angiogenesis, and the induction of ROS and ER stress. It also downregulates the SIRT1/FOXO1/NF- $\kappa$ B axis and inhibits survival signals such as AKT and ERK, resulting in apoptosis. This study is the first to evaluate the anti-cancer mechanisms of SIRT1 silencing by comparing 2D glioblastoma cells with 3D spheroids, emphasizing SIRT1 as a promising therapeutic target for GB and supporting the need for further clinical investigation.

Anahtar Kelimeler : Glioblastoma, SIRT1 silencing, 3D-spheroids, Apoptosis, Autophagy

Bildiri No : 6517

Ana Konu : Cell Biology and Functional Proteomics

Bildiri Türü : Oral Presentation

Sunucu : İrem Öğütçü

Eposta : iremogutcuu@gmail.com



## S009

### Screening the Nanotoxicity of Single-walled Carbon Nanotubes Using nLC-MS/MS Analysis

Ayimgül Uzunyol<sup>1</sup>, Nedim Hacıosmanoğlu<sup>2</sup>, Fatih İnci<sup>2</sup>

<sup>1</sup> Ostim Technical University, Biomedical Device Technology Faculty, Vocational School

<sup>2</sup> National Nanotechnology Research Center, Bilkent University

<sup>3</sup> Institute of Materials Science and Nanotechnology, Bilkent University

Screening the Nanotoxicity of Single-walled Carbon Nanotubes Using Nano-liquid Chromatography Mass-spectrometry (nLC-MS/MS) Analysis

**Background/aim:** Within the alluring wide range of application of single-walled carbon nanotubes(SWCNTs), use of SWCNTs has become under scrutiny due to their toxicity. Proteomic analysis gained great attention recently with the developed and advanced spectrometry tools to provide a deeper insight on the cellular pathways related to adverse effects of nanomaterials in cellular level. In this study, the cellular cytotoxic effects of SWCNTs on MCF-7 cells in vitro were investigated by comparative proteome analysis at the Proteomic level using Mass Spectrometry-based Proteomic approach.

**Methods:** Prior to the proteome analyses, SWCNTs and cells were incubated separately 3 different time intervals and protein digests were prepared. Then, the protein digests in different groups were subjected to nano-liquid chromatography mass-spectrometry (nLC-MS/MS) analysis, the abundances of the obtained proteins were compared with the control and their regulations, such as increase or decrease in protein profiles, were investigated.

**Results:** As a result of these analyses, a list of differentially regulated proteins were identified across all 3 different incubation periods. A change ranging from 1.5 to 20-fold in the abundance of regulated proteins, such as a significant increase or decrease, was observed in experiment versus control at different incubation times. In our study, a significant increase or decrease in regulation was obtained in a total of 70 protein profiles. Among these, a significant increase in the abundance of 45 proteins was observed, while a significant decrease in the abundance of 25 proteins was observed.

**Conclusion:** Overall, a list of differentially regulated proteins that were not previously known to interact or associate with the cytotoxicological effect of SWCNT on MCF-7 cell lines at the proteomic level was determined. It will establish a link between regulation of protein function/activity and cytotoxicity induced by SWCNT.

Anahtar Kelimeler : SWCNTs, MCF-7, Cytotoxicity, nLC-MS/MS, Proteomics

Bildiri No : 5580

Ana Konu : Proteomics in Model Organisms

Bildiri Türü : Oral Presentation

Sunucu : Ayimgül Uzunyol

Eposta : ayimgul.uzunyol@ostimteknik.edu.tr



## S010

### Antibacterial peptides from *Enterococcus hirae* strain against vancomycin-resistant enterococci

Aurèle A. Assou Koffi<sup>1</sup>, Baran Dingiloğlu<sup>2</sup>, Gizem Dinler Doğanay<sup>3</sup>, Nazmiye Özlem Şanlı<sup>4</sup>, Nazlı Arda<sup>5</sup>

<sup>1</sup> Istanbul University, Institute of Graduate Studies In Sciences, Department of Molecular Biology and Genetics, Istanbul, Türkiye

<sup>2</sup> Istanbul Technical University, Molecular Biology - Genetics and Biotechnology, Istanbul, Türkiye

<sup>3</sup> Istanbul Technical University, Molecular Biology - Genetics, Istanbul, Türkiye

<sup>4</sup> Istanbul University, Faculty of Science, Department of Biology, Section of Biotechnology, Istanbul, Türkiye

<sup>5</sup> Istanbul University, Faculty of Science, Department of Molecular Biology and Genetics, Istanbul, Türkiye

**AIM:** Enterococci are Gram-positive, facultative anaerobic bacteria responsible for nosocomial infections. A particularity of these bacteria is that they are naturally resistant to many antibacterial molecules. Indeed, they are resistant to aminoglycosides and  $\beta$ -lactam antibiotics. They were also reported as first microorganisms that developed resistance to glycopeptide-structured vancomycin antibiotics. Despite their ability to cause diseases, some enterococci were reported with antimicrobial activity due to their ability to produce antimicrobial peptides (AMPs). This study aimed to identify the active principle(s) found in cell-free supernatant from a new

*Enterococcus hirae*

strain, a potential antibiotic producer, to combat antibiotic-resistant bacteria.

**METHODS:** The antibacterial activity of the new *Enterococcus hirae* strain Y7 (isolated from a soil sample and identified by the next-generation sequencing method) was assessed against three pathogenic enterococci: *Enterococcus hirae* ATCC 10541, vancomycin-resistant enterococci (VRE): *Enterococcus faecium* ATCC 700221 and *Enterococcus faecalis* ATCC 51299. First, antibacterial activity of whole *E. hirae* Y7 cells was assessed with agar spot assay. As it is known that enterococci release AMPs, antibacterial activity assessments were performed as well on its cell-free supernatant (CFS) protein precipitate and on its non-proteinous part using agar well diffusion assay. In the purpose to identify antibacterial peptide(s), antibacterial activity of HPLC fractions from CFS protein precipitate were assessed using spot-on-lawn assay; and peptide mapping were conducted using LC-MS.

**FINDINGS:** While its non-proteinous fraction was inactive, CFS protein precipitate significantly inhibited the growth of all the pathogens including the two vancomycin-resistant ones. Unfortunately, the CFS protein precipitate fractions obtained from HPLC were inactive.

**CONCLUSION:** This work revealed that new *Enterococcus hirae* strain releases antibacterial peptide(s) against resistant bacteria. Further studies are in progress to determine which peptides are responsible from antibacterial activity, and to identify of the peptides(s) responsible for the activity.

Anahtar Kelimeler : Enterococci, antimicrobial peptide, cell-free supernatant, bacteriocin

Bildiri No : 6453

Ana Konu : Cell Biology and Functional Proteomics

Bildiri Türü : Oral Presentation

Sunucu : Aurèle A. Assou Koffi

Eposta : aurele1ers@gmail.com

## S011

### MetaproDB: A Collection of Biome-specific Protein Databases for Metaproteomics

Muzaffer Arıkan

İstanbul Üniversitesi Fen Fakültesi Biyoloji Bölümü Biyoteknoloji Anabilim Dalı

**Aim:** In metaproteomics research, creating a reference protein sequence database that balances comprehensiveness with manageable size is crucial for effective peptide identification. Consequently, the need for well-curated, extensive biome-specific reference databases is growing to ensure optimal performance in metaproteomics studies. In this study, I describe MetaproDB, an extensive resource offering biome-specific, ready-to-use protein sequence databases curated for diverse biomes.

**Methods:** I re-analyzed 50,854 previously published 16S rRNA gene amplicon sequencing samples across 29 biomes and identified the 50 most abundant bacterial genera within each biome. Then, using a custom version of the gNOMO2 microbiome multi-omics pipeline, I created a comprehensive catalog of biome-specific protein sequence databases, each containing protein sequences from the complete genomes of these most abundant genera.

**Results:** MetaproDB spans a wide range of environments, including human body regions, fermented beverages, wastewater, and soil, and currently provides ready-to-use reference protein sequence databases for 29 biomes. These databases, derived from 565 unique bacterial genera and their proteins across 212,534 genome assemblies, were generated from the 50 most abundant bacterial genera within each biome. Additionally, MetaproDB offers user-friendly scripts, enabling the creation of custom databases tailored to specific biomes and parameters.

**Conclusion:** MetaproDB serves as a comprehensive resource of customized reference protein sequence databases for metaproteomics studies.

Anahtar Kelimeler : metaproteomics, microbiome, protein database, amplicon sequencing

Bildiri No : 6406

Ana Konu : Bioinformatics

Bildiri Türü : Oral Presentation

Sunucu : Muzaffer Arıkan

Eposta : muzafferarikan@istanbul.edu.tr

## S012

### REVEL Scores Demonstrate Superior Performance Compared to AlphaMissense Scores

Mustafa Tarik Alay

Ankara Etlik City Hospital Department of Medical Genetic

**Aim:** To compare the pathogenic variant classification by contrasting the success rates of AlphaMissense and REVEL scores in hereditary cancers.

**Materials and Methods:** In this study, we assessed the efficacy of REVEL and AlphaMissense in categorizing pathogenic variants. We assessed the accuracy with which they classified variants identified by clinicians as pathogenic or likely pathogenic. We randomly selected 122 variants, all confirmed as unquestionably pathogenic by ClinVar (with a minimum of two stars), from a pool of approximately 5,000 patients who attended the Genetics Clinic at Ankara Etlik City Hospital.

**Results:** According to the study's results, REVEL correctly classified 95% of variants clinically classified as pathogenic/likely pathogenic, while AlphaMissense correctly classified only 78% of these variants. A statistically significant difference was observed between the classifications of these variants ( $p < 0.001$ ).

**Conclusion:** While advancements in protein 3D structures present significant potential, clinical findings are the most critical element in routine diagnostics, especially for complex conditions such as hereditary cancers.

Anahtar Kelimeler : Alphamissense, Revel, Hereditary Cancers

Bildiri No : 6442

Ana Konu : Clinical Applications

Bildiri Türü : Oral Presentation

Sunucu : Mustafa Tarik Alay

Eposta : mtarikalay@gmail.com

## S013

### Classification of RNA Sequence Data Using Machine Learning

Ferdi Güler, Melih Ağraz

Giresun Üniversitesi

**Title:** Classification of RNA Sequence Data Using Machine Learning

**Author:** Ferdi Güler

**Institution:** Department of Statistics, Giresun University Institute of Science, Giresun, Türkiye

**Purpose:** This study integrates RNA sequence data and proteomics to improve the accuracy of cancer prediction by classifying Kidney Chromophobe Renal Cell Carcinoma (KICH), a distinct subtype of kidney cancer. Accurate classification of KICH is crucial for enhancing diagnostic precision and guiding targeted treatments in proteomics-based research.

**Method:** The dataset includes RNA sequence data from 91 samples, consisting of 66 from KICH patients and 25 from healthy individuals. To address challenges posed by small sample sizes and high-dimensional RNA-Seq data, data augmentation and dimension reduction techniques were applied. Classification was conducted using XGBoost, Decision Trees, MLP, and the KAN neural network. SMOTE was used to augment the data, and feature selection from the best model was performed using Explainable AI (XAI) techniques.

**Findings:** The SMOTE-enhanced XGBoost model demonstrated superior performance, emphasizing the importance of data augmentation in RNA-Seq data classification. The KAN neural network also showed effective classification performance, with key genes identified through XAI-based feature selection. These genes are potential biomarkers for KICH in proteomics studies.

**Conclusion:** By incorporating RNA sequence data with proteomics, this study highlights the critical role of preprocessing, feature selection, and data augmentation in cancer classification. The results underscore the potential of these techniques to enhance RNA-Seq data model accuracy, contributing to the advancement of personalized medicine in oncology. Future research should focus on validating the selected genes in proteomics studies and incorporating them into scientific literature.

Anahtar Kelimeler : Proteomics, RNA-Sequence, Machine Learning, Classification, Feature Selection, Preprocessing, Dimension Reduction, Data Augmentation

Bildiri No : 6513

Ana Konu : Bioinformatics

Bildiri Türü : Oral Presentation

Sunucu : Ferdi Güler

Eposta : ferdiiguler@gmail.com



## S014

### Investigating the Effects of Freeze-Thaw Cycles on Isolated Protein Stability

Sertan Çevik<sup>1</sup>, Kübra Yıldız<sup>1</sup>, Dilara Ulusal Sevimli<sup>2</sup>, Eray Şimşek<sup>3</sup>

<sup>1</sup> Harran University, Faculty of Science and Letters, Department of Molecular Biology and Genetics, Şanlıurfa

<sup>2</sup> Harran University, Faculty of Science and Letters, Department of Biology, Şanlıurfa

<sup>3</sup> Harran University, Faculty of Agriculture, Department of Plant Protection, Şanlıurfa

#### OBJECTIVE

Isolated proteins are stored in laboratories by freezing for use in studies. Generally, it is believed in the literature that proteins frozen in large volumes and subsequently thawed and refrozen degrade, leading to observable activity losses. This study was conducted to observe whether protein extracts degrade as much as suggested in this regard. Additionally, the study aimed to determine whether the thawing temperature affects the degradation of the proteins.

