TuPA
Turkish Proteomics Association

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5th Turkish National Proteomics Congress

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HACETTEPE UNIVERSITY

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Turkish Academy of Sciences

ABSTRACT BOOK

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100th Anniversary of the Republic of Türkiye

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**Names are listed in surname's alphabetical order.*

October 13th 2023, Friday

09:00 - 09:30	**Opening Speeches**
09:30 - 11:15	**Session 1**
09:30 - 10:15	**Global Metabolomics and Lipidomics: Technology Development, Integration with Other Omics, and Applications** Prof. Liang Li *University of Alberta*
10:15 - 10:45	**COVID-19 Vaccines from Bench to Clinic** Prof. Serhat Ünal *Hacettepe University*
10:45 - 11:15	**PTMs in Cell Division** Asst. Prof. Nazlı Ezgi Özkan *KUTTAM Omics Core Facility*
11:15 - 11:30	**Coffee Break** 
11:30 - 12:30	**Session 2**
11:30 - 12:00	**Improving targeted mass spectrometry proteomics at the intact protein and peptide map levels by on-line aptamer affinity sample preparation** Prof. Fernando Benavente *University of Barcelona*
12:00 - 12:30	**Integrative Omics Approaches to Explore Extracellular Vesicles-Mediated Cell-Cell Communications** Assoc. Prof. Burak Derkuş *Ankara University*

October 13th 2023, Friday

12:30 - 13:30	**Oral Presentations**
12:30 - 12:40	**S01** **Molecular Analysis of Mammalian Cerebral Cortex Malformations Using Proteomics Approaches** Berfu Nur Yiğit, Donia Zaidi, Büşra Akarlar, Dilaray Tüfekçi, Fiona Francis, Nurhan Özlü
12:40 - 12:50	**S02** **Differential protein expression in Leishmania parasites: Visceral versus Cutaneous Leishmaniasis** Melike Dinç, Talat Yalçın, Merve Beyaz, İbrahim Çavuş, Ahmet Özbilgin
12:50 - 13:00	**S03** **Impact of Hydroxychloroquine-Mediated Autophagy Suppression on PC3 Cell Proteome** Sevinç Yanar, Merve Gülşen Bal Albayrak, Murat Kasap, Gürler Akpınar
13:00 - 13:10	**S04** **Boric acid and Calcium fructoborate effect in ovarian cancer** Ülkü Güler, Seda Sabah Özcan, Rehime Yapar, Bora Onat, Mehmet Korkmaz, Bekir Salih
13:10 - 13:20	**S05** **The Investigation of the Role of Keratin 8 in Cancer Cell Division** Ceyda Seren Ceyhan, Ali Yurtseven, Aybaran Olca Kebabcı, Büşra Akarlar, Nurhan Özlü
13:20 - 13:30	**S06** **Characterization of Lysine Glycation in Protein Therapeutics by Mass Spectrometry** Ceren Pamukçu, Ahmet Emin Atik
13:30 - 14:15	**Lunch Break**
14:15 - 14:45	**Satellite Symposium** **Making the impossible possible: new horizons of 4-D proteomics ecosystem with timsTOF Ultra and HT** Valeria Kuzyk *Bruker Daltonics*

October 13th 2023, Friday

14:45 - 15:45	**Panel**
14:45 - 15:05	**Novel transcriptional regulators and interaction partners in skeletal muscle differentiation** Prof. Çetin Kocaefe *Hacettepe University*
15:05 - 15:25	**Attributing a function to the CXXC5 protein through proximity biotinylation approach** Prof. Mesut Muyan *METU*
15:25 - 15:45	**A search for polyadenylation machinery partners** Prof. Elif Erson Bensan *METU*
15:45 - 16:00	**Coffee Break** 
16:00 - 16:45	**Poster Session**
16:45 - 18:15	**Session 3**
16:45 - 17:15	**Systems Proteomics of Cell Therapy for Heart Failure** Assoc. Prof. Kent Arrell *Mayo Clinic*
17:15 - 17:45	**A novel proteomic approach for in vitro identification of cell surface proteins: the use of purified exogenous TurboID/TurboID-Start** Prof. Murat Kasap *Kocaeli University*
17:45 - 18:15	**Evaluation of proteome profiles of Low, Intermediate and High Risk Neuroblastoma patients** Asisst. Prof. Ayşe Banu Demir *Izmir University of Economics*

October 14th 2023, Saturday

09:00 - 10:30	**Session 4**
09:00 - 09:30	**Probing the packaging of genetic material in giant-virus-dependent virophages using nano-mechanical mass spectrometry** Prof. Christophe D. Masselon *The French Alternative Energies and Atomic Energy Commission Interdisciplinary Research Institute of Grenoble*
09:30 - 10:00	**Artificial Intelligence Driven Approaches in Protein Data Science** Assoc. Prof. Tunca Doğan *Hacettepe University*
10:00 - 10:30	**Metabolomics; A Scope from diagnosis to therapy in Genetic Conditions** Prof. Fatih Ezgü *Gazi University*
10:30 - 10:45	**Coffee Break** 
10:45 - 12:30	**Session 5**
10:45 - 11:15	**Molecular Diagnostics in Personalized Health** **Dr. Sandra Goetze** *Clinical Proteotype Analysis Center, ETHZ*
11:15 - 11:45	**Detection of Spatial Protein Expression Changes in Neurodegeneration via MALDI-IMS** Prof. Ahmet Tarık Baykal *Acıbadem Mehmet Ali Aydınlar University*
11:45 - 12:15	**Proteomic analysis of a novel mitophagy model induced by bacterial HOK protein** Assoc. Prof. Gürler Akpınar *Kocaeli University*
12:15 - 12:30	**Pioneering large scale proteomics with high-throughput SomaScan Platform** Dr. Szabolcs Kokeny *Somalogic*

October 14th 2023, Saturday

12:30 - 13:15	**Lunch Break**
13:15 - 14:00	**Satellite Symposium** **Introduction to the new Orbitrap Astral mass spectrometer** Luka Milivojevic *RedoksLab*
14:00 - 15:40	**Oral Presentations**
14:00 - 14:10	**S07** **ER Proteome of P. teres maculata isolates Pinpoints Diverse Functions** Aslıhan Gunel, Ahmet, Caglar Ozketen, Merve Ulukapı,Faruk Selcuk, Ayse Andac Ozketen, Arzu Celık Oguz, Aziz Karakaya, Christof Rampıtsch, Melike Dinç, Talat Yalcin
14:10 - 14:20	**S08** **Characterization of Chryseobacterium sp. extract using LC-QTOF-MS and molecular networking** Umut Şahar, Didem Aksu, Mamadou Malick Diallo, Selin Sarıçayır, Pelin Sarıkaya, Güven Özdemir
14:20 - 14:30	**S09** **Investigation of OipA Effect on H.Pylori-Infected Gastric Organoid Model** Sümeyye Akçelik Deveci, Ali Durmuş, Sinem Öktem Okullu
14:30 - 14:40	**S10** **Bispecific Antibody Development for Enhanced Therapeutic Functionality in Immunotherapy** Aslı Semerci, Senem Şen, Melis Karaca, Elif Duman, Ebru Şahin Kehribar, Recep Erdem Ahan, Ebru Aras, Urartu Özgür Şafak Şeker
14:40 - 14:50	**S11** **Optimization of ranibizumab production in an engineered bacterial cell strain** Yeliz Dündar Orhan, Gamze Azgın, Sümeyra Nur Fuerkaiti, Serhat Öztürk, Bekir Salih, Ebru Şahin Kehribar, Elif Duman, Urartu Özgür Şafak Şeker
14:50 - 15:00	**S12** **Development of an analytical method for cTnI measurement in serum** Meltem Asicioglu, Nevin Gul Karaguler, Merve Oztug

October 14th 2023, Saturday

15:00 - 15:10	**S13** **Evaluation of DNA repair proteins in glioma patients by quantitative-proteomic-approach** Gamze Tuna, Meltem Kaya, Hande Tiraje Oguzhan, Sertac İslekel, Huray İslekel
15:10 - 15:20	**S14** **Quantification of DNA Repair Proteins in HT-29 Cells by LC/ID-HR-MS** Gamze Tuna, Selda Yilmaz, Meltem Kaya, Merve Yilmaz, Z. Gunnur Dikmen, Gul Huray Islekel
15:20 - 15:30	**S15** **Identification of Interaction Partners of H.pylori Outer Inflammatory Protein A** Sinem Oktem Okullu, Sümeyye Akcelik Deveci, Emel Timucin
15:30 - 15:40	**S16** **Structural Elucidation of FtsH-HflK-HflC Complex using XL-MS** Hatice Akkulak, Burak V Kabasakal, Süreyya Özcan
15:40 - 16:00	**Session 6**
15:40 - 16:00	**TUSEB R&D Project Supports Information Seminar** Büşra Gümüş *Health Institutes of Türkiye (TUSEB) - Department of Project Management and Support*
16:00 - 16:15	**Coffee Break** 
16:15 - 16:55	**Oral Presentations**
16:15 - 16:25	**S17** **BRD2 Interacts with SETD3 in Mouse Embryonic Stem Cells** Nihal Terzi Cizmecioglu
16:25 - 16:35	**S18** **Nuclear Localization and Functional Dynamics of Desmin intermediate Filament** Nilüfer Boustanabadimaralan Düz, Şehriban Büyükkılıç, Hani Alotaibi, Pervin Rukiye Dinçer

October 14th 2023, Saturday

16:35 - 16:45	**S19** **Proximity Proteomics Unveils Aebp1's Interactome, a Key Fibrotic Regulator** Hasan Basri Kılıç, Melis Şardan Ekiz, Ömür Çelikbıçak, Yusuf Çetin Kocaefe
16:45 - 16:55	**S20** **H3K36me2 in mESC pluripotency exit and lineage choice** Dersu Sezginmert, Nihal Terzi Çizmecioğlu
16:55 - 17:55	**Session 7**
16:55 - 17:25	**Neurodegenerative disorders and proteins** Prof. Ertuğrul Kılıç *Istanbul Medipol University*
17:25 - 17:55	**Navigating the Surface: Challenges and Prospects of Surface Proteomics** Asst. Prof. Şerife Ayaz Güner *Izmir Institute of Technology*
17:55 - 18:15	**Award Ceremony and Closing**

ORAL PRESENTATIONS ABSTRACTS

S01
Molecular Analysis of Mammalian Cerebral Cortex Malformations Using Proteomics Approaches

Berfu Nur Yiğit[1], Donia Zaidi[2,4], Büşra Akarlar[1], Dilaray Tüfekçi[1], Fiona Francis[2,3,4], Nurhan Özlü[1,5]

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[3] Sorbonne University, Umr-s 1270, F-75005 Paris, France
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[5] Koc University Research Center For Translational Medicine (kuttam), Istanbul, Turkey

The layered structure of the cerebral cortex is formed through a complicated sequence of highly controlled stages during corticogenesis. The perturbation of neuronal migration and cell division during this process can result in a rare disorder called cortical heterotopia. Patients with heterotopia can have recurrent epileptic seizures, developmental delay, mild-intellectual disabilities. This disorder is not well understood because the human mutations associated with the disease have often failed to give rise to the phenotype in mouse models.

EML1 is a heterotopia-associated gene where the perturbations cause heterotopia formation in humans as well as in mice. In this project, we aim to understand the pathological mechanisms involved in the formation of *EML1-related* heterotopia using proteomics approaches.

In this project, BioID proximity labeling, live-cell imaging approaches are used to understand mechanisms giving rise to *EML1-related* heterotopia, using mouse primary neuronal progenitor cells and a neural cell line.

Protein interaction partners of wild-type and mutant EML1 were identified by BioID with LC-MS/MS. According to these results, EML1 interacts with many microtubule, and cytoskeletal organization proteins including microtubule-associated proteins. Strikingly the interaction of many proteins related to the microtubule cytoskeleton is significantly impacted by a heterotopia-causing mutation in EML1. To determine differentially expressed proteins in mouse *Eml1* conditional knockout primary neuronal progenitor cells, the dimethyl labeling-based approach is used to compare the proteome of wild-type and *Eml1*-cKO-mouse models.

This study identified numerous novel interactors along with the known interaction partners of EML1 in neuronal cells. The interaction partner analysis revealed that the heterotopia-associated mutant form of EML1 loses many interactions in the cells. Our quantitative proteomics approach defined EML1-associated signaling pathways that provide important resources to study the underlying mechanism in brain development and heterotopia formation.

S02
Differential protein expression in Leishmania parasites: Visceral versus Cutaneous Leishmaniasis

Melike Dinç[1], Talat Yalçın[1], Merve Beyaz[1], İbrahim Çavuş[2], Ahmet Özbilgin[2]

[1] İzmir Yüksek Teknoloji Enstitüsü
[2] Manisa Celal Bayar University

Aim: Leishmaniasis is a parasitic disease that usually causes damage to the skin, and it becomes more destructive when mucosal tissue and internal organs are affected. Some certain species are directly associated with these severe forms of the disease. Among the most important questions in Leishmania research is why these species infect visceral organs, whereas others remain in the skin. Our goal was to investigate this phenomenon using comparative proteomic analysis.

Method: Label-free quantitative proteomic analysis was applied to 14 samples of *Leishmania tropica* iasolates. Total protein mixtures were digested with FASP method and desalted using ZipTip column tips.Peptide mixture was introduced to orbitrap fusion tribrid mass spectrometer (Thermo Scientific) from UltiMate™ 3000 RSLCnano System that was equipped with C18 0.075x250mm Acclaim PepMap100 separation column and 0.3x5mm PepMap100 trap column. Mass spectrometric data was analyzed with Proteome Discoverer program.

Results: Our findings revealed that membrane transporters and purine salvage pathway enzymes were expressed more in VL-causing parasites. Lactate catabolic process enzyme, D-lactate dehydrogenase-like protein was also upregulated in this group. In contrast, samples of CL were rich in calpains, as well as, ribosomal and antigenic proteins.

Conclusion: Totally 144 proteins were identified and quantified as differentially abundant in either the cutaneous leishmaniasis or visceral leishmaniasis group. Judging by the functions of these proteins, the overall picture tells us VL parasites are primarily focusing on ensuring their integrity while CL parasites are prone to produce energy for cell growth.

S03
Impact of Hydroxychloroquine-Mediated Autophagy Suppression on PC3 Cell Proteome

Sevinç Yanar[1,2], Merve Gülsen Bal Albayrak[1], Murat Kasap[1], Gürler Akpınar[3]

[1] Kocaeli University Faculty of Medicine, Department of Medical Biology
[2] Sakarya University Faculty of Medicine, Department of Histology and Embryology

Aim: Numerous preclinical and clinical studies have shown that autophagy inhibition holds a significant role as an adjunctive approach in the treatment of prostate cancer. Hydroxychloroquine (HCQ) has gained considerable attention due to its established role as an autophagy inhibitor across diverse cancer types. Although the mechanism of action of HCQ has been revealed to a certain extent, there is no study investigating its effect on the proteomic landscape. To unravel the therapeutic potential of autophagy modulation in the realm of prostate cancer, it is crucial to elucidate alterations in the proteome of cells. Therefore, this study aimed to investigate the effects of HCQ-mediated autophagy inhibition on the proteome of PC3 prostate cancer cells.

Methods: The cells were treated with 45μM HCQ for 72 hours and proteins were extracted. To identify differentially expressed proteins between treated and not-treated cells, the change in the proteome of cells was investigated by label-free quantification with nano-high performance liquid chromatography to tandem mass spectrometry (nHPLC-MS/MS). The proteins that showed significantly altered expression levels were evaluated using the bioinformatics tools STRING and PANTHER.

Results: Bioinformatic analyses revealed that HCQ induced significant alterations in key regulatory processes in PC3 cells. In particular, these changes predominantly occurred in cellular respiration, energy derivation, generation of precursor metabolites, oxidation-reduction process and metabolic pathways.

Conclusion: This study unveils for the first time that autophagy inhibitor HCQ can cause impaired cellular recycling, altered energy balance, and disrupted metabolic flux in prostate cancer cells. Understanding these consequences can provide insights into how autophagy inhibition influences cellular metabolism and its potential implications for cell function and viability.

Acknowledgment: This work was supported by a grant from Sakarya University (Grant No: 2022-9-32-26).

S04
Boric Acid and Calcium Fructoborate Effect in Ovarian Cancer

Ülkü Güler[1], Seda Sabah Özcan[2], Rehime Yapar[2], Bora Onat[3], Mehmet Korkmaz[2], Bekir Salih[1]

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Aim: We aimed to investigate the effects of Boric Acid (BA) and Calcium Fructoborate (CaF) on the proteomic profile of cells in terms of apoptotic pathways and DNA damage/repair pathways in A2780 ovarian cancer cell line.

Method: Cell viability and toxicity were determined by the MTT method. IC50 values were determined to be 1803 uM for BA and 2395 uM for CaF in 72h. Cells were treated with BA and CaF according to the MTT results and collected for protein isolation. A total of 50 µg of protein from each sample was first reduced with dithiothreitol, iodoacetamide was added allowing for protein alkylation. A methanol/chloroform precipitation protocol was performed The resulting precipitate was dissolved in a solution at a ratio of 1:100 (w/w, trypsin-LysC/protein) containing 8 M urea and 50 mM Tris buffer at pH 8.5 and diluted to 1 M urea concentration. Trypsin was added to the protein solution and incubated overnight. Trifluoroacetic acid was added. A Sep-Pak purification was performed. The purified samples were dried using a Speed-Vac and reconstituted in MilliQ water. Peptides were separated using an EASY-Spray column. The separation was performed on a nanosystem connected to a Q Exactive Plus mass spectrometer. Peptide fragmentation was performed through higher-energy collisional dissociation.

Results: The MS files were processed with the MaxQuant software and searched with Andromeda search engine against the human UniProt database. Comparison of groups were visualized through heatmaps that were prepared on the pHeatmap, an R function to draw heatmaps. Enriched pathways were determined through inputting the Uniprot codes of each protein on the online mapping tool path DIP. As a result, the CaF group was found to be associated with apoptosis and DNA repair pathways.

Conclusion: The potential of CaF as a natural molecule could support conventional ovarian cancer treatments.

S05
The Investigation of the Role of Keratin 8 in Cancer Cell Division

Ceyda Seren Ceyhan, Ali Yurtseven, Aybaran Olca Kebabcı, Büşra Akarlar, Nurhan Özlü

Koc University

Keratins are intermediate filaments distributed in epithelial cells and function to give tissue strength. They have diverse expression patterns in different cells and are used as markers in diagnostic tumor pathology. However, it is not well studied how these robust structures do not prevent cell division and how they are regulated during dynamic processes, such as cytokinesis. It is known that phosphorylation on Keratin proteins increases their solubility and alters their phenotype. In our study, we focus on Keratin 8 and aim to investigate its plasticity during cancer cell division by searching its phosphorylation profile.

In this project, MCF7, HeLa.S3, MDA-MB-231, and CAKI-2 cancer cells are arrested at interphase, mitosis, and cytokinesis stages. Phosphorylation profiles are investigated by stable isotope dimethyl labeling based quantitative phosphoproteomics approach by combining samples from different cell cycle stages for each cell line. To reduce the complexity of the labeled peptides, samples are fractionated by Strong Cation Exchange Chromatography, and low abundant phospho-peptides are enriched with TiO2 beads. Enriched phospho-peptides are subjected to LC-MS/MS analysis, and raw data files are processed with PD 2.3 and MaxQuant software.