#### METHOD

In this study, the potential degradation resulting from the repeated freezing and thawing of protein extracts obtained from barley was monitored using native-PAGE and SDS-PAGE. Protein was extracted from barley leaves without the use of an inhibitor, and the samples were divided into Eppendorf tubes, undergoing freezing and thawing up to nine times. The SOD activity of protein samples was observed using native-PAGE while the SDS-PAGE was used for separation of proteins based on their molecular weight.

#### RESULTS

Analysis revealed that proteins thawed at 25 °C degraded more than those thawed at 4 °C; however, the number of freeze-thaw cycles did not cause any significant degradation. It was observed that the least and most frequently frozen and thawed protein samples yielded similar results regardless of whether they were thawed at 25 °C or 4 °C.

#### CONCLUSION

This study demonstrates that repeated freezing and thawing of protein extracts obtained from barley doesn't cause degradation, contrary to the information available in the literature. However, the thawing temperature does significantly affect degradation. This result could be specific to the species, or the idea that proteins become unusable after the first thawing may have been disproven. In addition, the results have raised many uncertainties about the mechanism that leads to preservation of proteins during the freezing and thawing process, and the findings from this work serve as a preliminary study for future research.

Anahtar Kelimeler : barley, SDS-PAGE, native-PAGE, protein degradation, freezing and thawing

Bildiri No : 6520

Ana Konu : New Approaches in Proteomics (Other)

Bildiri Türü : Oral Presentation

Sunucu : Kübra Yıldız

Eposta : kubrayildiz@harran.edu.tr

# **POSTER PRESENTATIONS** **ABSTRACTS**

## P001

### Glycan Profiling of Non-Small Cell Lung Cancer by HPLC-HILIC-FLD

Betül Karabudak<sup>1</sup>, Fatih Karataş<sup>2</sup>, Hakan Yılmaz<sup>1</sup>, Bekir Salih<sup>3</sup>, Hacı Mehmet Kayılı<sup>1</sup>

<sup>1</sup> Karabük Üniversitesi, Mühendislik Fakültesi, Biyomedikal Mühendisliği Bölümü, Biyomedikal Mühendisliği Ana Bilim Dalı, Karabük

<sup>2</sup> Karabük Üniversitesi, Tıp Fakültesi, Dahili Tıp Bilimleri Bölümü, Tıbbi Onkoloji Ana Bilim Dalı, Karabük

<sup>3</sup> Hacettepe Üniversitesi, Fen Fakültesi, Kimya Bölümü, Fiziko Kimya Ana Bilim Dalı, Ankara

**Aim:** The study aimed to profile glycan abundances in non-small cell lung cancer (NSCLC) patients and explore the potential of machine learning for diagnostic classification.

**Method:** Glycan profiles were analyzed using High-Performance Liquid Chromatography (HPLC) coupled with Hydrophilic Interaction Liquid Chromatography (HILIC) and Fluorescence Detection (FLD). Machine learning algorithms were then applied to classify NSCLC patients and healthy controls based on the glycosylation data.

**Results:** Significant changes in glycan abundances were observed in NSCLC patients compared to healthy controls. Machine learning classification yielded promising preliminary results in distinguishing between the two groups.

**Conclusion:** This pre-data study suggests that glycan profiling, combined with machine learning, holds potential for NSCLC diagnosis and patient stratification. Further validation with larger sample sizes is required for conclusive results.

Anahtar Kelimeler : lung cancer, glycosylation, glycomics, machine learning

Bildiri No : 6518

Ana Konu : Multi-omics Approaches

Bildiri Türü : Poster Presentation

Sunucu : Betül Karabudak

Eposta : betulkarabudak@karabuk.edu.tr

## P002

### Distinctive protein expression in reprogrammed and ancestral bladder cancer cells

Banu Iskender Izgi, Bengi Su Rumeysa Barlak, Zeynep Sude Kongur, Mehmet Sarihan, Gurler Akpinar, Murat Kasap

Kocaeli Üniversitesi Tıbbi Biyoloji Anabilim Dalı

**Purpose:** The term ‘reprogramming’ involves reversing the terminally differentiated state of a cell to a pluripotent state by ectopically expressing transcription factors that regulate stemness characteristics. Cells from different origins can be reprogrammed into a pluripotent state, but the combination of defined factors and reprogramming enhancers varies greatly. Certain cancer cell lines have also been shown to revert to an undifferentiated state. However, reprogramming a malignant cell into a benign state is challenging and requires understanding the current state of the cell's functional components while fine-tuning the reprogramming factors. Therefore, we aimed to compare the protein content of ancestral bladder cancer cells with that of reprogrammed cancer cells to assess the feasibility and success of cancer cell reprogramming.

**Method:** Non-genome integrating Sendai virus system utilised for reprogramming. ‘Yamanaka factors’ were used to transduce grade 4 bladder cancer cell line HTB-5. Transduced HTB-5 cells were transferred onto vitronectin-coated dishes and cultured in a feeder-free human-induced pluripotent stem cell culture system. Reprogrammed cells were characterised for pluripotency markers. The nHPLC LC-MS/MS system was employed for protein identification.

**Findings:** LC-MS/MS peptide peak data was searched against the entire proteome database, identifying over 3000 proteins. Processed protein data exhibiting significant change among the samples were analysed using PANTHER and STRING. Significant changes were noted in the proteins associated with stem cell maintenance, epithelial-mesenchymal transition, cell adhesion, cell motility and differentiation.

**Conclusion:** Detecting the reprogramming-associated changes in protein expression is crucial, not only for establishing a standardised protocol for reprogramming cancer cells but also for elucidating the mechanisms underlying the fine balance of benignity and malignancy. In this study, we reprogrammed a grade 4 bladder cancer cell line and demonstrated, for the first time, the dramatic changes in protein expression that occur upon reprogramming which will be validated through our ongoing research in this area.

Anahtar Kelimeler : Reprogramming, HTB-5, Proteomics, Bladder Cancer

Bildiri No : 6436

Ana Konu : Cell Biology and Functional Proteomics

Bildiri Türü : Poster Presentation

Sunucu : Banu Iskender Izgi

Eposta : banu.iskender@kocaeli.edu.tr



## P003

### Plasma Phosphoproteomics Analysis of HG-PT1 Bladder Cancer

Andrea Henze<sup>1</sup>, Büşra Ergun<sup>2</sup>, Tunay Doğan<sup>3</sup>, Asif Yıldırım<sup>4</sup>, Aysel Özpınar<sup>5</sup>

<sup>1</sup> Institute of Agricultural and Nutritional Sciences, Martin-luther-universität Halle-wittenberg/almanya

<sup>2</sup> Department of Biochemistry and Molecular Biology, Institute of Health Sciences, Acibadem Mehmet Ali Aydınlar University, Istanbul/turkey

<sup>3</sup> Department of Medical Laboratory Techniques, Vocational School of Health Services, İstinye University, Istanbul/turkey

<sup>4</sup> Department of Urology, Istanbul Medeniyet University Goztepe Prof. Dr. Suleyman Yalcin City Hospital, Istanbul/turkey

<sup>5</sup> Department of Memedical Biochemistry, School of Medicine, Acibadem Mehmet Ali Aydınlar University, Istanbul/turkey

**Introduction:** Among all bladder tumors, high-grade T1 stage tumors (Hg-pT1) are known to have clinically significant heterogeneity, recurrence, progression, and mortality. Proteomic approaches are useful to elucidate the invasion of cells constituting bladder tumor tissue at the molecular level. Protein phosphorylation is a critical, rate-limiting step for regulating signaling pathways in numerous biological events. The phosphorylation status of a protein can provide important information about the functional and mechanistic changes caused by bladder cancer. Therefore, this study aimed to analyze the phosphoproteome of serum samples from Hg-pT1 (n=10) and control samples (n=10).

**Methods:** For the phosphoproteome analysis, n=20 serum samples in total were used. Using an LC-MS/MS-based phosphoproteomics approach, altered phosphoproteomic pathways potentially regulating the Hg-pT1 phenotype were identified in bladder cancer serum samples. Phosphopeptides were enriched by titanium dioxide chromatography after tryptic digestion of proteins and were fractionated by high-pH LC to improve the mass-spectrometric analysis.

**Results:** Plasma proteomic analysis of bladder cancer identified 534 proteins and 180 phospho-sites. Principal component analysis was performed to analyze the differences between bladder cancer and control groups. In a scatter plot, 175 proteins were shown to be significantly regulated proteins. According to the total correlation plot of 445 quantified proteins extracellular vesicular exosome, response to oxygen radical proteins has been identified as proteins with a high correlation between bladder cancer and control groups. Hierarchical clustering analysis of significantly regulated proteins showed that proteins involved in lipid metabolic process and transport differed between bladder cancer and control groups. Additionally, differences were observed between the two groups concerning the hierarchical clustering of 3 significantly regulated phospho-sites (APOB, PLG, F5).

**Conclusions:** Our previous work on bladder cancer tissue proteomics and preliminary data obtained from serum samples provided great knowledge about Hg-pT1 bladder cancer. Furthermore, supports future biomarker discovery work for the diagnosis of bladder cancer.

Anahtar Kelimeler : Plasma, Bladder Cancer, Proteomics

Bildiri No : 6457

Ana Konu : Clinical Applications

Bildiri Türü : Poster Presentation

Sunucu : Aysel Özpınar

Eposta : aysel.ozpinar@acibadem.edu.tr

## P004

### Evaluation of Peptide Profiles of Breast-Cancer Tissues by Proteomics Methods

Yasemin Ucal<sup>1</sup>, Busra Ergun<sup>2</sup>, Cavit Kerem Kayhan<sup>3</sup>, Fatma Tokat<sup>4</sup>, Halil Kara<sup>5</sup>, Taner Korkmaz<sup>6</sup>, Cihan Uras<sup>5</sup>, Umit Ince<sup>4</sup>, Aysel Özpınar<sup>7</sup>

<sup>1</sup> Eu-openscreen, Junior Scientific Project Manager, Berlin/germany

<sup>2</sup> Department of Biochemistry and Molecular Biology, Institute of Health Sciences, Acibadem Mehmet Ali Aydınlar University, Istanbul/turkey

<sup>3</sup> Acibadem Health Group, Acibadem Maslak Hospital, Pathology Laboratory, Istanbul/turkey

<sup>4</sup> Department of Medical Pathology, School of Medicine, Acibadem Mehmet Ali Aydınlar University, Istanbul/turkey

<sup>5</sup> Department of General Surgery, School of Medicine, Acibadem Mehmet Ali Aydınlar University, Istanbul/turkey

<sup>6</sup> Department of Internal Diseases, School of Medicine, Acibadem Mehmet Ali Aydınlar University, Istanbul/turkey

<sup>7</sup> Department of Medical Biochemistry, School of Medicine, Acibadem Mehmet Ali Aydınlar University, Istanbul/turkey

**Introduction:** Multigenic tests(PAM50,OncotypeDx,MammaPrint,EndoPredict and Mammosrat) determine the treatment strategies and prognosis of early-stage breast cancer with hormone-receptor-positive patients. Based on the results of test, patients are evaluated in different risk groups. However, these tests have limitations. There remains obstacle to determine the prognosis and treatment options in a intermediate-risk-group. Additionally, it is not every patient is evaluated by multigene tests due to high costs. Therefore, it is important to identify potential protein biomarkers in order to establish cost-effective immunohistochemistry panels. The aim of this study is to investigate potential protein biomarkers in breast-cancer tissues defined in five different risk groups according to PAM50 test.

**Methods:** 10 of tissues were selected as a low-risk-luminal-A-group, 20 as an intermediate-risk-group(n=10 luminal-A;n=10 luminal-B), and 12 as a high-risk-group(n=2 luminal-A;n=10 luminal-B). Tissues were sectioned at 3µm thickness on ITO slides. Peptides were analyzed by MALDI-MSI. Results were evaluated with the number of monoisotopic peptides, peptide localizations, hierarchical clustering and principal component analysis using SCiLSLab version 2016b(Bremen, Germany). For protein identifications, samples were prepared by FASP method and analyzed by LC-MS/MS. Analysis of data was evaluated by ThermoDiscoverer software, protein identifications were evaluated by Sequest&Mascot software.