To understand the phosphorylation profile of Keratin 8 during cancer cell division, triplex dimethyl labeling is performed, and cell cycle stage-specific phospho-sites are analyzed for each cell line. Inter-cell type comparisons revealed commonly regulated phospho-sites in all cell lines at the head domain of Keratin 8. According to intra-cellular phosphorylation analysis, MCF7 cells have the highest number of phospho-sites with the highest Keratin 8 expression levels. The number of phospho-sites decreases as the Keratin 8 expression levels decrease through epithelial to epithelial-mesenchymal cells.

Our findings revealed upregulated phospho-sites of Keratin 8 at specific cell cycle stages. There are commonly regulated and cell-type unique phospho-sites that are potential marker candidates for cell division and tumor diagnosis.

S06
Characterization of Lysine Glycation in Protein Therapeutics by Mass Spectrometry

Ceren Pamukcu[1], Ahmet Emin Atik[1,2]

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[2] Acıbadem Mehmet Ali Aydınlar University, Faculty of Engineering and Natural Sciences, Department of Natural Sciences, İstanbul, Turkey

Aim: Glycation is a non-enzymatic PTM generally observed in therapeutic monoclonal antibodies (mAbs). It may occur during the manufacturing process and/or long-term storage in sucrose-containing formulations. In the current study, a hybrid mass spectrometer (QTof-MS) was used to compare the glycation level of one originator mAb with its three biosimilars under forced glycation stress.

Method: For the forced glycation study, samples were incubated at 37°C with 500 mM of D-glucose solution for 1, 3, and 7 days. Samples were analyzed via intact mass and peptide mapping level with an ultra-performance liquid chromatography coupled to a Xevo G2–XS QTof mass spectrometer (Waters Corporation). The instrument was equipped with an ESI source operating in positive ion mode with full MS scan mode over a mass range of 400 – 4000 m/z and with MSE mode over a mass range of 50 – 2000 m/z, for intact mass and peptide mapping analysis, respectively.

Results: Intact mass data demonstrated that total glycation levels are identical for originator and biosimilar samples by having three glucose additions to the five prominent glycoform peaks (G0F/G0F, G0F/G1F, G1F/G1F, G1F/G2F, and G2F/G2F) after 7 days at 37°C. The deconvoluted intact mass spectra of the samples showed a mass shift of 3 x 162 Da between the forced glycated and unstressed samples. Intact mass analysis results also revealed that the level of glycation increased with prolonged incubation time. Peptide mapping analysis identified that glycation occurred at three lysine (Lys, K) residues with relative abundances of more than 10%.

Conclusion: These results demonstrate that intact mass and peptide mapping analyses can be used for the quantification of glycation levels in mAbs. The former analysis is fast and can be implemented as a routine testing to monitor the extent of glycation for mAbs.

S07
ER Proteome of P. teres maculata isolates Pinpoints Diverse Functions

Aslihan Gunel[1], Ahmet Caglar Ozketen[3], Merve Ulukapı[2], Faruk Selcuk[2], Ayse Andac Ozketen[4], Arzu Celık Oguz[3], Ayse Andac Ozketen[4], Aziz Karakaya[5], Christof Rampıtsch[6], Melike Dinç[7], Talat Yalcin[7]

[1] Kirsehir Ahi Evran University, Department of Chemistry-biochemistry, Kirsehir-turkey
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Spot form net blotch (SFNB) is one of the major threats to global barley production annually. The causative agent of the disease is a necrotrophic fungal pathogen *Pyrenophora teres* f. *maculata* (Ptm). Virulence and avirulence-associated genes and their mechanism of interaction with the host are studied yet it remains to be discovered. Proteomics is a versatile and robust strategy to enlighten the host-pathogen interaction however only a limited number of virulence and avirulence-associated genes are revealed. One of the possible reasons for this obstacle is cataloging the associated genes with only cytosolic proteome excludes certain proteins such as membrane-bound proteins. Therefore, subcellular proteomics is a solution to pinpoint spatial proteome. For this purpose, here we conducted proteomics analysis on the isolated endoplasmic reticulum (ER) of Ptm for the first time. We scrutinize rough ER and smooth ER proteins from highly virulent 13-122 and 13-127Hsp isolates, and low virulent 13-128Hb and 13-177. We have detected a total of 740 proteins and annotated them using different tools to decipher functional roles. We executed different bioinformatics analyses to predict secreted proteins, effectors, and their subcellular localization patterns. Among the predicted 50 effectors, 35 (34.0%) of them enlisted as possible apoplastic effectors. 21 of the predicted effectors have also shown significant homologies to known virulence and avirulence genes from public host-pathogen interaction databases, with emphasis on their potential for future studies as promising candidates.

S08
Characterization of Chryseobacterium sp. Extract Using LC-QTOF-MS and Molecular Networking

Umut Şahar[1], Didem Aksu[2], Mamadou Malick Diallo[1], Selin Sarıçayır[1], Pelin Sarıkaya[1], Güven Özdemir[1]

[1] Ege University, Faculty of Science, Department of Biology, Izmir
[2] Ege University, Application and Research Center For Testing and Analysis, Izmir

Aim: This study was aimed to characterize the chemical constituents of *Chryseobacterium* sp. by an untargeted strategy using LC-QTOF-MS combined with Molecular Networking (GNPS), identify potential target proteins (pharmacophore) for these molecules and monitor the antimicrobial activity.

Method: The cells were removed from the media by centrifugation, supernatant was acidified and kept overnight at 4°C. Solution was extracted using chloroform: methanol. After extraction, supernatant and the dried extract were analyzed by LC-QTOF-MS. Untargeted MRM screening with gradient HPLC program on C18 column was performed. After file conversion for obtained spectra, molecular network was generated on GNPS platform. Pharmmapper was used to discover the probable inhibitors among these metabolites. The results were visualized on Cytoscape for complex network analyses. Antimicrobial activity of the extract against gram positive/negative bacteria was determined by disc diffusion method.

Results: The untargeted LC-QTOF-MS/MS method was successfully applied for profiling of extracts. After molecular networking of LC-QTOF-MS/MS spectra, a total of 116 metabolites with distinctive fragment spectra were detected. Between these compounds, 28 amino acids/peptides and other small molecules e.g., fatty acids, alkaloids, were reported using GNPS platform. Bioinformatic results was revealed that some of dipeptides have high binding affinity (Normfit>0.90) values with several target bacterial proteins. The in vitro test results showed that the extract has antibacterial activity against all the five bacterial species and highest antibacterial activity was observed against *B. cereus and B. subtilis*

Conclusion: Bacterial metabolites are a major source of bioactive compounds. LC-QTOF-MS/MS and GNPS database combination was found to be a powerful method for rapid analysis of bioactive materials from *Chryseobacterium* sp. extracts without the help of reference standards. Using PharmMapper, possible antibacterial targets were identified, i.e., monomeric sarcosine oxidase (uniprot: P40859) from Bacillus sp. could be a target for N-butanoyl-DL-tryptophan with high fit score.

S09
Investigation of OipA Effect on H.Pylori-Infected Gastric Organoid Model

Sümeyye Akçelik Deveci[1], Ali Durmuş[2], Sinem Öktem Okullu[3]

[1] Acıbadem Mehmet Ali Aydınlar University, Department of Medical Biotechnology, Institute of Health and Science
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Aim: *Helicobacter pylori (H.pylori)* is a pathogenic bacterium that causes gastric diseases by inflammation in the stomach. One of the outer membrane proteins of *H.pylori* is outer membrane protein A (OipA) which promotes bacterial colonization in the stomach and is intimately associated with infection. However, our knowledge of the effects of this protein on host cells is insufficient. The aim of this study is understand the role of OipA in the pathogenesis of *H.pylori* by post-infection proteomic analysis of human gastric organoid models infected with *H.pylori* strains; wild type G27 and mutated G27-ΔoipA

Methods: The constructed human gastric organoid model was infected with *H.pylori* G27 and G27-ΔoipA. The proteins isolated from infected and control organoids were identified by LC-MS/MS analysis. After statistical normalization using Thermo Proteome Discoverer, the label-free quantification (LFQ) method was employed to calculate the differential expressional changes. Proteins were considered differentially expressed only if they met the following criteria: minimum of two unique peptides and a fold change of at least 1,3. The OipA-effected human proteins were determined by comparison of both control and ΔoipA-G27 infection groups, and a protein-protein interaction (PPI) map was generated by Cytoscape software.

Results: As a result of the LC-MS/MS analysis, out of the identified 3650 proteins, 1922 of them have equal or more than two unique peptides, and they were quantified by LFQ. Ninety-seven proteins were significantly affected by OipA protein presence during *H.pylori* infection. PPI map reveals the significant cellular pathways affected by *H.pylori* infection through the OipA protein as rRNA processing, apoptosis, and metabolism of amino acids pathways.

Conclusions: This study examined the effect of the OipA protein on the gastric organoid model during *H.pylori* infection, identified intracellular target proteins and pathways of the OipA protein in the prognosis of *H.pylori*-related diseases.

S10
Bispecific Antibody Development for Enhanced Therapeutic Functionality in Immunotherapy

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Antibodies play a crucial role in the immune system by recognizing inflammation and mounting a targeted response against specific stimuli, bolstering the body's defense mechanisms. Modern technology enables the production of synthetically designed antibodies outside organisms, rendering them more effective and safer. Bispecific antibodies represent a relatively new-era in development, achieved through enhanced molecular biology techniques and synthetic biology tools. These molecules provide multiple cellular ligand recognition activities, evoking simultaneous responses in the body to stimulate treatment.

In this study, we present a novel platform for the rapid and precise development of bispecific antibodies. In our system, each antibody fragment is independently regulated with dedicated promoter regions on the responsible plasmid. Equal contributions are provided for equidimensional protein production of each fragment. Additionally, antibody stability is fortified through specific amino acid modifications in heavy fragments, and the variable chains are interchanged between heavy and light chains. The designed and produced molecules are capable of stimulating two different selected ligands and humoral responses concurrently within the body.

Our study targets Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), human epidermal growth factor receptor 2 (HER2), and Anti-Integrin alpha V (CD51) ligands, combined with epidermal growth factor receptor (EGFR). EGFR, known for stimulating cell growth, is often overexpressed on cancer cell surfaces. Combining EGFR targeting with T-cell specific markers (anti-CTLA4) allows for the simultaneous stimulation of humoral responses, T-cell-mediated cellular responses, and apoptosis in tumor cells. The combination with other cancer markers (HER2 and CD51) further supports robust stimulation of the apoptosis mechanism in tumor cells. Both approaches are complemented by the humoral response, facilitating practical tumor cell destruction during therapy.

Our technical approach is presented as an adaptable tool, supporting the development of various bispecific antibodies. The developed bispecific molecules and the system's potential offer a promising avenue for advancing bispecific antibody development.

S11
Optimization of Ranibizumab Production in an Engineered Bacterial Cell Strain

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Aim: Our study aims to enhance the production of ranibizumab, a vital non-glycosylated protein used in treating conditions like age-related macular degeneration and macular edema. Currently produced in Escherichia coli as inclusion bodies, it incurs high purification costs and decreased production efficiency upon refolding. Our primary objective is to not only develop a ranibizumab biosimilar but also optimize production processes for increased yield of both total protein and soluble ranibizumab through innovative approaches.

Method: In this research, various ranibizumab variants were expressed in bacterial systems in soluble form and subsequently purified, aiming to achieve greater production efficiency at reduced costs. Signal peptides were employed to guide antibody fragments to the periplasmic space, facilitating proper protein folding. Additionally, the study involved disrupting an outer membrane structural gene, resulting in a leaky strain that permitted the extracellular purification of the antibody fragment.

Results: The utilization of signal peptides effectively directed ranibizumab variants to the periplasm, resulting in significantly improved solubility and production levels within leaky *E. coli* cells. The proper folding of the antibody fragments was confirmed through circular dichroism (CD) analysis, while size-exclusion chromatography (SEC) was employed to compare molecular weight distributions with a commercial product (Lucentis®). LC/MS analysis conclusively verified the molecular size of the produced protein, aligning with existing literature. Protein activity was validated through VEGF binding ELISA, and anti-proliferative activity was established via MTT assays conducted in human retinal endothelial cells.

Conclusion: The study successfully achieved two critical objectives: the efficient production of biologically active ranibizumab with proper folding and cost-effective purification, all within a leaky *E. coli* strain. This groundbreaking approach facilitates the extracellular production of antibody fragments and opens doors to the cost-effective production of biosimilar drugs that naturally form inclusion bodies.

S12
Cardiac Troponin I Measurement in Serum Using Targeted Proteomics

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Aim: Cardiac troponin I is a biomarker protein used in the diagnosis of acute myocardial infarction. However, there is no measurement procedure currently available that can ensure the metrological traceability of cTnI results in clinical measurements. Our goal in this study is to develop an isotope dilution-liquid chromatography tandem mass spectrometry (ID-LC/MSMS) based analytical method using a targeted proteomics approach to ensure metrological traceability.

Method: To isolate cTnI from human serum, an antibody specific to cTnI was immobilized onto magnetic nanoparticles. Using the synthesized complex, cTnI enrichment from serum was achieved through immunoaffinity enrichment. Following trypsin digestion, multiple tryptic peptides were monitored via parallel reaction monitoring mass spectrometry (PRM-MS).

Results: The developed method successfully quantified cTnI in the range of 0.7-24 ng/ml. Method validation was done by analysing three samples at each concentration on four different days. The quantification limit of the developed ID-LC-MS/MS method was determined to be 1.8 ng/ml. The uncertainty of the validated method was also calculated.

Conclusion: The presented analytical technique showed high performance in precisely measuring the levels of cardiac troponin I in human serum. This method holds promise in establishing reference values for secondary certified materials, and ensuring dependable cTnI measurements.

S13
Evaluation of DNA Repair Proteins in Glioma Patients by Quantitative-Proteomic-Approach

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Aim: Gliomas are derived from glial cells and are the most frequent malignant tumors caused by mutations in the brain. Isocitrate dehydrogenase-1 *(IDH1)* mutation status has an important role in the classification as well as prognosis of gliomas. The majority of cancers are caused by unrepaired DNA damage, and defects in the repair system are also associated with inherited forms of cancer. Base excision repair (BER) is the most common DNA repair mechanism. Human apurinic/apyrimidinic-endonuclease-1 (hAPE1) is a multifunctional protein that plays central role in cellular response to free radicals and oxidative stress. Human poly(ADPribose)-polymerase-1 (hPARP1) plays major role in the repair of DNA strand breaks. It is known that DNA repair capacity increases in malignant tumours and it has a positive correlation with drug resistance. For these reasons, the main purpose of this study is to evaluate hAPE1 and hPARP1 proteins in *IDH1*-wildtype (n=17) and *IDH1*-mutant (n=14) glioma patients' brain tissues with liquid chromatography/ isotope-dilution high resolution mass spectrometry (LC/ID-HR-MS) method.
Method: Proteins were extracted from tissues and digested with trypsin. hAPE1 and hPARP1 were analyzed by LC/ID-HR-MS. A fully ^{15}N-labeled analogue of hAPE1 and Lys-$^{13}C_6$,$^{15}N_2$–r Arg-$^{13}C_6$,$^{15}N_4$–labeled tryptic peptides of hPARP1 were used for the quantitative measurements.

Results: In the wildtype group, hPARP1 levels were significantly higher than in the mutants (p=0.037). There was no significant difference between the two groups regarding hAPE1 concentration in samples (p=0.321). In all the glioma patients, a positive correlation was found between hPARP1 and hAPE1 levels (p=0.001, r=0.590).

Conclusion: This result may explain the significant propensity for cancer cells to develop treatment resistance by improving their DNA repair capacity against chemotherapeutic drugs in *IDH1*-wild type glioma patients at the molecular level.

S14
Quantification of DNA Repair Proteins in HT-29 Cells by LC/ID-HR-MS

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Aim: Living cell DNA is continuously damaged internally or externally, and most of these DNA lesions are repaired through the base excision repair (BER) pathway. Recent years, DNA repair proteins have emerged as predictive, prognostic, and therapeutic targets in cancer. BER begins when DNA glycosylase enzymes like 8-oxoguanine DNA glycosylase-1(hOGG1) and endonuclease 8-like 1(hNEIL1) recognize damaged sites, cleaving the glycosidic bond between the damaged base and sugar, separating the base from the nucleotide. Apurinic/apyrimidinic endonuclease-1(hAPE1) then breaks the phosphodiester bonds in the resulting baseless region. Poly(ADP)ribose polymerase-1(hPARP1) binds to the broken DNA ends, safeguarding them and activating BER enzymes. Finally, DNA polymerase β(hPOLβ) fills the gap, and DNA ligase catalyzes phosphodiester bond formation. This study directly quantified hAPE1, hPARP1, hOGG1, hPOLB, and hNEIL1 in human colorectal adenocarcinoma(HT-29) cells using label-based high-resolution quantitative proteomic approach.

Method: Proteins were extracted from HT-29 cell sample. A fully ^{15}N-labeled analogue of hAPE1, hOGG1, hNEIL1, hPOLβ and Lys-$^{13}C_6$, $^{15}N_2$– or Arg-$^{13}C_6$, $^{15}N_4$–labeled tryptic peptides of hPARP1 were used for the quantitative measurements. Cell sample digested with trypsin. All five proteins were simultaneously analyzed by liquid chromatography/ isotope-dilution high resolution mass spectrometry (LC/ID-HR-MS)(Thermo Scientific Exactive Plus Orbitrap MS).

Results: Six peptides for hAPE1, hNEIL1 and hPARP1, seven peptides for hPOLβ and three peptides for hOGG1 were identified, matching a subset of the theoretically predicted hAPE1, hAPE1, hOGG1, hNEIL1, hPOLβ and hPARP1 tryptic peptides. The amount of proteins studied was calculated at the ng/μg protein level in the cell.

Conclusion: The results obtained showed that the LC/ID-HR-MS measurement method is highly suitable for the multiple analysis of these proteins in biological samples. Thus, by absolute quantification of these DNA repair proteins, the nature of DNA repair can be explained in more detail.

S15
Identification of Interaction Partners of H.pylori Outer Inflammatory Protein A

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Aim: OipA is one of the outer membrane proteins of *H. pylori* that induces pro-inflammatory signals and secretion of IL-8 when bacteria attach to the gastric epithelial layer. Although the functions of this protein have been defined, the interaction partner(s) in the gastric epithelial cell layer has not been identified. In this study, it was aimed to identify gastric cell surface proteins that OipA interacts.

Method: Recombinant OipA protein was produced and gastric epithelial cell surface proteins were extracted from AGS cell line. OipA protein was used as a bait protein to pull down prey proteins from gastric cell surface proteins that interact with OipA. The obtained proteins were analyzed with LC-MS/MS to identify the predicted interaction partners.

Results: Proteomic analysis showed 704 unique binding partners of OipA. Inspection of this partner list led to a collection of a small set of OipA binders that were selected based on subcellular localization and biological relevancy. This small set includes HGF, MET, CA-II, AGRB1, VAP-1, TETN, CYTM, CLCA2, HBA1, RAB4A, NHRF1, FBLN3, NECP2, SDCB1,CAD13, MAPK3. All 16 protein partners were extensively analyzed by molecular modeling methods, providing 16 distinct atom-resolution complex structures of OipA.