**Results:** Average peptide detected in low-risk-luminal-A, intermediate-risk-luminal-A, intermediate-risk-luminal-B, high-risk-luminal-B and high-risk-luminal-A groups were 156, 204, 270, 106, and 210 respectively. According to LC-MS/MS results, 1102 peptides were found to be common among the groups. The proteins belonging to the peptides in MALDI-MSI data matched with LC-MS/MS data have been examined. As a result of ROC analysis, 4 proteins (STAB1,ARMC10,TACC1,CLIC6) that showed differences between groups and had an AUC value >0.70 were selected as candidate biomarkers.

**Conclusions:** The identification and validation of 4 potential protein biomarkers may be important in the prognosis of hormone-positive-breast-cancer will support the determination of the immunohistochemistry panel that we aim to develop in the long term.

Anahtar Kelimeler : FFPE, Breast Cancer, MALDI-MSI, LC-MSMS, Peptide

Bildiri No : 6456

Ana Konu : Clinical Applications

Bildiri Türü : Poster Presentation

Sunucu : Busra Ergun

Eposta : busra.ergun@live.acibadem.edu.tr

## P005

### Evaluation of IGF-1 and other possible protein biomarkers in serum

Ebru Uçaktürk<sup>1,2</sup>, Emirhan Nemutlu<sup>1,2</sup>

<sup>1</sup> Hacettepe University, Turkish Doping Control Center, Ankara, Sıhhiye, Turkey

<sup>2</sup> Hacettepe University, Faculty of Pharmacy, Department of Analytical Chemistry, Ankara, Sıhhiye, Turkey

**Purpose:** The use of insulin like growth factor 1 (IGF-1) and recombinant human growth hormone (rhGH) in sport is prohibited by World Anti-Doping Agency. Some serum proteins can be used as possible biomarkers for the administration of IGF-1 and rhGH [1]. In the study proposed here, detection of IGF-1 and the other possible protein markers in serum have been investigated using different depletion methods.

**Methods:** Immunoaffinity resins (Immunodep2 and Immunodep12) and acetonitrile precipitation have been tried to deplete abundant serum proteins. The obtained peptides after in-solution tryptic digestion of depleted samples have been analyzed in two dimensional nano-LC-Q Exactive with data dependent acquisition mode. IGF-1 (free and total), IGF-2 (free and total), IGF binding proteins (IGFBP), leucine-rich a-2- glycoprotein (LRG), IGF acid labile subunit (IGFALS) in serum were evaluated.

**Findings:** IGF-1 (free), and other reported blood biomarkers [IGF-2 (free), IGFBP2, IGFBP3, IGFBP4, ALS and LRG1] have been successfully detected when Immunodep12 has been used for depletion. However, IGF-1 (free), and IGFBP4 could not have been found using Immunodep2. In order to assess the total amount of IGF-1 and IGF-2, acidified ACN (75% ACN containing 2% acetic acid) was tried for depletion. In this condition, only IGF-2 and IGFBP3 have been determined. In addition, further depletion has been performed with Immunodep2 and Immunodep12 after acidified ACN depletion. The results showed that some of the biomarkers, IGF-1 (total), IGF-2 (total), IGFBP2, IGFBP3 and LRG1 but except IGFALS, have been successfully identified.

**Conclusion:** Investigated untargeted methods in here allow us the monitoring and relative quantitation of the mentioned biomarkers. In addition, these untargeted methods will be used for retrospective analysis of individual's serum protein profile.

#### References

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Anahtar Kelimeler : insulin like growth factor 1, Doping, Biomarker

Bildiri No : 6445

Ana Konu : New Approaches in Proteomics (Other)

Bildiri Türü : Poster Presentation

Sunucu : Ebru Uçaktürk

Eposta : ebru9679@gmail.com



**P006**

**Sodium Pentaborate Pentahydrate Enhances Paclitaxel-induced Cytotoxicity in Hormone-dependent Prostate Cancer**

Sevinc Yanar<sup>1</sup>, Tuğcan Korak<sup>2</sup>, Merve Gulsen Bal Albayrak<sup>2</sup>, Murat Kasap<sup>2</sup>

<sup>1</sup> Sakarya Üniversitesi Histoloji ve Embriyoloji Anabilim Dalı

<sup>2</sup> Kocaeli Üniversitesi Tıbbi Biyoloji Anabilim Dalı

**Aim:** Prostate cancer remains a significant health challenge, necessitating innovative therapeutic strategies to improve treatment outcomes. Sodium pentaborate pentahydrate (SPP), a boron-based compound with potential anticancer activity, has previously demonstrated inhibitory effects on the proliferation of hormone-dependent LNCaP prostate cancer cells. Paclitaxel (Pac), a widely used chemotherapeutic agent, is effective against various cancers but is often associated with significant side effects, limiting its therapeutic potential. To mitigate these side effects, combination therapies are frequently explored to achieve better efficacy at lower doses. In this study, we aimed to explore the combined effects of SPP and Pac on LNCaP cells.

**Methods:** Cytotoxicity of SPP and Pac on LNCaP and healthy prostate epithelial cells PNT1a was assessed using the WST-1 assay. Drug interactions were analyzed using CompuSyn software to calculate the combination index (CI) and dose reduction index (DRI). One-way analysis of variance (ANOVA) followed by Tukey's test was carried out to perform statistical analysis.

**Results:** The results revealed that the combination of SPP and Pac significantly increased toxicity in LNCaP cells ( $P < 0.01$ ) without causing harm to healthy prostate cells, PNT1a. The strongest synergistic effect was observed when cells were treated with 250  $\mu\text{g}/\text{mL}$  SPP and 10 nM Pac for 24 hours (CI = 0.347; DRI = 5.628).

**Conclusion:** The findings indicate, for the first time, that SPP and Pac combination provides a synergistic anticancer effect, allowing for reduced Pac dosage and potentially minimizing its side effects. Further studies are required to explore the mechanisms behind this synergy and its potential clinical application.

Anahtar Kelimeler : Sodium pentaborate pentahydrate, paclitaxel, combination, synergistic, prostate cancer

Bildiri No : 6535

Ana Konu : Cell Biology and Functional Proteomics

Bildiri Türü : Poster Presentation

Sunucu : Sevinc Yanar

Eposta : sevincsecer@gmail.com



## P007

### Creation of a Cell Line Expressing SplitTurboID for Nuclear-Proteome Studies

Ebru Durur, Mehmet Sarihan, Murat Kasap, Gürler Akpınar

Kocaeli Üniversitesi, Tıp Fakültesi, Tıbbi Biyoloji Anabilim Dalı, Kocaeli

**AIM:** The nucleus is the center where the regulation of gene synthesis and cellular physiological processes is controlled. Therefore, it is crucial to study the nuclear proteome to understand the mechanisms and effects of that specifically target gene synthesis. We have developed a novel approach to nuclear proteome study involving regulated expression of Split-turboID, using MDA-MB-231 breast cancer cell line..

**METHOD:** In this study, MDA-MB-231 cells, a highly aggressive breast cancer cell line, was genetically engineered to express split TurboID enzyme in a three-step process. First, cells were transfected with the pCDNA6/TR plasmid, and selected with blasticidin to stably express the TetR protein. . Next, the TetR+ cells were transfected with pcDNA4/TO-FKBP-V5-Split TurboID N plasmid, encoding the N-terminal fragment of Split-TurboID, followed by zeocin selection to generate a monoclonal cell line expressing the N-terminal fragment. Finally, the cells were transfected with the pcDNA3.1-HA-FRB-Split TurboID C-NLS plasmid, expressing the C-terminal fragment, and selected with geneticin to produce a monoclonal cell line expressing both the N- and C-terminal domains. After the transfection steps, tetracycline and rapamycin were added to the culture media to generate active Split-TurboID and biotinylate of nuclear proteins. The success of biotinylation was confirmed via immunofluorescence microscopy.

**FINDINGS:** A successfully engineered metastatic breast cancer cell line was developed. This cell line harbored active Split TurboID in its nucleus causing biotinylation of nuclear proteins and proteins transiently associating with the nucleus under tetracycline control.

**RESULT:** In this study, a tetracycline-inducible monoclonal cell line that expressed split-TurboID in the nucleus was developed, and validated through IF and Western blot analyses. The data was analyzed using bioinformatic tools

Anahtar Kelimeler : Nuclear Proteome, Breast Cancer, TurboID, SplitTurboID, Proteomics

Bildiri No : 6332

Ana Konu : New Approaches in Proteomics (Other)

Bildiri Türü : Poster Presentation

Sunucu : Ebru Durur

Eposta : ebrudurur2@gmail.com

## P008

### Reprogramming Changes the Protein Content of UM-UC-3 Bladder Cancer Cells

Bengi Su Rumeysa Barlak, Mehmet Sarihan, Zeynep Sude Kongur, Gurler Akpinar, Murat Kasap, Banu Iskender İzgi

Kocaeli Üniversitesi Tıbbi Biyoloji Anabilim Dalı

**Purpose:** Metastasis and resistance to drugs are two significant challenges in cancer treatment. Harnessing the cancer cell genome through reprogramming offers great potential for both understanding cancer initiation and developing novel therapies. Increasing evidence indicates that introducing reprogramming factors, namely Yamanaka factors, enables certain cancer cell types to be reprogrammed, express pluripotency-associated markers and alter malignant morphology and cell behaviour. In this study, we aimed to analyse the molecular changes in the bladder cancer cell line UM-UC-3 following reprogramming with previously defined factors.

**Method:** UM-UC-3 cells were reprogrammed using a Sendai-virus-based approach after determining the appropriate virus titer. Both ancestral and reprogrammed cells were cultured for at least 10 passages before proteins were extracted for further analysis. Protein samples were enzymatically digested and the concentrated peptide samples were analysed using label-free quantification with nLC-MS/MS.

**Findings:** After reprogramming, UM-UC-3 cells were cultured under feeder-free conditions on vitronectin or Geltrex-coated plates. However, the majority of the cell population exhibited morphological changes that resulted in a shift from adherent culture to suspension culture. Proteomics data suggested alterations in the protein content of reprogrammed UM-UC-3 cells compared to ancestral UM-UC-3 cells in various pathways regulating stem cell maintenance, epithelial-mesenchymal-transition and stem cell differentiation.

**Conclusion:** The reprogramming technology presents an opportunity to redirect terminally differentiated cell fates back to an undifferentiated state, which, in theory, could be utilised to revert malignant cancer cells to a more manageable form. However, the limitations in reproducibility of cancer cell reprogramming due to cancer-specific epigenome impede its potential application in diagnosis and treatment. Here, we demonstrated that reprogrammed UM-UC-3 cells and ancestral bladder cancer cells exhibit changes in critical proteins. Moreover, the atypical growth preference of reprogrammed bladder cancer cells may stem from partial reprogramming necessitating further investigation.

Anahtar Kelimeler : UM-UC-3, Reprogramming, Proteomics

Bildiri No : 6434

Ana Konu : Cell Biology and Functional Proteomics

Bildiri Türü : Poster Presentation

Sunucu : Bengi Su Rumeysa Barlak

Eposta : bengisubarlak7@gmail.com

**P009**

**Proteomic Investigation of V493F-FTO-overexpression suggest FTO's role Ubiquitin-Dependent ERAD Pathway**

Aylin Kanlı, Murat Kasap, Gürler Akpınar, Mehmet Doğan Gülkaç

Kocaeli Üniversitesi Tıbbi Biyoloji Anabilim Dalı

**Aim:** Demethylase fat mass and obesity-related protein (FTO), which belongs to the AlkB homologous (ABH) family, are associated with various neurological diseases and cancers, particularly obesity. This protein, which contains many structurally and functionally different regions, contains a COOH-terminal domain whose function, unlike other ABH members, is not fully understood. We aimed to investigate the effects of the exonic V493F mutation in this region of FTO on the soluble proteome.

**Method:** For this aim, SH-SY5Y cells stably overexpressing wild-type (WT-FTO) or mutant FTO (V493F-FTO) proteins under the control of the Tet promoter were created and used. More than 500 protein spots were compared in 2DE gels, and changes in the expression levels of 10 protein spots were detected when two-fold regulation criteria were taken into account. Four of these proteins were regulated after WT-FTO and six were regulated after V493F-FTO overexpression. Proteins were subsequently identified using MALDI-TOF/TOF analysis. Results: Although the regulation ratios for the differentiated proteins were less pronounced, overexpression of WT-FTO still affected mainly cell cycle-associated DNA replication and repair processes (such as PCNA). Exogenous production of V493F-FTO altered the expression levels of proteins that respond to cellular stress (such as HSPA4), as well as proteins involved in the regulation of misfolded proteins in the ubiquitin-dependent ERAD pathway (such as ARHGDI1 and VCP).