Conclusion: The binding of OipA protein and candidate interaction partner is an important step in the attachment of *H. pylori* to the gastric mucus layer. The interaction partners can be targeted to prevent this infection. Hence, we conclude that the reported interaction partners of OipA that were determined by a proteomic analysis could be highly promising for future studies aiming to examine the molecular mechanism(s) of OipA in *H. pylori* infection.

S16
Structural Elucidation of FtsH-HflK-HflC Complex using XL-MS

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Cross-linking mass spectrometry (XL-MS) is an emerging structural proteomics method that is integrative and complementary to conventional structural biology methods, such as Cryo-EM, X-ray Crystallography, and NMR. The specific residues of proteins in close proximity are covalently bound via cross-linkers to get inter- and intra-protein interactions. The connections obtained within and between proteins provide us with the information to elucidate the structure of proteins and protein complexes. Here we investigated the composition and structure of the FtsH-HflK-HflC E. coli membrane protein complex via XL-MS.

Briefly, proteins were produced in *E. coli* recombinantly, inner membranes were solubilized, and the target proteins were purified using affinity chromatography. An MS-cleavable cross-linker, DSBU, was used for the cross-linking reactions in the solubilized membrane and purified protein samples. The cross-linked proteins were then digested yielding peptide and cross-link pairs. The chromatographic separation was followed by high-resolution MS. The raw MS data containing precursor and fragment ions were analyzed through cross-linking software to identify interaction points. The interactions identified by the software were examined on raw MS data, PDB, AlphaFold, and PyMOL.

The cross-link search analysis yielded 30 inter-protein and 119 intra-protein interactions. The connections of DSBU with not only lysine residue but also serine, threonine, and threonine residues were detected. The interactions were in agreement with partially known Cryo-EM structures. In addition, 6 new interactions points were identified on both periplasmic and cytoplasmic regions of FtsH-HflK-HflC.

These findings contribute valuable insights into the structure of the FtsH-HflK-HflC complex towards the understanding of the full structure and function of this mega membrane complex.

S17
BRD2 Interacts with SETD3 in Mouse Embryonic Stem Cells

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Aim: Early embryonic development is coordinated through complex gene expression alterations guided by chromatin and transcription factors. With self-renewal and pluripotency features, embryonic stem cells (ESC) are used to model early development in vitro. Our earlier results identified a SET domain-containing methyltransferase SETD3 as a critical epigenetic factor for meso/endoderm differentiation of mouse ESCs (mESCs). We aimed at understanding its mechanism of action through proteomic approaches coupled with cell biology.

Methods: Immunoprecipitation followed by mass spectrometry from mESC nuclear extracts revealed SETD3 interaction partners including a bromodomain protein, BRD2, that can associate with acetylated histones. Co-immunoprecipitation and proximity ligation assay (PLA) confirmed this interaction. To identify its molecular mechanism, we generated SETD3 and BRD2 domain-deletion constructs using overlapping PCR. HEK293FT cells were transiently transfected with these constructs. Protein localization was assessed using immunocytochemistry and subcellular fractionation. PLA experiments were then conducted in a combinatorial manner to investigate the critical interaction domains in each protein.

Results: Mass spectrometry identified BRD2 as a SETD3 interaction partner. This interaction was confirmed through co-immunoprecipitation and PLA experiments. All domain-deleted constructs of SETD3 or BRD2 showed expected localization patterns. Deletion of the Rubisco-Substrate-Binding domain of SETD3 and the Bromodomain-2 domain in BRD2 led to significantly reduced signal intensity in PLA.

Conclusion: Our findings demonstrate a nuclear interaction between two epigenetic factors, SETD3 and BRD2. This interaction is facilitated by the Rubisco-Substrate-Binding domain of SETD3 and the Bromodomain-2 domain of BRD2. Ongoing effort is on how this interaction affects the chromatin environment and differentiation-specific gene expression program.

S18
Nuclear Localization and Functional Dynamics of Desmin intermediate Filament

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Aim: Desmin, a muscle specific protein, plays a role in shaping the network of intermediate filaments in different types of muscles like cardiac, skeletal, and smooth muscles. Recent studies indicate that apart from Lamin proteins, other types of intermediate filaments might also enter the cell nucleus. The C-terminal tail domain of Desmin intermediate filament contains a binding domain for Lamin B, and disruption of this domain has been linked to myofibrillar myopathy-1. This phenomenon highlights Desmin as a potential candidate for nucleocytoplasmic interactions. The aim of this study is to investigate the nuclear localization of Desmin in skeletal muscle progenitor cells, thereby offering new insights into its functional dynamics.

Method: We utilized immunofluorescence (IF) co-staining techniques, simultaneously labelling Desmin with two distinct nuclear markers: DAPI and Lamin B. To provide additional validation of Desmin's nuclear localization, we conducted subcellular fractionation experiments followed by immunoblotting analysis. In order to elucidate the modified molecular pattern associated with the potential role of Desmin within the nucleus we merged proteomic data with an extended transcriptomic dataset.

Result: Our co-staining analysis, which focused on Pearson's Correlation Coefficient, revealed increased Desmin signals along the nuclear periphery. Furthermore, the detection of Desmin within the nuclear fraction, as confirmed by Western blotting, offers secondary validation of Desmin's nuclear translocation within skeletal muscle progenitor cells. Through the implementation of *in-silico* analysis using omics data acquired from both AB wild-type zebrafish and Desmin knockout mice, it is indicated that Desmin could potentially impact gene expression in an indirect manner.

Conclusion: Our research has shed light on a previously unexplored and valuable dimension related to nuclear Desmin in skeletal muscle precursor cells. This insight makes a noteworthy contribution to the ongoing scientific discussions about nucleocytoplasmic communication pathways within cells.
This work was supported by TÜBİTAK (Grant numbers: 222Z082 and 123Z114).

S19
Proximity Proteomics Unveils Aebp1's Interactome, a Key Fibrotic Regulator

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The extracellular matrix (ECM) is essential for the structure and function of multicellular organisms, offering physical and chemical cues regulate the organization and behavior of cells. Degenerative changes in ECM cause fibrosis which is irreversible accumulation of ECM proteins leads tissue dysfunction. Fibrosis is the key component of multi-systemic complex disorders and aging. Understanding ECM maturation is key to delay or halt fibrosis – that is yet impossible. Aebp1 is an ECM protein that is presumed to regulate collagen organization. AEBP1 gene mutations cause Ehlers-Danlos syndrome, characterized by skin hyper-elasticity. Although, exact function is unknown, we showed Aebp1 upregulation accompanying muscle differentiation and injury repair in relevant models. Proximity-based proteomics is a paradigm-shifter technology enables investigation of protein-protein interactions in their spatial organization within cellular context. To enlighten the function of Aebp1 in ECM, we aimed to utilize proximity proteomics to define its protein partners.

We constructed horseradish peroxidase (HRP) fusion vectors as biotin conjugating enzymes, and achieved stable, conditional expressing (TetON) myoblasts (C2C12) along with appropriate controls. Single cell cloning was performed by both limiting dilution and on semisolid methylcellulose. HRP positive clones were selected and verified via biotinylation by immunofluorscence and blotting. Biotinylation and streptavidin enrichment was performed in selected colonies. Following tryptic digestion, peptides were purified with C18stagetips and mass spectrometry was performed with maXisII ETD nLC/LC-QTOF. Raw data were analyzed using MaxQuant 2.4.0. software.

Aebp1-HRP proximity candidate list was generated and compared to controls. 66 unique proteins were identified in replicates where 16 are ECM proteins such as collagen modifying enzyme Lox, Htra1 serine peptidase and Igf binding Igfbp2. These preliminary results pinpoint Aebp1 as an ECM modifier that may not directly interact with collagens but a candidate regulator of collagen modifiers. Once verified, these results identify Aebp1 as a potential drug target to combat fibrosis.

S20
H3K36me2 in mESC Pluripotency Exit and Lineage Choice

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Aim: Gene expression changes during embryonic stem cell (ESC) differentiation is regulated by epigenetic mechanisms. Understanding these can help uncover how cell fate decisions are made during early embryonic development. Comparison of chromatin of ESCs with lineage-committed cells can implicate chromatin factors functional in exit from pluripotency and the choice of proper lineages. Therefore, we quantitatively analyzed histone modifications in mouse ESC differentiation towards neuroectoderm and endoderm.

Method: We cultured mouse ESCs (mESCs) and differentiated them towards neuroectoderm or endoderm lineages and performed mass spectrometry on total histones. Subsequent Western blots verified significantly altered H3K36me2. RT-qPCR analyses showed changes in H3K36-specific methyltransferases, demethylases, and readers at the mESC stage or during neuroectoderm/endoderm commitment. Knock-out studies revealed a list of H3K36-methylation-related genes critical for neuroectoderm differentiation.

Results: We present quantitative histone modification levels in mESCs and lineage-committed cells. H3K36me2 increased specifically in neuroectoderm compared to mESCs or endoderm-committed cells. Regulation of H3K36 methylation might have a role in pluripotency exit and/or differentiation. *Nsd2*, *Dnmt3b*, and *Zmynd11* increased during differentiation regardless of lineage. Conversely, mESCs had higher *Kdm4c* and *Msh6* expression than differentiated cells. Comparing neuroectoderm and endoderm-committed cells, we revealed that *Nsd1*, *Setd5*, and *Dnmt3a* had lineage-specific expression patterns. Among these, *Nsd1* and *Setd5* were found to be critical for neuroectoderm differentiation.

Conclusions: Our results show quantitative changes in histone modifications during mESC lineage commitment and implicate H3K36me2 regulation for not only pluripotency exit but also lineage choice. Its regulatory proteins show stage (mESC vs. committed) or lineage (neuroectoderm vs. endoderm) dependent expression changes. Further work will be needed to discover their possible involvement in cell fate decisions and target genes.

POSTER PRESENTATIONS ABSTRACTS

P01
The Use of TurboID for Cell Surface Proteome Analysis in Breast Cancer

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Aim: The extracellular matrix (ECM) and cell junction (CJ) proteins provide structural and functional support to the cells and play several crucial roles e.g., cell adhesion, cell migration, cell signaling and communication under various processes. Elucidation of changes in ECM and CJ proteomes is important since they play crucial roles in the development, progression, and metastasis of various cancers, including breast cancer (BC). Therefore, we used a novel approach to study these proteomes of three BC cell lines to discover candidate biomarkers. For this, ECM and CJ proteins were labelled with TurboID, enriched via streptavidin and identified with nLC-MS/MS.

Method: TurboID was produced in E. coli and purified. The active enzyme was added to the culture media to label ECM and CJ proteins of MCF-10A, MCF-7 and MDA-MB-231 cells. Cell-free extracts were prepared and used for enrichment of biotinylated proteins using streptavidin-coated beads. The enriched proteins were subjected to label-free quantification using nLC-MS/MS.

Findings: Western blot analysis using HRP-conjugated streptavidin demonstrated biotinylation in enriched fractions. Biotinylation of ECM and CJ proteins were demonstrated with western blot and immunflorescent analyses using Streptevidin-HRP and Streptavidin-TexasRED in all cell lines. A total of 73 significantly regulated CJ and ECM proteins were found among the cell lines.

Results: STRING and GO enrichment analyses; nitrogen metabolism, amide biosynthesis, adhesion organization, cell regulation and developmental processes were affected during the carcinogenesis. ECM and CJ proteins were successfully identified and their relative abundance levels were determined among the breast cancer cell lines. The attractiveness of this study came from the fact that the experimental approach that was used here was novel, easy to implement and reproducible to allow comparative proteome analysis. It simply involved addition of purified TurboID to the culture medium to label ECM and CJ proteins and enrichment of the labelled proteins for label-free quantification.

P02
Temporal Progression of Senescence In Mef Cells

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Increasing rates of age-related diseases have been plaguing the world's aging population. To tackle this, we need a better understanding of what causes the associated pathologies. Senescence is a process of cellular aging that involves cell cycle arrest. It has been linked to age-associated illnesses, and many underlying mechanisms are involved in the process. Senescence detection calls for the analysis of single cells and multiple mechanisms at once.

To address the central question of how we can measure cellular processes to understand the progression of senescence, I developed a mass cytometry-based method for monitoring the expression of senescence biomarkers at different stages of the process in mouse embryonic fibroblasts (MEFs). Mass cytometry, or cytometry by time-of-flight, involves the detection of heavy metal isotopes that are used as antibody tags. Senescence was induced with a genotoxic drug, and senescence protein biomarkers were labeled with lanthanide-tagged antibodies. Treated samples were dosed for 48 hours with Etoposide, followed by a 0-6 day incubation period. Similarly, control samples were dosed with DMSO for 48 hours and incubated for as long as the corresponding treated sample.

After correction for non-specific binding, bulk analysis showed that treated samples exhibited higher levels of p21 and GAPDH compared to corresponding controls; β-Gal showed the opposite trend. However, data analysis using mean absolute deviation (MAD) showed that cells with high levels of p21 also exhibited high levels of β-Gal. The next steps entail running the single-cell data through a trajectory-predicting algorithm Wanderlust.

Once complete, the method would enable us to infer which senescence-related mechanism changes at each stage of the process. This could allow for an extended health span and the design of better therapeutic methods.

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P03
Microtubule Plus End Tracking Protein p150(Glued) is Dysregulated in SMA

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Aim: Spinal muscular atrophy (SMA) is a rare neurodegenerative disease, caused by the absence of functional SMN protein. SMN loss results in some impairments of the cytoskeleton, including microtubules. We previously demostrated alterations in several microtubule-associated proteins (MAPs) and reduced microtubule stability in SMA.However, the contribution of MAPs to microtubule dysregulations is not fully understood. In this study, we created an interaction network of SMN with MAPs using bioinformatic tools,and analyzed microtubule plus end tracking p150Glued in vitro.

Method: The list of MAPs was retrieved from databases to construct the protein network. Direct and indirect interactions of SMN with MAPs with high confidence were collected and created as a network. p150Glued was visualized as one of the hub proteins and it's level was investigated by Western blot in SMN knock down motor neuron-like NSC34 cells. Quantitative image analysis was performed to evaluate p150Glued comet structures, subsequent to the immunofluorescence stainings and confocal imaging.

Results: Bioinformatic analysis revealed several candidate MAPs, having potential to functionally interact with SMN. p150Glued gained attention, since it interacts with 22 MAPs in the network. In SMN-depleted cells, we identified a significant upregulation in p150Glued,which form dash-like comet structures mostly at growing microtubule tips. Quantitative microscopic analysis showed no change in comet sizes;however, comet numbers were significantly increased at both proximal and distal parts of the neurites in SMN knock down cells.

Conclusion: Our findings indicate that levels and comet numbers of p150Glued was altered in SMN deficiency. p150Glued dysregulations could affect both microtubule dynamics and retrograde transport, since it is the major subunit of dynactin. Studies are ongoing to understand the effects of SMN reduction on microtubule dysregulations in SMA. This study is supported by The Scientific and Technological Research Council of Turkey (TÜBİTAK,Project number; 121S884)

P04
Proteomic Analysis of Exosomes Produced by Endothelial Cells in Culture

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Aim: Atherosclerosis is a progressive vascular disease; inflammation plays an essential role in its mechanism. Phenotypic changes occur in endothelial cells (EC) and Vascular Smooth Muscle Cells (VSMC), the primary cells of the vascular wall. Exosomes, vesicles released from cells, play an important role in intercellular communication. In this study, we focus on the changes in the protein content of exosomes released from EC in culture. We use two agents, TNF-α and lipopolysaccharide (LPS), as molecules mimicking the low-level inflammation during atherosclerosis.We aim to compare the proteomic content of exosomes in response to inflammatory stimuli with controls to observe the dose-dependent effect of exosomes on the recipient cells.

Methods: We treated EC (Human umbilical vein endothelial cells used) with TNF-α (10 ng/ml) or LPS (50 ng/ml) for 72 hours using these agents as a low-level inflammation model, while non-treated cells served as controls. The exosomes from cell culture supernatants are isolated by ultracentrifugation and characterized by Western blotting and nanoparticle tracking analysis (NTA). The exosomes of the two treated and control groups (75 ug protein) are further subjected to trypsin digest and analyzed in LC-MS/MS. The raw data are processed with MaxQuant and Perseus software.

Results: The yield of exosomes from EC treated with TNF-α or LPS (expressed as exosomes per cell number) by NTA measurement is comparable. Both treatments result in exosomes with identical marker content (CD63 and CD81) by Western blotting. We identified approx. 150 proteins from each sample. Gene ontology analysis revealed that exosome proteins involve different cellular components and biological processes.

Conclusion: The analyses have resulted in a general profiling of the proteins in the EC-derived exosomes in cells treated with TNF-α or LPS. A comprehensive analysis of the proteomics results may reveal the potential regulatory functions of the exosomes in EC-VSMC communication.

P05
Revealing Dynamic Proximity Interaction Profiling of YPEL2

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Aim: YPEL2, a member of the evolutionarily conserved YPEL family, has been implicated in various cellular processes such as proliferation, mobility, differentiation, senescence, and apoptosis. However, the underlying mechanism through which YPEL2, as well as YPEL proteins in general, exert their effects remains elusive. Since proteins function within the context of a dynamically changing network of interacting protein partners, this study aims to shed light on the functional role of YPEL2 via uncovering potential interaction partners of YPEL2.

Methods: To elucidate the functional role of YPEL2 by identifying its dynamic putative interaction partners, we employed dynamic TurboID-coupled mass spectrometry analyses. By inducing transgene expression in COS7 cells, we investigated the dynamic proximity interaction partners of YPEL2 in a time-dependent manner. This approach allowed us to infer the potential functions of YPEL2 through the identification of its interacting proteins.

Results: Our findings from inducible transgene expression experiments in COS7 cells, wherein YPEL2 is synthesized, revealed that the proximity interaction partners of YPEL2 are primarily involved in key RNA metabolic processes and ribonucleoprotein complex biogenesis, particularly in cellular stress responses. Notably, we observed an interaction between YPEL2 and the RNA binding protein ELAVL1, which is located in stress granules, as well as the selective autophagy receptor SQSTM1. Furthermore, our study demonstrated YPEL2's involvement in events associated with the formation and disassembly of stress granules in response to oxidative stress.

Conclusion: We identified interaction partners associated with essential RNA-related processes, stress response mechanisms, and autophagy. The interaction with RNA binding protein ELAVL1 and the involvement in stress granule dynamics suggest that YPEL2 may play a role in stress surveillance mechanisms. By unraveling these interaction networks and shedding light on YPEL2's potential functions, our work establishes a starting point for further investigations into the structural and functional features of YPEL2.

P06
Role of TRIM33 in CXXC5 Mediated Gene Expression

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Aim: CXXC5 protein, as a member of the zinc-finger CXXC family, is regulated through estrogen (E2) signaling. Deregulated CXXC5 expression may contribute to the initiation/progression of E2-target tissue malignancies. Although CXXC5 participates in many processes from cell proliferation to death, the functional features and mechanisms of action of CXXC5 are unclear. Our studies identified CXXC5 as an unmethylated CpG dinucleotide binding protein that lacks intrinsic transcription regulatory properties but is capable of regulating gene expressions. To address how CXXC5 mediates its effects, we utilized BioID-coupled mass spectrometry to explore proximity interaction partners of CXXC5. We found that CXXC5 modulate gene expressions as a nucleation factor through functional association with various nuclear proteins, including E3 ligases and/or transcription co-regulators TRIM25 and TRIM33. We aimed here to assess the role of TRIM33 and TRIM25 in CXXC5 mediated gene expressions.