**Conclusion:** Overall, our findings suggest that FTO may have an additional role in triggering the response to intracellular stress and may also have a role in the ubiquitin-dependent ERAD pathway. Further in vivo and in vitro investigations should be conducted to determine the potential link between the FTO protein and the ubiquitin-dependent ERAD pathway.

Anahtar Kelimeler : FTO protein, V493F mutation, SH-SY5Y cells, Proteomics

Bildiri No : 6496

Ana Konu : Cell Biology and Functional Proteomics

Bildiri Türü : Poster Presentation

Sunucu : Aylin Kanlı

Eposta : aylin.kanli@kocaeli.edu.tr



## P010

### BioID2-based Screening of ERAD Reveals Inner Mitochondrial Membrane Protein Prohibitin-2

Baran Dingilolu<sup>1</sup>, Davod Khalafkhany<sup>3</sup>, Nesrin Ozoren<sup>3</sup>, Gizem Dinler Doganay<sup>2</sup>

<sup>1</sup> Istanbul Technical University, Molecular Biology-genetics & Biotechnology, Istanbul, Turkey

<sup>2</sup> Istanbul Technical University, Molecular Biology-genetics, Istanbul, Turkey

<sup>3</sup> Bogazici University, Molecular Biology and Genetics, Istanbul, Turkey

**Background/aim:** The protein quality control (PQC) system is directly activated when newly synthesized proteins emerge from the ER lumen. Chaperones assist in protein folding and prevent misfolded proteins from evading PQC. Properly folded proteins are transported from the ER lumen through the secretory pathway, whereas misfolded or unfolded proteins are redirected to the cytosol via retro-translocation and ER-associated degradation (ERAD). Derlin-1, a critical component of the retrotranslocon embedded in the ER membrane, is essential for the degradation of both soluble and integral membrane ERAD substrates. Another key player, p97/VCP, is responsible for the identification and removal of misfolded or improperly assembled proteins from the endoplasmic reticulum (ER). This study aims to identify novel ERAD substrates using a triple-point BioID2 labeling system (Derlin1/ Derlin1-170/ p97) that encompasses the ER lumen, membrane, and cytosolic regions.

**Materials and methods:** Derlin1, Derlin1-170, and VCP/p97 fused with biotin ligases were cloned into mammalian expression vectors. In-situ labeling assays were conducted in MCF-7 cells, with overexpression and biotinylation verified by immunoblotting. Protein localization was confirmed via immunocytochemistry (ICC). Biotinylated proteins were captured with streptavidin pull-down, digested with trypsin, and identified by LC-MS/MS (Waters Synapt G2-Si). Data analysis was performed using Progenesis QIP. Cycloheximide chase assays were used to investigate ERAD candidates in both cancerous and healthy breast cell lines.

**Results:** Out of a total of 171 proteins detected by LC-MS/MS, 12 were found to be common across all 3 groups. Upon examining 3 of these proteins, it was observed that the PHB2 protein behaves like an ERAD substrate according to complementary experiments.

**Conclusion:** It has been demonstrated through complementary experiments that the PHB2 protein is involved in the ERAD pathway, although the reason for its presence in this pathway is still under investigation.

**Acknowledgement:** This study was supported by ITU BAP (TDK-2021-43343).

Anahtar Kelimeler : BioID, ERAD, PQC

Bildiri No : 6523

Ana Konu : Cell Biology and Functional Proteomics

Bildiri Türü : Poster Presentation

Sunucu : Baran Dingilolu

Eposta : barandingilolu@gmail.com



## P011

### Investigation of Novel Bag-1S:VCP/p97 Interaction Through Bag-1S Mutants

Ezgi Basturk<sup>1</sup>, Hatice Dila Boncuk<sup>2</sup>, Baran Dingiloglu<sup>1</sup>, Gizem Dinler Doganay<sup>2</sup>

<sup>1</sup> Istanbul Technical University, Molecular Biology - Genetics & Biotechnology

<sup>2</sup> Istanbul Technical University, Molecular Biology & Genetics

**Background/Aim:** Bag-1S is one of the anti-apoptotic Bag-1 isoforms of BAG family proteins that have multiple functions and have been preserved throughout the evolutionary process. Bag-1S protein has been shown to be involved in processes such as apoptotic pathways, neuronal differentiation, transcription regulation, cell proliferation and proteasomal degradation to ensure intracellular homeostasis. In the recent studies carried out by our group, novel interaction surfaces of Bag-1S and VCP/p97 which participates endoplasmic reticulum-associated degradation (ERAD) pathway were identified by HDX-MS analysis. Investigation of Bag-1S and VCP/p97 interaction thought to be contribute to the development of biosimilar therapeutics for diseases that assist by unfolded or misfolded protein accumulation in the ER. The aim of this study is purification of mutant Bag-1S T45A and Bag-1S T166A proteins in stable, folded forms for the further possible contribution on detailed identification of Bag-1S and VCP/p97 interaction interface.

**Methods:** Recombinant production process of wild type, T45A and T166A mutant Bag-1S proteins in E.coli BL21(DE3) was optimized. Affinity purification with Ni-NTA resins through N-terminal 8xHis tags of Bag-1S proteins was applied. Purified proteins' characterization was carried out by orthogonal methods. For the effect of mutants on the interaction interface with VCP/p97; pre-incubated proteins were crosslinked with DSS and in vitro pull-down assay was applied.

**Results:** Bag-1S T45A and T166A mutants recombinant production processes were optimized. Orthogonal analyses of purified proteins were resulted that mutant Bag-1S proteins were purified as in properly folded and monomeric forms for the further interaction surface investigations with VCP/p97. In vitro pull-down assay results were supportive to the claimed interaction surface.

**Conclusion:** In conclusion, it is thought that examination of Bag-1S:VCP/p97 interaction can assist to the treatment of diseases caused by misfolded protein degradation in the long term.

**Acknowledgements:** This study was supported by TÜBİTAK 119Z261 and 2209-A.

Anahtar Kelimeler : Bag-1S, VCPp97, ERAD, HDX-MS

Bildiri No : 6528

Ana Konu : Structural Proteomics

Bildiri Türü : Poster Presentation

Sunucu : Hatice Dila Boncuk

Eposta : ezgibasturk@gmail.com

## P012

### Designing Liposomal Delivery System for Novel Inhibitor Targeting Bag-1/C-Raf Interaction-Interface

Yağız Akbaş<sup>1</sup>, Serra Arat<sup>1</sup>, Baran Dingiloğlu<sup>1</sup>, Özge Tatlı<sup>2</sup>, Abdulhalim Kılıç<sup>1</sup>, Gizem Dinler Doğanay<sup>1</sup>

<sup>1</sup> Istanbul Technical University, Faculty of Science and Literature, Department of Molecular Biology-genetics & Biotechnology

<sup>2</sup> Istanbul Medeniyet University, Faculty of Engineering and Natural Sciences, Department of Molecular Biology and Genetics

**Aim:** Breast cancer is the leading cause of mortality for women globally and current cancer treatments are inadequate due to the development of drug resistance. Identifying and targeting critical protein-protein interactions (PPIs) that contribute to drug resistance is crucial for developing novel therapeutic strategies. Bag-1/C-Raf interaction plays a pivotal role in the survival and proliferation of breast cancer cells. The Bag-1/C-Raf interaction surface was previously identified, and a specific inhibitor disrupting the formation of this protein complex was developed. Due to the inability of the inhibitor to penetrate cells effectively, we used liposomal encapsulation to enhance cellular uptake. The present study aims to target this interaction by using a novel inhibitor that we had developed and to use liposomal encapsulation to enhance cellular uptake.

**Methods:** MCF-7 breast cancer cells were treated with liposomes containing Pep-3 peptide. Cell viability was assessed by MTT assay to determine the effective concentration (IC<sub>50</sub>) of the inhibitor and the treatment effect of the inhibitor was tested by analyzing the expression levels of proteins in the Raf kinase pathway by western blotting.

**Results:** MTT analyses showed a significant decrease in cell viability and immunoblotting analyses revealed a substantial reduction in the activation of downstream targets in the Raf kinase pathway, indicating effective disruption of the Bag-1/C-Raf interaction.

**Conclusion:** Our data support the efficiency of the liposomal delivery system in enhancing the cellular uptake of our novel inhibitor, demonstrating that targeting PPIs through liposomal delivery systems could be a promising strategy for overcoming drug resistance in breast cancer treatment. Acknowledgement: This study is funded by TUBITAK 1001 Research Funds (117Z848).

Anahtar Kelimeler : Breast Cancer, Liposome, Targeted Therapy, Protein-Protein Interactions PPIs, Peptide Inhibitor

Bildiri No : 6529  
Ana Konu : Cell Biology and Functional Proteomics  
Bildiri Türü : Poster Presentation  
Sunucu : Yağız Akbaş  
Eposta : akbasyagz@gmail.com

## P013

### Identification of the Interaction Between Foxo3a and Lipid Metabolism Proteins

İlke Sarak, Yiğit Efe Aydın, Pelin Özfiliz Kılbaş

İstanbul Kültür Üniversitesi, Moleküler Biyoloji ve Genetik Anabilim Dalı, Moleküler Biyoloji ve Genetik Bölümü, İstanbul

#### Purpose

To investigate the interaction of proteins involved in fatty acid metabolism and tumor suppressor FoxO3a protein, our study aims to identify proteins interacting with FoxO3a among various candidates involved in lipid metabolism using in silico docking and in vitro immunoprecipitation studies.

#### Method

**1-Obtainment of 3D structures of proteins and evaluation of model quality:** 3D structures of FoxO3a and potential interaction candidates were obtained from Colab alphafold database. Model quality was evaluated with ERRAT, PROCHECK and MolProbity.

**2-Evaluation of Intrinsic Disorder:** Intrinsic disorder of FOXO3A and potential interaction candidates was examined using Composition Profiler, RIDAO, D2P2 and IUPRED3 platforms.

**3- Molecular Docking:** Detection of possible binding sites between FoxO3a and potential interaction partners involved in fatty acid metabolism was performed using HawDock and HDOCK servers and the best models were determined.

**4-MD Simulation:** MD simulation was conducted and analyzed on the best models created using Gromacs software.

**5- Interaction with FoxO3a and selected lipid metabolism candidates were validated through co-immunoprecipitation techniques.**

**Findings** The models of FoxO3a and its potential interaction partners were evaluated in terms of quality. Based on the results obtained from this evaluation, each selected protein was subjected to intrinsic disorder evaluations and it was shown that the proteins contained intrinsic disorder elements. Molecular docking between FoxO3A and interaction candidates and MD simulation was performed for the best models obtained.

#### Conclusion

This study showed that FoxO3a and various proteins known to participate in fatty acid metabolism have intrinsic disorder regions. In addition, molecular docking, MD simulation, and immunoprecipitation validation can provide an idea about the interaction of Foxo3a and various proteins known to participate in fatty acid metabolism and these regions can be used as targets in future studies.

Anahtar Kelimeler : intrinsic disorder, molecular docking, molecular dynamics simulation, FOXO3A, lipid metabolism

Bildiri No : 6534

Ana Konu : Bioinformatics

Bildiri Türü : Poster Presentation

Sunucu : İlke Sarak

Eposta : sarakilke@gmail.com



## P014

### Effect of Mechanical Energy Application on Protein-Nanoparticle Interactions

Dilsu Taşkın, Emre Birol, Bekir Salih

Hacettepe University, Department of Chemistry

Nanoparticles (NPs) can be used as drug carriers. Efficiency of drug carriers depend on their interactions with biomolecules present within body fluids, proteins in particular. Protein layer formed around NPs is called protein corona. Probing properties of protein-NP interactions is possible through use of ultrasound (US) waves on protein NP complexes as a source of mechanical energy, outcome can be monitored via bottom-up proteomics approaches using high resolution mass spectrometry.

**Aim:**The aim of this study is the quantitative measurement of protein adsorption on Silica NPs upon incubation with human serum in presence and absence of US application. The final goal is to infer knowledge on binding properties of proteins to Silica NPs based on quantitative changes, which could be used to assess usefulness of NPs for further pharmaceutical applications.