Methods: To identify proximity interaction partners of CXXC5, we employed BioID-coupled mass spectrometry. We investigated the interaction between TRIM25 and TRIM33 by Co-ICC, Co-IP and PLA approaches. For elucidating the ubiquitination of CXXC5, we used protein pull-down approaches and investigated the involvement of TRIM25 and TRIM33 proteins in this process using siRNA knockdown experiments. To investigate the regulatory roles of them, we utilized mRNA-sequencing approach to find the common target genes.

Results: Our findings revealed that CXXC5 interacts with TRIM33 but not with TRIM25. Although CXXC5 is ubiquitinated, TRIM33 does not play role in CXXC5 ubiquitination. We then asked whether interaction between CXXC5 and TRIM33 is critical for CXXC5 target gene expression using mRNA-sequencing approach. Results implied that CXXC5 and TRIM33 regulate a set of gene expressions interdependently.

Conclusion: Our study suggests that functional interaction between CXXC5 and TRIM33 could be critical for the target gene expression independently of the ubiquitination processes.

P07
Investigation of the Regulatory Role of Palmitoylation During Cell Division

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Koc Unıversıty

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 prilimenery 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.

P08
Investigation of ROCK Inhibitor on the Senescent-Mesenchymal Stem Cell Secretome

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Aim: Mesenchymal Stem Cells (MSCs) enter senescence following stress or as they lose their regenerative potential with age. Senescent cells exhibit a senescence-associated secretory profile (SASP) linked to age-related pathologies. Concurrently, senescent cells have a deleterious effect on their tissue microenvironment through the SASP. Eliminating the senescent cells or modifying the composition of the SASP could be a novel therapeutic strategy. Previous studies showed that the Rho-associated protein kinase (ROCK) inhibitor reduces the secretion of pro-inflammatory cytokines like IL-1β and IL-6, which are commonly associated with cancer development, without affecting permanent cell growth arrest. In this study, we aimed to investigate the impact of ROCK inhibitor on the secretome of stress-induced senescent cells.

Method: It was hypothesized that the inhibition of ROCK could modulate the secretome of senescent cells and mitigate the negative effects of complications caused by these cells. MSCs were induced into senescence by incubating them with 300 mM H2O2 for 30 minutes, and the cells were allowed to recover for 48 hours. Then, the cells were cultured in the presence and absence of 10 µM Y-27632 (ROCK inhibitor) for an additional 48 hours. Subsequently, the secretome of control (s-hMSC) and inhibitor-treated (s-hMSC-Y27632) senescent cells were collected using StrataClean beads. After on-bead digestion, LC-MS/MS analysis was conducted using AB-SCIEX 5600+ mass spectrometry.

Results: When we compared the secretome of the control and Y-27632 treated senescent cells, as expected in the control group, we observed enrichment in senescence-associated pathways like negative regulation of cellular catabolic processes, positive regulation of endopeptidase activity, and mRNA stabilization. In the ROCK inhibitor-treated group, positive regulation of macromolecule metabolic processes, gene expression, and regulation of cell mobility were enriched.

Conclusion: This preliminary finding, upon further validation, could denote a senomorphic role of ROCK inhibitor on senescent cells.

P09
Optimization of Expression and Purification of Eml1 Protein

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The EML1 protein, part of the EML (echinoderm microtubule-associated protein-like) family, is involved in forming the mitotic spindle and microtubule network during cell division. Its crystal structure, resolved at 2.6 Å, consists of two β-propeller domains connected by a hydrophobic core. The HELP motif, which aids in protein-protein interactions, is integrated into the hydrophobic core. The HELP motif in EML1 is a hydrophobic region that is located between the two beta propellers in the protein. It is crucial for the microtubule binding. The T243A mutation is a specific genetic mutation within the EML1 gene. The T243A mutation impacts the protein's ability to interact with microtubules and is associated with the disease called ribbon-like heterotopia. The reasons behind the impaired interaction between the mutant protein and microtubules remain unknown. Studying the structural biology of EML1 is instrumental in unraveling its intricate relationship with microtubules.

In this study, we optimized the expression and purification of the EML1 protein in bacteria to resolve the structure of the EML1 T243A mutant protein by X-ray crystallography. Different lysis buffers and bacterial strains were tested for their suitability in protein expression and purification. The bacterial strains utilized for optimization included Bl21 Star, Bl21 Rosetta-gami, Bl21 OverExpress c43, and Bl21 Rosetta 2. The optimal condition for the EML1 T243A mutant protein purification will be presented.

Resolving the structural biology of EML1 will provide important insight into EML1-microtubule interaction and its association with heterotopia. This knowledge can advance our understanding of cellular processes, disease mechanisms, and potential therapeutic strategies related to microtubule-associated disorders.

P10
Cell Cycle Resolved DUB Activitome Profiling in MCF7 Cells

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Aim: Deubiquitinating enzymes (DUBs) are proteases that can remove ubiquitin tags from substrate proteins and, therefore involved in various cellular functions by regulating the stability, activity, and localization of their substrates. The DUB family consists of 105 members in the human genome and is frequently de-regulated in various cancers including breast cancer. Despite the importance of DUBs in cellular proteostasis, there has not been a high-throughput effort to analyze the activity of DUBs in a cell cycle-resolved manner. To investigate the DUB activitomes in cell cycle phases, we used the MCF7 breast cancer cell line as a model to conduct a chemoproteomics approach by using DUB-specific activity-based probes (ABPs) combined with proteomics.

Method: MCF7 cells synchronized to enrich specific cell cycle phases (G1, S, G2, and M) using pharmacological methods. Synchronized cell populations were first analyzed using flow cytometry and Western Blotting to confirm enrichment. Consequently, a chemically synthesized ABP that can form a covalent linkage with the active site of DUBs is used to capture the active DUBs in cell lysates. DUB-ABP complexes precipitated using a neutravidin-based pull-down assay and processed for proteomics analysis (KUPAM, Koc University).

Results: Flow cytometry and Western Blot analysis showed successful synchronization of cell cycle phases in MCF7 cells. The specificity of pull-down assays capturing DUB-ABP complexes was confirmed using a-biotin antibody immunoblotting. Finally, analysis of proteomics results has identified 5 DUBs that show mitosis-specific activity in the MCF7 cell population.

Conclusion: Here, we successfully synchronized MCF7 cell populations and performed DUB activitome analysis using a chemoproteomics approach. Our preliminary findings have shown that we can identify cell cycle phase-specific DUB activities. We are aiming to confirm our findings using cell biology methods by focusing on individual DUBs.

P11
Pro-Inflammatory Response of Schwann Cells in Metastatic Breast Cancer

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Aim: Schwann cells are glial cells of peripheral nervous system, which are responsible from protection, and repair of axons. They are also activated by factors released from cancer cells and may enhance cancer growth and metastasis. There are however, limited information regarding the role of Schwann cells in cancer metastasis. Up to our knowledge, there is no study examining the interactions between Schwann cells and metastatic breast carcinoma cells directly. Minocycline, a tetracycline antibiotic, inhibits glial cell activation. We aimed to determine the effects of factors secreted by different metastatic cells such as 4TBM and 4TLM on Schwann cell activation.

Method: We previously characterized 4T1 breast carcinoma cells that are metastasized to brain (4TBM) and liver (4TLM) and demonstrated that these cells have both common and differential features (1,2,3). Conditioned mediums from metastatic and non-metastatic 67NR breast cancer cells were used to activate primary Schwann cells. Schwann cells were prepared from sciatic nerves of Balb-c mice. CCL2 (MCP-1), a chemokine and the cytokine IL-6 enhance the aggressiveness and metastatic potential of breast cancer. CM from 4TBM and 4TLM cells but not from 67NR cells markedly enhanced IL-6 secretion from Schwann cells. Liver metastatic subset of 4T1 breast carcinoma (4TLM) cells also markedly enhanced CCL-2 secretion from Schwann cells.

Results: Minocycline pre-treatment markedly inhibited increases in IL-6 and CCL-2 secretion. IL-17 is another cytokine involved in inflammation and anti-tumoral immunity. Schwann cells under culture secreted high levels of IL-17 that was not altered further.

Conclusion: These results demonstrated that factors derived from metastatic breast carcinoma cells activate Schwann cells enhancing secretion of inflammatory cytokines/chemokines. Minocycline can partly prevent Schwann cell activation due to factors secreted by cancer cells. Hence, prevention of Schwann cell activation within tumor microenvironment might be an effective approach to decrease aggressiveness and metastatic potential of breast cancer.

P12
BMP-1 Inhibition and Its Effect on Metastatic Breast Carcinoma

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Aim: Triple negative breast cancer (TNBC) is the most malignant subtype of breast cancer in which drug resistance is common. Transforming growth factor-β (TGF-β) by inducing epithelial and mesenchymal transition (EMT) and cancer stem cell population leads to multidrug resistance. Bone morphogenetic protein-1 (BMP-1) is a metalloprotease involved in release of TGF-β. Heterogeneous group of cells form carcinomas and only a small percentage can form distant metastasis. Cells that metastasize to distant organs are treatment resistant, hence new treatments should be explored.