**Method:**Nanoparticles were incubated with human serum. On one set of experiments, using a bath type sonicator operating at 37 kHz ultrasound frequency, formed protein-NP complexes were subjected to mechanical stimulation. Collected complexes were digested on particle. Peptides obtained from both US treated and untreated samples were identified using high resolution Orbitrap Mass Spectrometer. Identification and quantitative analysis of proteins based on raw mass spectrometry data was carried out using MaxQuant computer software.

**Results:** Relative quantitative information was successfully obtained from mass spectrometry raw data and subsequent use of MaxQuant software suite. Changes in percent abundance of proteins was detected in between raw serum, NPs incubated with serum and NPs incubated with serum followed by US application.

**Conclusion:** US waves can be used to alter binding patterns of proteins to NPs which can be utilized to decorate NP surface in different ways. They also provide a way to assess characteristics of binding such as strengths of binding and point to possible cooperative adsorption properties.

Anahtar Kelimeler : Nanoparticles, Protein Corona, Ultrasound Waves, Orbitrap Mass Spectrometry

Bildiri No : 6521

Ana Konu : New Approaches in Proteomics (Other)

Bildiri Türü : Poster Presentation

Sunucu : Dilsu Taşkın

Eposta : dilsutaskinn@gmail.com



## P015

### Cell-Specific Prediction of Protein-Protein Interaction Networks in Huntington's Disease

Ekin Sonmez<sup>1</sup>, Tuna Birgun<sup>3</sup>, Huseyin Cimen<sup>1,2</sup>

<sup>1</sup> Gebze Technical University, Institute of Biotechnology

<sup>2</sup> Gebze Technical University, Central Research Laboratory Application and Research Center, Gtumar

<sup>3</sup> Marmara University, Bioengineering Department

**Aim:** Huntington's disease (HTT) is a progressive neurodegenerative disorder caused by a CAG trinucleotide repeat expansion in the HTT gene, resulting in an irregularly extended polyglutamine expansion in the huntingtin protein. This mutation mainly affects neurons in the striatum, leading to motor dysfunction and cognitive impairment. This study aims to identify and characterize the cell-specific protein-protein interaction networks in the striatum of a mouse model for Huntington's disease to reveal potential prognostic and diagnostic targets.

**Method:** Single-cell and bulk RNA-seq datasets were collected from the publicly available GEO database. After performing quality control, clustering, and cell annotation, the datasets were integrated employing the Seurat package in R. Subsequent downstream analyses, including differential expression analysis, Gene Ontology (GO), and KEGG pathway analysis, were conducted. Cell-specific protein-protein interaction networks were then examined using the KeyPathwayMiner tool in Cytoscape.

**Results:** Differentially expressed genes were identified in each cell cluster for disease state. Distinct and predicted protein-protein interaction networks were mapped for each specific cell cluster within the striatum of a mouse model for Huntington's disease.

**Conclusion:** This study highlights that each cell type in the Huntington's disease model exhibits predicted networks for a unique protein interactome. These predicted or identified proteins are expected to be novel cell-specific therapeutic targets for Huntington's disease. Future work will focus on validating these findings through multi-omic analysis in vitro

Anahtar Kelimeler : huntington, single cell, transcriptomics, rnaseq, ppi

Bildiri No : 6412

Ana Konu : Bioinformatics

Bildiri Türü : Poster Presentation

Sunucu : Ekin Sonmez

Eposta : ekinsonmez@gtu.edu.tr

## P016

### Elucidating the Mechanisms of Nucleus-Mitochondria Communication

Beyzanur Çobanoğlu, Gürler Akpınar, Murat Kasap

Kocaeli Üniversitesi Tıbbi Biyoloji Anabilim Dalı

**Aim:**We aimed to create a novel cellular model through which an increased cross-talk between the two organelles occurred. To create the model, a bacterial toxin protein, named HOK, was expressed and directed to inner mitochondrial membrane in HEK293 cells which was previously engineered by us to express Split-TurboID in the nucleus.

**Methods:**The gene encoding GFP-tagged Hok protein was synthesized and cloned into pCDNA4/TO, which was transfected into HEK293T-TetR+-Split-TurboID-N-C cells to create a monoclonal stable cell line. To confirm the proper functioning of the established cell line, the intracellular localization of Split-TurboID was demonstrated using V5 and HA primary antibodies, and the biotinylation of nuclear proteins was shown using Texas Red-conjugated neutravidin antibody via immunofluorescence microscopy. Mitochondrial depolarization by monitoring mitochondrial morphology using GFP-HOK expression. Nuclear proteins biotinylated by Split-TurboID was enriched and identified by nHPLC-LC-MS/MS analysis.

**Results:**Induction of HOK-GFP expression resulted in mitochondrial depolarization. Immunofluorescence analysis allowed monitoring of the whole process and indicated that an increased cross-talk has occurred between mitochondria and nucleus. A comparative specific nuclear proteome analysis by utilizing the activity of Split-TurboID revealed identities of the proteins that were not previously known to be involved in the cross-talk process between mitochondria and nucleus.

**Conclusions:**The study developed a novel cellular model to investigate mitochondria-to-nucleus communication by expressing a bacterial toxin protein (HOK) in HEK293 cells, causing mitochondrial disruption and increased contact between mitochondria and the nucleus. An increased number of mitochondrial proteins in the nuclear proteome which were previously unknown was observed.

Anahtar Kelimeler : Proteomics, Mitochondria, Nucleus

Bildiri No : 6433

Ana Konu : Cell Biology and Functional Proteomics

Bildiri Türü : Poster Presentation

Sunucu : Beyzanur Çobanoğlu

Eposta : beyzanurcbngl@gmail.com

**P017**

**HDX-MS Combined with MD Simulations Reveals the Bcl-2/Beclin 1 Interface**

Miray Türk<sup>1</sup>, Umut Çağan Uçar<sup>1</sup>, Baran Dingiloğlu<sup>1</sup>, Gizem Dinler Doğanay<sup>1,2</sup>

<sup>1</sup> Molecular Biology, Genetics-biotechnology, Graduate School, Istanbul Technical University, 34469 Istanbul, Turkey

<sup>2</sup> Department of Molecular Biology and Genetics, Istanbul Technical University, 34469 Istanbul, Turkey

Autophagy is a crucial cellular process responsible for degrading unwanted proteins and dysfunctional cellular components, helping to maintain homeostasis. Disruption in the balance between autophagy, apoptosis, and the ubiquitin-proteasome system can lead to a variety of diseases, including neurodegenerative disorders and cancer. Beclin 1 is essential in forming the pre-autophagosomal complex, a key step for initiating autophagy. Its interaction with Bcl-2 inhibits Beclin 1's activity by blocking its association with other autophagic complex members. Consequently, the Bcl-2/Beclin 1 complex plays a central role in regulating both apoptosis and autophagy, impacting overall cellular homeostasis.

Despite significant research, the full-length structures of Bcl-2 and Beclin 1 are not fully understood. Although certain interaction points have been identified, targeting the Bcl-2/Beclin 1 complex for therapeutic purposes has been challenging, largely due to the lack of detailed structural data. This emphasizes the need for further investigation into their interaction surface(s).

Our study aimed to map the binding interfaces of the Bcl-2/Beclin 1 complex. Full-length Bcl-2 and Beclin 1 proteins were produced, affinity-purified, and characterized, with their activities confirmed through in vitro interaction assays. Using Hydrogen-Deuterium Exchange Mass Spectrometry (HDX-MS), we analyzed deuterium incorporation into Beclin 1 in the presence or absence of Bcl-2. Molecular dynamics (MD) simulations were also performed to visualize the HDX-MS data due to the lack of available full-length structures.

Our results revealed that, beyond the traditional BH3 domain-ligand binding interaction, additional interaction surfaces on Beclin 1 were identified. These novel surfaces may offer new therapeutic targets for modulating the Bcl-2/Beclin 1 interaction.

Anahtar Kelimeler : apoptosis, autophagy, Bcl-2, Beclin 1, HDX-MS, Hydrogen-Deuterium Exchange Mass-Spectrometry

Bildiri No : 6526

Ana Konu : Structural Proteomics

Bildiri Türü : Poster Presentation

Sunucu : Miray Türk

Eposta : turkm16@itu.edu.tr



## P018

### Effect of epetraborole's target overexpression on Escherichia coli proteome

Anara Babayeva<sup>1,2</sup>, Esra Dibek<sup>3</sup>, Murat Kasap<sup>4</sup>, Gürler Akpınar<sup>4</sup>, Bekir Çöl<sup>1</sup>

<sup>1</sup> Mugla Sıtkı Kocman University, Faculty of Science, Department of Biology

<sup>2</sup> Mugla Sıtkı Kocman University, Graduate School of Natural and Applied Sciences, Department of Biology

<sup>3</sup> Mugla Sıtkı Kocman University/köyceğiz Vocational School of Health Services, Pharmacy Services Program

<sup>4</sup> Kocaeli University, Faculty of Medicine, Proteomics Laboratory

**Abstract** LeuS catalyzes the transfer of leucine to the corresponding tRNA<sup>Leu</sup>, a crucial step in protein synthesis. Epetraborole (EP) is a boron-containing antibiotic that inhibits bacterial leucyl-tRNA synthetase (LeuS), affecting protein synthesis and preventing bacterial growth. This study investigates the effect of increased LeuS expression levels on the total proteome of Escherichia coli.

**Aim:** The aim of this study was to investigate the effect of artificial overexpression of the LeuS enzyme on the E. coli proteome in the presence of epetraborole using LC-MS/MS-based proteomics.

**Method:** The control strain (AG1(pCA24N)) and the leuS-overexpressing strain AG1(pCA24N::leuS) were exposed to EP (0.25 µg/ml) for one hour. Cytoplasmic proteins were then prepared and subjected to nHPLC followed by LC-MS/MS proteomic analysis. Label-free quantification analysis showed that 72 proteins showed differential regulation between the two groups. The proteins were then classified according to their role in metabolic pathways using bioinformatics tools.

**Findings:** The results of LC-MS/MS analysis revealed that 4 and 68 genes were up- and down-regulated in response to increased LeuS levels in the presence of EP in E. coli. The Lac-T5 promoter induced by IPTG caused a 329-fold increase in the level of LeuS protein. The Cat gene encoded by the recombinant plasmid was upregulated about 15-fold, as expected. The other upregulated genes dnaJ and rpsO encode chaperone protein-DnaJ and 30S ribosomal protein-S15, respectively. However, a general decrease was detected in the protein levels of genes related to protein synthesis such as elongation factors, aminoacyl-tRNA synthetases and ribosomal proteins.

**Conclusion:** The study presents the differentially regulated genes in Escherichia coli using LC-MS/MS proteome analysis in response to target (LeuS) overexpression in the presence of the antibiotic epetraborole, and provides information on its impact on bacterial physiology.

Anahtar Kelimeler : proteomic, antibiotic, LeuS, epetraborole

Bildiri No : 6420

Ana Konu : Proteomics in Model Organisms

Bildiri Türü : Poster Presentation

Sunucu : Anara Babayeva

Eposta : anara.babazade@gmail.com



## P019

### Proteomic Analysis of *Escherichia coli* Cytoplasmic Proteins with Trans-Cinnamic Acid

Merve Sezer Kürkçü<sup>1, 5</sup>, Hatice Öztürkel Kabakaş<sup>2</sup>, Ronayi Baykuşak<sup>3</sup>, Kadriye Aslıhan Onat Taşdelen<sup>2</sup>, Bekir Çöl<sup>4, 5</sup>

<sup>1</sup> Muğla Sıtkı Koçman University, Research and Application Center For Research Laboratories (alm Research Building), Muğla, Türkiye

<sup>2</sup> Muğla Sıtkı Koçman University, Graduate School of Natural and Applied Sciences, Biology Program, Muğla, Türkiye

<sup>3</sup> Muğla Sıtkı Koçman University, Faculty of Science, Department of Molecular Biology and Genetic , Division of Biotechnology, Muğla, Türkiye

<sup>4</sup> Muğla Sıtkı Koçman University, Faculty of Science, Department of Biology, Division of Biotechnology, Muğla, Türkiye

<sup>5</sup> Muğla Sıtkı Koçman University, Biotechnology Research Center (alm Research Building), Muğla, Türkiye

**Aim:** Transcinnamic acid has a variety of biochemical and biological functions in both plants and humans. Hydroxycinnamic acids have been shown to have biological activities such as antibacterial, anti-fungal, anti-viral, anti-oxidant, anti-cancer, anti-inflammatory, anti-diabetic, anti-melanogenic. The fact that these compounds are widely found in plants, that they are natural, and that they have many biological activities provides an advantage in terms of study. Therefore, its role in nature and medicine is quite important. There are not enough studies addressing the role or effect of this compound on bacteria at the molecular level to address its biological importance. Therefore, in this study, we aimed to identify some of the proteins whose regulation differs in response to transcinnamic acid by a proteomic approach. For this, we chose the bacterium *Escherichia coli* as a model organism.

**Method:** We found that the sublethal concentration against transinamic acid was 0.5mg/ml in rich media of *E.coli* strains. *E.coli* strain BW25113 was grown to log phase in Luria-Broth medium and exposed for one hour to none and 0.5mg/ml TCA. The cytoplasmic protein extracts were prepared. The protein extracts obtained were made purer by precipitation. Extracts determined by Bradford assay protein concentrations analyzed via 2D gel electrophoresis.

**Results:** This study investigated the proteomic response of *Escherichia coli* to transcinnamic acid, revealing significant regulatory changes in protein expression. Out of the 24 proteins identified with altered regulation, 16 were downregulated and 8 were upregulated in the presence of transcinnamic acid.

**Conclusion:** These findings highlight the impact of transcinnamic acid on bacterial protein profiles and suggest potential pathways through which it exerts its biological effects. Further analysis of the up/downregulated proteins using MALDI-TOF-TOF and identification through Mascot and Swissprot databases will provide deeper insights into the specific molecular mechanisms and potential applications of transcinnamic acid in microbial and therapeutic contexts.

Anahtar Kelimeler : trans cinnamic acid, *Escherichia coli*, proteomic, protein regulation

Bildiri No : 6438

Ana Konu : Proteomics in Model Organisms

Bildiri Türü : Poster Presentation

Sunucu : Kadriye Aslıhan Onat Taşdelen

Eposta : kadriyeaslihanonat@posta.mu.edu.tr

## P020

### HRI Activator and Proteasome Inhibitor Combination Effects on Bortezomib-Resistant Cells

Semih Şeker<sup>1</sup>, Emrah Okur<sup>2</sup>, Azmi Yerlikaya<sup>1</sup>

<sup>1</sup> Department of Medical Biology, Faculty of Medicine, Kutahya Health Sciences University, Kutahya, Turkey

<sup>2</sup> Department of Biology, Faculty of Art and Sciences, Kutahya Dumlupınar University, Kutahya, Turkey

**Aim:** Heme-Regulated Inhibitor (HRI) kinase, a crucial serine-threonine kinase that regulates eIF2 $\alpha$  phosphorylation. Initially identified in erythroid cells responding to heme deficiency, recent studies indicate HRI's activation in non-erythroid cells by various intracellular signals. Modulating eIF2 $\alpha$  phosphorylation by HRI presents a dual potential in pharmacology: Offering cytoprotective effects against stress-induced damage but also potentially triggering cell death pathways.

**Methods:** In this study, we explored the effects of HRI activators and inhibitors, particularly the HRI activator BTdCPU, on bortezomib-resistant PC3 prostate cancer cell lines.

**Results:** Our findings suggest that BTdCPU, either alone or combined with proteasome inhibitor bortezomib, presents a promising therapeutic strategy for cancers resistant to bortezomib. Furthermore, analysis using bioinformatic tools such as UALCAN has highlighted the critical role of HRI in metastatic processes across both mutant and non-mutant p53 cancers.

**Conclusion:** The current research underscores the potential of targeting HRI in cancer therapy, highlighting the need for further comparative studies to elucidate its full therapeutic impact.

**Acknowledgements:** The study was funded by the Health Institutes of Türkiye (TUSEB) with programme code 2022-ACİL-08 and project no 21313.

Anahtar Kelimeler : Bortezomib-Resistant Cells, HRI, Cancer Therapy

Bildiri No : 6435

Ana Konu : Cell Biology and Functional Proteomics

Bildiri Türü : Poster Presentation

Sunucu : Emrah Okur

Eposta : emrah.okur@dpu.edu.tr

## P021

### Comparison of Human Blood and Saliva IgG N-Glycosylation by HPLC-HILIC-FLD

Sena Aksoy<sup>1</sup>, Betül Karabudak<sup>1,3</sup>, Hakan Yılmaz<sup>1</sup>, Bekir Salih<sup>2</sup>, H. Mehmet Kayılı<sup>1</sup>

<sup>1</sup> Karabük Üniversitesi Biyomedikal Mühendisliği

<sup>2</sup> Hacettepe Üniversitesi Fiziko Kimya Anabilim Dalı

<sup>3</sup> Karabük Üniversitesi Sağlık Meslek Yüksekokulu

**Aim:**The study aimed to compare the N-glycosylation profiles of Immunoglobulin G (IgG) in human blood and saliva, exploring saliva as a potential non-invasive alternative for glycosylation studies.

**Method:**IgG was extracted from human blood and saliva, and its N-glycosylation profiles were analyzed using High-Performance Liquid Chromatography (HPLC) coupled with Hydrophilic Interaction Liquid Chromatography (HILIC) and Fluorescence Detection (FLD).

**Results:**The analysis revealed remarkably similar N-glycosylation profiles for IgG in both blood and saliva. Identical patterns were observed for major glycan structures such as fucosylated, galactosylated, and sialylated species, with only minor differences in low-abundance glycans.

**Conclusion:**Saliva exhibits a glycan profile comparable to blood, suggesting its potential as a non-invasive alternative for IgG glycosylation studies. This opens new avenues for biomarker discovery and immune monitoring through saliva-based diagnostics.

Anahtar Kelimeler : glycosylation, IgG, Saliva

Bildiri No : 6504

Ana Konu : Multi-omics Approaches

Bildiri Türü : Poster Presentation

Sunucu : Sena Aksoy

Eposta : senaaksoy@karabuk.edu.tr



## P022

### Storage Effects on Ethyl Esterified Glycans Stability: Insights from MALDI-TOF

Furkan Şahin<sup>1,2,4</sup>, Zelal Zuhul Kaya<sup>3</sup>, Mustafa Serteser<sup>1,4</sup>, Hasan Ümit Öztürk<sup>2</sup>, Ahmet Tarık Baykal<sup>1,4</sup>

<sup>1</sup> Biyokimya ve Moleküler Biyoloji Bölümü, Acıbadem Üniversitesi, İstanbul

<sup>2</sup> Medikal Biyoteknoloji Grubu, TÜBİTAK Marmara Araştırma Merkezi, Kocaeli

<sup>3</sup> Tıbbi Biyokimya Anabilim Dalı, Nişantaşı Üniversitesi, İstanbul

<sup>4</sup> Tıbbi Biyokimya Anabilim Dalı, Acıbadem Üniversitesi, İstanbul

**Aim:** Glycosylation is a post-translational modification where glycan groups are added to proteins, impacting their structural and functional properties. Managed by around 200 glycosyltransferase enzymes and influenced by environmental factors, this process is vital for Immunoglobulin G (IgG) glycoproteins, which are key antibodies in immune responses. Glycosylation motifs in the Fc region of IgGs are essential for targeting immune cells.

Ethyl esterification is a derivatization technique that improves the ionization of sialic acid groups, enhancing the accuracy of MALDI-TOF mass spectrometry. This method is less chemically reactive than others, preserving glycan integrity and specifically targeting 2,3- and 2,6-sialic acids for reliable analyses.

This study investigates the stability of ethyl esterified glycans at different storage temperatures (+4°C, -20°C, and -80°C) to determine how long they can be reliably stored after derivatization.

**Methods:** For this study, Immunoglobulin G (IgG) was isolated from serum samples. Following isolation, the glycans attached to IgGs were cleaved using PNGase F. The released glycans were then derivatized through ethyl esterification with EDC and HOBT. Subsequently, the derivatized glycans were purified using handmade cotton columns and stored at three different temperatures (+4°C, -20°C, and -80°C). The samples were analyzed at various time points using a Bruker Rapiflex MALDI-TOF mass spectrometer.

**Results:** Ethyl esterified glycans show changes after day 0 when stored at +4, -20 and -80 degrees. Bisectic glycans decreased, while fucoses increased and decreased. Galactosylated glycans also increased, while sialylated glycans increased and decreased.

**Conclusion:** Despite the limited sample size, it was observed that glycans, following ethyl esterification, did not maintain stability across different storage temperatures. Therefore, it is recommended that analyses be conducted immediately after the ethyl esterification reaction to achieve more reliable experimental results.

Anahtar Kelimeler : Glycomics, Proteomics, MALDI-TOF, Mass Spectrometry, Derivatization

Bildiri No : 6446

Ana Konu : Post Translational Modification Analysis

Bildiri Türü : Poster Presentation

Sunucu : Furkan Şahin

Eposta : furkan.sahin1416@gmail.com



**P023**  
**AI in Glyco-Science: GPT-4's Role in Data Analysis**

Hacı Mehmet Kayılı

Karabük University, Engineering Faculty, Biomedical Engineering Department, Karabük, Türkiye.

**Aim:**This study aimed to develop AI-driven software tools, leveraging GPT-4, to improve the analysis of glycoproteomics and glycan data derived from liquid chromatography-mass spectrometry (LC-MS) and HPLC-HILIC-FLD, respectively.

**Method:**Two Python-based tools were developed. The first tool, for glycoproteomics, utilizes GPT-4 to compare experimental MS/MS glycopeptide fragments with theoretical ones, improving the identification of glycopeptide precursors. The second tool, designed for glycan analysis, processes HPLC-HILIC-FLD chromatograms, identifies glycan peaks, and calculates their areas and relative abundances.

**Results:**The AI-assisted tools significantly enhanced both glycopeptide detection accuracy and the efficiency of glycan analysis. These improvements accelerated data processing, improved precision, and reduced the time required for glycoproteomics and glycan research.

**Conclusion:**By incorporating GPT-4 into the development of these software tools, researchers have created a robust AI-driven solution that streamlines glycomics and glycoproteomics data analysis, offering improved speed and accuracy for future advancements in the field.

Anahtar Kelimeler : glycomics, glycoproteomics, bioinformatics, Artificial Intelligence

Bildiri No : 6506

Ana Konu : Bioinformatics

Bildiri Türü : Poster Presentation

Sunucu : Hacı Mehmet Kayılı

Eposta : h.mehmetkayili@gmail.com

## P024

### Proteomic Analysis of Thiourea-Mediated Tolerance to Salinity Stress in Maize

Emre Pehlivan, Mustafa Yıldız, Hakan Terzi

Afyon Kocatepe University, Department of Molecular Biology and Genetics

**Aim:** Thiourea (TU) is involved in systemic regulation of plant adaptation to salinity stress but the underlying molecular mechanisms still remain largely unknown. The aim of present study was to investigate the changes of growth parameters as well as leaf proteome changes of maize (*Zea mays* L.) seedlings in response to salinity stress and exogenous TU application.

**Methods:** Maize seedlings were hydroponically treated with three treatments including control, NaCl (100 mM NaCl), and TU+NaCl (35  $\mu$ M TU + 100 mM NaCl). After 10 days, the effects of salinity stress and exogenous TU on seedling growth were analyzed. Label-free quantification by nanoscale liquid chromatography coupled with tandem mass spectrometry (nLC-MS/MS) was employed to relative protein quantification.

**Results:** Exogenous application of TU significantly alleviated NaCl-induced growth inhibition. Furthermore, proteomic analysis showed that out of 572 identified proteins, on comparison with control 386 proteins were up-regulated and 186 proteins were down-regulated due to NaCl treatment. Moreover, TU+NaCl treatment enhanced the abundance of 77 proteins and down-regulated 63 proteins when compared to NaCl treatment. Gene ontology (GO) and KEGG pathways analysis revealed that most of the proteins were found to be involved in metabolic pathways, biosynthesis of secondary metabolites, biosynthesis of amino acids, carbon metabolism, and photosynthesis.

**Conclusion:** This study may be helpful to further understand the role of TU in maize seedlings under salinity stress.

Anahtar Kelimeler : nLC-MSMS, Proteomics, Salinity, Thiourea, *Zea mays* L

Bildiri No : 6423

Ana Konu : Plant/ Animal/ Nutrition

Bildiri Türü : Poster Presentation

Sunucu : Emre Pehlivan

Eposta : mustafa\_yildizus@yahoo.com

## P025

### Proteomic Analysis Reveals Thiourea Mediated Amelioration of Cadmium in Maize

Nurgül Sönmez, Mustafa Yıldız, Hakan Terzi, Emre Pehlivan, Saliha Aydın

Afyon Kocatepe University, Department of Molecular Biology and Genetics

**Aim:** Cadmium (Cd) is a non-essential element that is highly toxic to living organisms. Thiourea (TU) is a sulfur-rich plant growth promoter that protects plants from oxidative damage caused by environmental stresses. However, it remains largely unknown how TU regulates proteins in response to Cd stress in maize (*Zea mays* L.). The aim of this study was to determine the effects of TU on alleviation of Cd stress in the leaves of maize seedlings.

**Methods:** The hydroponically grown maize seedlings were exposed to Cd (50 mM) and TU (35 mM) for 10 days in the following manner: control (no Cd and TU), Cd (only 50  $\mu$ M Cd), and TU+Cd (35  $\mu$ M TU and 50  $\mu$ M Cd). Growth responses were recorded after treatments. Proteome changes in leaf tissues were assessed using a label-free proteome approach (nLC-MS/MS).

**Results:** Exogenous application of TU significantly alleviated Cd-induced growth inhibition. Additionally, with a threshold of fold-change cutoff of 2.0-fold for increased accumulation and <0.5-fold for decrease, 1573 differentially expressed proteins (DEPs) were identified in Cd- and/or TU+Cd-treated leaves. Among these DEPs, 1237 proteins were up-regulated and 336 proteins were down-regulated after treatments. Gene ontology (GO) and KEGG pathways analysis revealed that most of the DEPs were found to be involved in metabolic pathways, biosynthesis of secondary metabolites, carbon metabolism, biosynthesis of amino acids, citrate cycle, biosynthesis of cofactors, cysteine and methionine metabolism, and glutathione metabolism.

**Conclusion:** The current research provided novel insights into TU-mediated tolerance of maize seedlings against Cd stress at the proteome level.

Anahtar Kelimeler : Cadmium, nLC-MSMS, Proteomics, Thiourea, *Zea mays* L

Bildiri No : 6424

Ana Konu : Plant/ Animal/ Nutrition

Bildiri Türü : Poster Presentation

Sunucu : Saliha Aydın

Eposta : hakanterzi81@gmail.com

**P026**

**Decoding the Unique Metabolome of Turkish Monofloral Honeys Using TimsTOF-MS**

Malik Oğuz Arslanoğlu<sup>1</sup>, Selinay Özel<sup>1</sup>, Melis Şardan Ekiz<sup>2</sup>, Ömür Gencay Çelemlı<sup>3</sup>, İdil Yet<sup>4</sup>, Ömür Çelikbıçak<sup>1,2</sup>

<sup>1</sup> Hacettepe University, Faculty of Science, Department of Chemistry, Ankara, 06800, Türkiye

<sup>2</sup> Hünitek - Hacettepe University Advanced Technologies Application and Research Center, Ankara, 06800, Türkiye

<sup>3</sup> Hacettepe University, Faculty of Science, Department of Biology, Ankara, 06800, Türkiye

<sup>4</sup> Hacettepe University, Institute of Health Sciences, Department of Bioinformatics, Ankara, 06230, Türkiye

**Aim:** This study aims to profile various honey types found in Turkey using metabolomic approaches. Turkey produces diverse types of honey across different regions. Numerous studies have investigated the chemical composition of these honeys and their effects on human health. However, there are relatively few studies that examine the complete metabolome of honey. Existing research has primarily focused on flavonoids and volatile compounds. Through the bioinformatic analysis of metabolomic data obtained in this study, clearer and more interpretable information regarding the classification and biological characterization of Turkish honeys will be achieved. This will also enable a more reliable determination of Turkish honey's global standing, its health benefits, and its characteristics in relation to food safety standards. Furthermore, this study aims to contribute new insights to the scientific literature on honey classification and analysis, through the original methodologies and findings developed during the project.

**Method:** Honey samples were extracted using a methanol:water mixture and subsequently analyzed with an ultra- performance liquid chromatography (UPLC) system coupled with timsTOF (trapped ion mobility time-of-flight) tandem mass spectrometry. The identified metabolites were processed using MetaboScape software, and bioinformatic analyses were conducted via R software.

**Results:** The metabolite extraction method was optimized, and this method was applied to 27 different monofloral honey samples. The identified metabolites were examined using bioinformatic analysis techniques, and distinct metabolite profiles were established for each honey type.

**Conclusion:** The metabolite profiles of regional Turkish monofloral honeys were successfully elucidated through ion mobility mass spectrometry. Classification studies were conducted by considering factors such as geographical regions, floral origins, climatic conditions, and environmental factors.

Anahtar Kelimeler : honey, metabolomics, mass spectrometry, trapped ion mobility

Bildiri No : 6425

Ana Konu : Plant/ Animal/ Nutrition

Bildiri Türü : Poster Presentation

Sunucu : Malik Oğuz Arslanoğlu

Eposta : malikoguzx2@gmail.com



**P027**

**Unveiling The Proteomic Signature of Turkish Monofloral Honey**

Selinay Özel<sup>1</sup>, Melis Şardan Ekiz<sup>2</sup>, İdil Yet<sup>3</sup>, Ömür Gencay Çelemlı<sup>4</sup>, Ömür Çelikbıçak<sup>1</sup>

<sup>1</sup> Hacettepe University, Faculty of Science, Chemistry Department, Ankara

<sup>2</sup> Hacettepe University, Advanced Technologies Application and Research Center, Ankara

<sup>3</sup> Hacettepe University, Institute of Medical Sciences, Bioinformatic Department, Ankara

<sup>4</sup> Hacettepe University, Faculty of Science, Biology Department, Ankara

**Aim:** Bees provide essential food sources that contribute to both healthy nutrition and health protection. These products include honey, royal jelly, and propolis, with honey being the most widely known and consumed. Honey contains approximately 200 different components, though its protein content is relatively low compared to other components. Over the last decade, proteomic approaches have been employed to analyze honey proteins and explore its biological activity at the proteome level. However, due to its low protein content (0.1–3.3%), the full proteome of honey has yet to be comprehensively elucidated. A review of the literature reveals insufficient information on the honey proteome, and no studies have been conducted on this subject in Turkey. The aim of this study is to determine the protein content of various monofloral honey types.

**Method:** A total of 25 monofloral honey samples were collected from two different harvest periods. Sample preparation involved protein extraction, followed by denaturation, reduction, alkylation, trypsin digestion, desalting, and clean-up procedures prior to proteomic analysis. The resulting peptide mixtures were analyzed using a nano-LC coupled with an ultra high resolution ESI-QTOF-MS/MS system.

**Findings:** The protein content of the monofloral honey samples was successfully determined, and subsequent proteomic analyses identified 106 proteins across the 50 samples. The identified proteins originated from both bee and plant sources. Bioinformatic studies were conducted to associate these proteins with the regional characteristics of the honeys.

**Conclusion:** Turkey is remarkably rich in honey variety, yet no previous studies have focused on determining the protein content in its honeys. The information obtained through this study is crucial in highlighting regional differences in honey types. Additionally, it is anticipated that the findings will make significant contributions to the scientific literature, particularly in areas such as honey classification, authentication, quality assessment, and verification of originality.

Anahtar Kelimeler : proteomic, bioinformatic, honey proteom, monofloral honey

Bildiri No : 6444

Ana Konu : Plant/ Animal/ Nutrition

Bildiri Türü : Poster Presentation

Sunucu : Selinay Özel

Eposta : selinayozel95@gmail.com

**P028**  
**Production, Purification, and Characterization of VEGF165**

Serra Arat, Yagız Akbas, Gizem Dinler Doganay

Istanbul Technical University

**Background:** Angiogenesis and vasculogenesis are both regulated by vascular endothelial growth factors (VEGFs) and receptors (VEGFRs). There are many members of the VEGF family, such as VEGF-A, VEGF-B, and VEGF-C. VEGF-A is overexpressed in strongly vascularized tumors. VEGF pre-mRNA is produced from a single, eight-exon gene and it is expressed as seven different isoforms. VEGF165, also called as VEGF-A, is the most expressed and primarily active isoform in humans. VEGF165 stimulates angiogenesis and plays role in the development of various human disorders including cancer and rheumatoid arthritis. The production of recombinant VEGF165 is essential for development of therapeutic anti-VEGF drugs targeted many disorders related to pathological angiogenesis, especially in cancers.

**Aim:** The aim of this study is to produce soluble recombinant VEGF165, purify, characterize, and show its activity.

**Methods:** Optimal bacterial host, growth media, temperature conditions, IPTG concentrations were selected to produce soluble VEGF165. Immobilized metal affinity chromatography (IMAC) and anion exchange chromatography (AEX) were performed to purify the protein. Size exclusion chromatography (SEC-HPLC) and circular dichroism (CD) were performed for the characterization. To assess the functional potential of our purified VEGF, cellular activity was observed in MCF-7 mammalian breast cancer cell line using MTT assay.

**Results:** Our results show that growth of E. coli Rosetta strain at 17°C for 22 hours after 0.1 mM IPTG induction in 2X-YT media is the optimal condition to produce soluble recombinant VEGF165. SEC-HPLC and CD methods revealed that successfully purified VEGF165 was correctly folded, and MTT assays showed its cellular activity.

**Conclusion:** We presented a method to produce soluble VEGF165 and purify it in folded and active forms, which has potential to be used in the development of therapeutic anti-VEGF drugs for cancer.

Anahtar Kelimeler : VEGF165, Recombinant protein production, Angiogenesis

Bildiri No : 6530

Ana Konu : Structural Proteomics

Bildiri Türü : Poster Presentation

Sunucu : Serra Arat

Eposta : aratse19@itu.edu.tr

**P029**

**Construction of Recombinant scFv-Fc Structure Against Hepatitis-B Surface Antigen**

Sude Nur Arslan<sup>1,2</sup>, Muhammed Hamza Müslümanoğlu<sup>1</sup>, Aylın Özdemir Bahadır<sup>2</sup>

<sup>1</sup> Department of Molecular Biology and Genetics, Yıldız Technical University

<sup>2</sup> Vice President of Life Sciences, Tubitak Marmara Research Center

**Aim:** The aim of this work is to construct and express a single-chain variable fragment (scFv) that is fused to an Fc region, which can act as a neutralising antibody against the Hepatitis B Virus (HBV).

**Method:** Anti-HBsAg surface antigens which were improved in previous studies scFv gene amplified by PCR and cloned into a mammalian expression vector. This recombinant plasmid transformed into Escherichia coli (E. coli) cells by chemical transformation method. Recombinant clones were detected by colony PCR and leaky expression of the construct was controlled by SDS-PAGE and Western blot analysis.

**Results:** High amounts of leaky expression for this scFv-Fc protein were detected in E.coli without the addition of isopropyl  $\beta$ -D-1-thiogalactopyranoside (IPTG) for protein production stimulation. The Western blot using an anti-Fc antibody verified that the scFv-Fc protein was in its full form, and SDS-PAGE analysis confirmed protein expression. Even without external inducers, we detected the structure of the whole recombinant antibody fragment.

**Conclusion:** Following the establishment of plasmids with a specific scFv-Fc sequence, plasmids will transfect Chinese Hamster Ovary (CHO) cells for polyclonal production. Furthermore, in future studies, we plan to choose colonies for production to specifically bind the Hepatitis-B Surface Antigen.

Anahtar Kelimeler : Antibody, Therapeutic Proteins, Recombinant Antibody

Bildiri No : 6291

Ana Konu : Clinical Applications

Bildiri Türü : Poster Presentation

Sunucu : Sude Nur Arslan

Eposta : sudenurarslan9@gmail.com



## P030

### Structural and Stability Analysis of CHEK2 VUS Detected in Turkish Population

Nisan Denizce Can, Yağız Akbaş, Gizem Dinler Doğanay

Istanbul Technical University, Molecular Biology - Genetics and Biotechnology

**Aim:** Chk2 tumor suppressor protein is activated by homodimerization and autophosphorylation upon the formation of double-strand DNA breaks. Active Chk2 triggers DNA repair, cell cycle arrest, and apoptosis. Genomic screening of Turkish breast and colorectal cancer patients and healthy controls revealed CHEK2 is most VUS-carrying cancer susceptibility gene. Recurrent CHEK2 c.1053G>T (p.Glu351Asp), c.480A>G (p.Ile160Met), c.1427C>T (p.Thr476Met), c.549G>C (p.Leu183Phe) were considered as worthy to be studied in the protein level regarding the crucial role of Chk2 in DNA damage checkpoint.

**Methods:** Wild-type and mutant Chk2 proteins were produced in *E. coli* BL21 cells and purified by IMAC. Secondary structures of the proteins were investigated by circular dichroism (CD). The impact of the variants on the protein stability was examined by thermal melting analyses and time-dependent trypsinolysis assays. Stoichiometry of proteins was analyzed following non-hydrolyzable ATP and lambda phosphatase treatment by SEC. To observe the effect of variants on the cell viability, and expression and phosphorylation of the Chk2 downstream targets, MTT assay and immunoblotting were performed on Chk2 WT and mutant overexpressing MCF-7 cells.

**Results:** The CD spectrum showed that mutants did not significantly impact the secondary structure. Thermal melting analyses revealed the loss of one of the folding units of Chk2<sup>L183F</sup> and Chk2<sup>I160M</sup>, whose reduced stability was shown by limited trypsinolysis. SEC-HPLC showed Chk2<sup>L183F</sup> and Chk2<sup>I160M</sup> have the highest tendency to dimeric forms after lambda phosphatase treatment. L183F and I160M decreased the viability of cells, and immunoblots confirmed the reduced stability of especially L183F mutation.

**Conclusion:** Among the CHEK2 VUS detected repeatedly in the Turkish population, L183F and I160M were found to impair the protein stability, which may be due to their being trapped in dimeric structure. In light of our findings, L183F was re-classified as likely pathogenic.

Anahtar Kelimeler : checkpoint kinase 2, breast cancer, colorectal cancer, variant of unknown significance

Bildiri No : 6431

Ana Konu : Cell Biology and Functional Proteomics

Bildiri Türü : Poster Presentation

Sunucu : Nisan Denizce Can

Eposta : nisandc@gmail.com



## P031

### Conformational Analysis of Polyelectrolytes for Therapeutic Oligonucleotide Delivery

Ömer Şenocak<sup>1</sup>, Buğra Balaban<sup>1</sup>, Mehmet Emrah Yaman<sup>2</sup>, Tuğba Arifioğlu<sup>1</sup>, Mehmet Atakay<sup>1</sup>, Bekir Salih<sup>1</sup>

<sup>1</sup> Hacettepe University Faculty of Science Department of Chemistry

<sup>2</sup> Atatürk University Faculty of Pharmacy Department of Analytical Chemistry

Polyelectrolytes are charged polymer chains that can form complexes with biomolecules, making them valuable for biopharmaceutical applications, including therapeutic oligonucleotide delivery. To better understand their dynamics, the structure of polyelectrolytes must first be characterized.

**Aim** The goal of this study is to characterize the structural properties of poly-L-lysine (PLL) and polyethylenimine (PEI) polyelectrolytes used in therapeutic drug delivery. By investigating their mass-to-charge ratio ( $m/z$ ) and collision cross-section (CCS) values, the study aims to understand how these charged polymers behave structurally, offering a basis for future biopharmaceutical applications.

**Method** Polyelectrolytes were analyzed with a trapped ion mobility spectrometry-time of flight-mass spectrometer (TIMS-TOF-MS) instrument with electrospray ionization capability. In parallel, molecular dynamics (MD) simulations were conducted to model their conformations in various conditions. Theoretical CCS values were calculated from the simulations and compared with experimental data.

**Results** The TIMS-TOF-MS technique successfully measured the  $m/z$  and CCS values of PLL and PEI, providing detailed structural information. MD simulations offered complementary insights by modeling the polymers' conformational behavior in solution, allowing for a theoretical determination of CCS values.

**Conclusion** The theoretical CCS values obtained from molecular dynamics simulations closely matched the experimental CCS data ( $\sim 0.5 - 2.0$  %) demonstrating the accuracy and reliability of the simulation models. This agreement confirmed the compatibility of the theoretical and experimental approaches, offering deeper insight into the structural behavior and dynamics of the polyelectrolytes under various conditions.

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Anahtar Kelimeler : Therapeutic Oligonucleotide Delivery, Polyelectrolytes, Ion Mobility-Mass Spectrometry, Molecular Dynamics Simulation

Bildiri No : 6429

Ana Konu : New Approaches in Proteomics (Other)

Bildiri Türü : Poster Presentation

Sunucu : Ömer Şenocak

Eposta : mehmetatakay@gmail.com

P032

## Diagnostic Utility of Salivary Biomarkers in Assessment of Oral Leukoplakia

Alican Kuran, Enver Alper Sinanoğlu

Kocaeli Üniversitesi Ağız Diş ve Çene Radyolojisi Anabilim Dalı

**Objective:** Oral cancers are typically diagnosed at an advanced stage, which can have a significant impact on the patient's prognosis and lead to poor outcomes. Epithelial precursor lesions, such as oral leukoplakia (OL), may precede the development of oral cancer. Therefore, that is necessary to enable the timely identification of these precursor lesions to make the early diagnosis of cancer. Nevertheless, the gold standard for diagnosing OL remains the histopathological examination of tissue obtained via biopsy. However, saliva containing abundant proteins offers an accessible, secure and non-invasive approach for disease detection. There are studies aiming to identify a series of potential biomarkers for the diagnosis of OL by evaluating salivary proteins through proteomic analysis. The objective of this study is to provide a brief review of the findings and limitations of these studies in the literature and to identify potential salivary biomarkers for the diagnosis and monitoring of disease progression in OL.

**Methods:** A preliminary search was first conducted in PubMed and Scopus using the keywords "proteomics," "oral leukoplakia," "salivary biomarkers," and "diagnosis/prognosis." Upon completion of this search, an additional article search was performed manually in the Google Scholar database using the same keywords.

**Results:** This brief review encompasses seven studies that collectively seek to elucidate the underlying mechanism of OL development and to enhance diagnostic processes through the utilization of salivary proteins and proteomic analysis.

**Conclusion:** Salivary proteins have the potential to serve as a biomarker for the early diagnosis of OL, obviating the need for biopsy. Nevertheless, a globally applicable standardized protocol is imperative for these studies. The validation of salivary proteins as biomarkers for OL, in conjunction with more innovative detection techniques and the identification of novel biomarkers, will facilitate the establishment of salivary biomarkers as a pivotal early diagnostic method for OL.

Anahtar Kelimeler : Oral leukoplakia, Proteomics, Saliva

Bildiri No : 6443

Ana Konu : Clinical Applications

Bildiri Türü : Poster Presentation

Sunucu : Alican Kuran

Eposta : alican.kuran@outlook.com

### P033

## The Effect of FoxO3a Overexpression on Breast Cancer Metabolism through Orlistat Treatment

Yiğit Efe Aydın, Mustafa Atakan, Buket Özcan, Pelin Özfiliz Kılbaş

Kültür Üniversitesi

**Background :** Breast cancer is the most common type of cancer among women worldwide. The transcription factor FoxO3a, which has been studied in various types of cancer, is known to be associated with cellular mechanisms such as apoptosis, autophagy, and endoplasmic stress. However, the role of FoxO3a in fatty acid synthesis metabolism, which plays a pivotal role in the development of breast cancer, remains unclear.

**Aim:** In this project, breast cancer cell lines with different hormone receptor levels, such as BT474 and MDA-MB-231, will be used to determine the changes in fatty acid metabolism induced by FoxO3a overexpression and orlistat treatment, and their effects on apoptotic processes will be investigated.

**Method:** MTT assay, Growth assay, Colony assay, Western blot, Fluorescent staining,

**Results and Conclusion:** To determine the IC<sub>50</sub> values in cells, MTT assay and growth assay were performed. The doses found were 75  $\mu$ M for the BT474 cell line and 150  $\mu$ M for the MDA-MB-231 cell line. Changes in the expression of fatty acid metabolism proteins within the cells were analyzed by Western blot. It was observed that the expression levels of proteins such as FASN, ACC, and ACL decreased following Orlistat treatment. Additionally, an increase in FoxO3a expression was associated with a decrease in ACSL-1 expression levels. Consequently, it was observed that apoptosis was induced in the cells.

Anahtar Kelimeler : FoxO3a, Breast cancer, Lipid metabolism, Orlistat

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Ana Konu : Cell Biology and Functional Proteomics

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Sunucu : Yiğit Efe Aydın

Eposta : yigitefeaydinnn@gmail.com



**P034**

**Investigating the Regulatory Role of Palmitoylation During Cell Division**

Gamze Nur Yapıcı, Nazlı Ezgi Ozkan, Daniel J. Geiszler, Nurhan Ozlu

Koc University

**Background/aim:** Protein palmitoylation is a reversible lipid modification that plays a crucial role in regulating the localization and function of membrane-associated proteins. The modification has been shown to affect protein-protein interactions, membrane trafficking, and signaling pathways. SILAC-based proteomic analysis and the process of cell division to study the role of palmitoylation and enzymes that transfer palmitoyl groups during cell division.

**Methods:** To prepare the cells for analysis, we synchronized HeLa cells at two key cell cycle stages: interphase and mitosis, using a well-established protocol. SILAC labeling was applied to create "heavy" and "light" cell populations, where heavy cells were metabolically labeled with 17-ODYA and light cells with palmitic acid, and vice versa in biological replicates. A clickable palmitic acid analog, 17-ODYA (17-octadecynoic acid), is incorporated into palmitoylation sites through a metabolic labeling method. Subsequently, the cells are lysed, and the click reaction is performed using biotin azide or rhodamine azide. The cells were cultured in DMEM medium devoid of lysine and arginine amino acids, supplemented with isotopically labeled lysine and arginine for heavy labeling and natural isotopes for light labeling. Subsequently, we performed click chemistry on the lysed SILAC-labeled cells, followed by biotinylation of palmitoylated proteins using streptavidin-coated beads. After enrichment, proteins were subjected to trypsin digestion, and resulting peptides were analyzed using mass spectrometry. The acquired data were processed using MaxQuant and Proteome Discoverer software against the Swiss-Prot human proteome database.

**Results and conclusions:** As our preliminary data using SILAC-labeled heavy and light samples, we have identified proteins with significantly altered SILAC ratios in samples labeled with 17-ODYA and palmitic acid. Upon further analysis of these proteins in the SwissPalm database, it was observed that the palmitoylated protein ratios exhibited a substantial increase. These findings confirm the successful identification of palmitoylated proteins. This data provides insights into the impact of palmitoylation on mitosis.

Anahtar Kelimeler : Palmitoylation, click chemistry, cell division, proteomics, post translational modifications

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Ana Konu : Post Translational Modification Analysis

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Sunucu : Gamze Nur Yapıcı

Eposta : gyapici22@ku.edu.tr



**P035**

**Proteomic Investigation of V493F-FTO-overexpression suggest FTO's role Ubiquitin-Dependent ERAD Pathway**

Aylin Kanlı, Murat Kasap, Gürler Akpınar, Mehmet Doğan Gülkaç

Kocaeli Üniversitesi Tıbbi Biyoloji Anabilim Dalı

**Aim:** Demethylase fat mass and obesity-related protein (FTO), which belongs to the AlkB homologous (ABH) family, are associated with various neurological diseases and cancers, particularly obesity. This protein, which contains many structurally and functionally different regions, contains a COOH-terminal domain whose function, unlike other ABH members, is not fully understood. We aimed to investigate the effects of the exonic V493F mutation in this region of FTO on the soluble proteome.

**Method:** For this aim, SH-SY5Y cells stably overexpressing wild-type (WT-FTO) or mutant FTO (V493F-FTO) proteins under the control of the Tet promoter were created and used. More than 500 protein spots were compared in 2DE gels, and changes in the expression levels of 10 protein spots were detected when two-fold regulation criteria were taken into account. Four of these proteins were regulated after WT-FTO and six were regulated after V493F-FTO overexpression. Proteins were subsequently identified using MALDI-TOF/TOF analysis. **Results:** Although the regulation ratios for the differentiated proteins were less pronounced, overexpression of WT-FTO still affected mainly cell cycle-associated DNA replication and repair processes (such as PCNA). Exogenous production of V493F-FTO altered the expression levels of proteins that respond to cellular stress (such as HSPA4), as well as proteins involved in the regulation of misfolded proteins in the ubiquitin-dependent ERAD pathway (such as ARHGDI1 and VCP).

**Conclusion:** Overall, our findings suggest that FTO may have an additional role in triggering the response to intracellular stress and may also have a role in the ubiquitin-dependent ERAD pathway. Further in vivo and in vitro investigations should be conducted to determine the potential link between the FTO protein and the ubiquitin-dependent ERAD pathway.

**Anahtar Kelimeler :** FTO protein, V493F mutation, SH-SY5Y cells, Proteomics

**Bildiri No :** 6496

**Ana Konu :** Cell Biology and Functional Proteomics

**Bildiri Türü :** Poster Presentation

**Sunucu :** Aylin Kanlı

**Eposta :** aylin.kanli@kocaeli.edu.tr



**Turkish Proteomics Association**