Method: We previously observed that level of BMP-1 in exosomes of metastatic murine breast cancer cells is higher compared to non-metastatic cells (1). We here used subset of 4T1 cells that were metastasized to liver and the brain (4TLM and 4TBM respectively). These cells are highly aggressive and have both common and distinct phenotypical features as reported before (2). We found that inhibition of BMP-1 activity decreases proliferation of 4TLM and 4TBM cells 72 hours after treatment. We wanted to evaluate the chronic effects of BMP-1 inhibitor on metastatic potential of breast carcinoma cells.We used a BMP-1 selective inhibitor called UK383367. The 4TBM and 4TLM cells were treated with various concentrations of the inhibitor for 6-8 weeks and changes in vimentin and TWIST-1 levels were determined using western blot. Vimetin and TWIST-1 are considered as markers and mediators of EMT. Since BMP-1 is involved in extracellular matrix deposition,changes in extracellular matrix proteins were also determined.

Results: Chronic treatment with BMP-1 inhibitor markedly suppressed vimentin and TWIST-1 expression in both cell lines. We,however, didn't observe any changes in secretion of extracellular proteins. Specifically, levels of trombospondin-1, collagen VA1 and laminin beta-1 wasn't changed following long-term treatment with BMP-1 inhibitor.

Conclusion: To conclude, inhibition of BMP-1 activity may revert EMT phenotype and enhance chemosensitivity of the metastatic breast carcinoma.

P13
Direct Determination of Surface Proteins in Leishmania

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Leishmaniasis is a neglected tropical disease caused by leishmania parasite. Due to immigration to our country, effective population of the disease has increased recently. The lesion, which has visceral and cutaneous forms, can be lethal when it acts on internal organs. Surface proteins are crucial part of the parasite and host interaction. The parasite attaches to the host cell through surface proteins, enters the cell, suppresses the immune system, allows many other biological functions. It is the most critical research part in biomarker discovery. Generally, cell surface biotinylation and cationic colloidal silica beads with which the surface is coated are used to analyze surface proteins. These methods break down the cell therefore contain contaminating proteins from cytosolic proteins. In addition, high hydrophobicity of plasma membrane proteins reduces their solubility in water, while their embeddedness in the membrane makes it difficult to identify. In this study, instead of the commonly used cell surface analysis methods, a faster and less experimental workflow, aiming to cut plasma membrane proteins without breaking the cell, was tried in Leishmania species *(L. Tropica, L. Infantum, L. Major, L. Donovani)*.

This method digest surface proteins by treating the cell surface with a proteolytic enzyme for a short time. Thus, it is expected to contain fewer contaminants and unwanted proteins. As a result of this method analysis with the Fusion Orbitrap Mass Spectrometer, the rate of surface protein defined in4 different species was 9. 34% in *L. Tropica*, 7. 55% in *L. Major*, 7. 9% in *L. Infantum*, 7. 52% in *L. Donovani*. Consistent with the literature and candidates for biomarker ISCL, KMP-11, Leishmanolysin, PSA-2, ABC transporter, and lanosterol 14α demethylase, proteins were identified. With this method, plasma membrane proteins were identified more easily and quickly. Improvement of the method in future studies may increase the number of plasma membrane proteins identified in Leishmania.

P14
Dynamic Comparison of Histphilus Somni Outer Memrane Vesicles (OMVs)

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Aim: Histophilus somni is a clinicaly important gram-negative pathogen that causes thromboembolic meningoencephalitis, pneumonia, polyarthritis and acute myocarditis in cattle, as well as infections of the urogenital tract and abortions and fetal septicemia in pregnant animals. Outer membrane vesicles (OMVs) are non-replicating and highly immunogenic spherical nanoparticles derived by Gram-negative bacteria. These particles effectively transmit virulence factors to the host, increasing pathogenesis and activating antigen-presenting host cells. In this project, the immunogenic and protective potentials of OMVs is investigated with the proteomic and RNA sequenscing analyzes.

Methods: H. somni standart strain ATCC 43625 have been cultured in 5% sheep blood agar for 24 hours twice and transferred to Brain heart Infusion Broth supplemented with 0.1% tris-base, and 0.001% thiamine hydrochloride. Liquid culture samples are collected at the different stages of logaritmic growth phase and cells were pelleted. Acquired supernatant is subjected to series of ultracentrifugation to isolate OMVs. Concentration and size of OMVs analyzed with Nanoparticle Tracking analyses(NTA) and sample concentrations are equalized. Proteins are purified from whole OMV samples and identified by Liquid Chromatography-Tandem Mass Spectrometry. Similarly RNAs are isolated from whole OMVs with affinity column based purification method and single-read sequencing is conducted to acquire small RNA libraries.

Results: It is found that OMVs are packed with many of the known surface antigens as well as structural proteins and porins with varying expression levels. similarly the unique small RNA content that are found in OMVs differs in terms of relative expression through different stages of logaritmic growth.

Conclusion: With this dynamic comparison study it is concluded that the OMV contents show high similarity to the antigenic surface of the pathogen. However content of OMVs changes during the logaritmic growth of the bacteria in terms of relative abundance. Therefore it is important to consider the sample collection time.

P15
Evaluation Of Biocompatible Microextractive Probes For Metabolomic Studies

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Aim: Solid phase microextraction (SPME) is a versatile sample preparation and sampling technique that has applicability for in-vivo, in-vitro, and on-site applications. A universal SPME sampler that is suitable for both solvent and thermal desorption, being capable of extracting polar and nonpolar analytes, volatiles and non-volatiles is in high demand; however, such device has not been developed yet. This study aims to develop and optimize SPME fibers that meet the abovementioned requirements for an ideal SPME sampler.

Method: Hydrophilic lipophilic balanced (HLB) polymer embedded in amorphous fluoropolymer PTFE/AF 2400 was used as a SPME sampler. The evaluation of HLB/PTFE probes for extraction of non-volatile analytes was performed in direct immersion mode followed by LC-MS analysis while for volatile analytes was performed by placing the probe in the head space of sample followed by GC-MS analysis. For these evaluations, volatile and non-volatile analytes reported as clinically important markers or metabolites in various cell lines studies were used.

Results: SPME-LC-MS results showed that 60 min of extraction, 120 min of desorption with ACN/MeOH/H2O (40:40:20, v:v:v) provided the optimum conditions for the study of non-polar analytes with limit of quantitation (LOQ) ranging between 150 and 500 ng/mL. In case of SPME-GC-MS, the optimum conditions were found as 10 min of extraction and 5 min of desorption which provided 0.5 ng/mL LOQ for all tested analytes.

Conclusion: The developed probe demonstrated good compatibility with solvent (LC-MS) and thermal (GC-MS) desorption. HLB enabled extraction of a wide range of analytes, while PTFE/AF provided sufficient stability for solvent and thermal desorption.

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P16
Alteration of Serum N-Glycome in Patients with Knee Osteoarthritis

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Aim: Osteoarthritis (OA) is the most prevalent type of arthritis in the world, and its prevalence rises with age. It usually involves the knee joint, called knee OA (KOA). Early detection of this condition, which results in socioeconomic losses, is crucial. In this study, we aimed to determine the serum N-glycan profiles of KOA patients using the MALDI-TOF-MS method and to observe the change in N-glycans by comparing them with the healthy control group.

Material and Methods: Forty-eight patients diagnosed with KOA and 57 healthy individuals with similar demographic characteristics were included in our study.We performed a methodology involving linkage-specific ethyl-esterification of released N-glycans. Released N-glycans were purified with a HILIC-based approach and analyzed by MALDI-TOF-MS. A total of 63 N-glycans and 67 specific traits were statistically investigated for serum N-glycans that had been released during enzymatic digestion.

Results: The statistical analysis revealed significant differences between patients and controls, including 24 N-glycan compositions ($p<0.05$). Ten of them were discovered to be up-regulated in patients, while 14 N-glycans were discovered to be down-regulated. 33 distinct N-glycan traits were also found to differ significantly.

Conclusion: The results suggest that serum N-glycan levels will guide new strategies to be developed for the diagnosis and treatment of KOA.

P17
PI3K/Akt Pathway Regulates NPM-1 Phosphorylation levels

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Aim: Activation of Class I PI3 kinases(PI3K) through growth factors is a key event in cell survival and proliferation. Class I PI3Ks have four isoforms, and only two (p110α and p110β) are expressed ubiquitously in all tissues. Although p110α and p110β are redundant to each other for many cellular functions, previous studies have shown that different physiological contexts can yield different dominant PI3K isoforms. For example, PTEN-null cancers depend on the p110β isoform, while cancers with activating mutations of p110 α depend only on p110α. Therefore, to intervene in isoform-specific PI3K activity occurring during carcinogenesis, it is crucial to determine isoform-specific effectors of PI3K. In this study, we aimed to uncover the isoform-specific phosphorylation alterations occurring downstream of p110α activity.

Methods: We developed a genetically modified p110α-dependent mouse embryonic fibroblast(MEF) line. We starved and induced our p110α-dependent transgenic line with PDGF. We identified differential phosphorylation responses through mass spectroscopy-based phosphoproteomics after this stimulation. At the opposite end, we treated the p110α-dependent MEF line with the isoform-specific PI3K inhibitor(BYL-719) and again carried out phosphoproteomics to validate the specificity of phosphopeptides determined after stimulation.

Results: Amongst many other candidates, the S125 phosphosite of Nucleophosmine 1(NPM1) was found to be a target of p110α. Then, we treated p110αH1047R MEFs-bearing an activating mutation of p110α- with BYL-719 and observed a reduction in the phosphorylation levels of NPM1. Furthermore, a comparable reduction at the phosphorylation levels of NPM-1 was observed when p110α activating mutation-carrying breast cancer cell lines -T47D and MCF7- were treated with BYL-719.

Conclusion: In summary, NPM1 is a promising downstream target for PI3K through its known/unknown effectors.

P18
Phosphoproteome Analysis of Parkin Expressing SH-SY5Y Cells by 2-D Electrophoresis

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Background/aim: A 52 kD of Parkin protein regulates breakdown of protein via E3 ubiquitin ligase activity. In recent years, Parkin protein is placed at the center of scientific research due to the involvement in early-onset Parkinson's disease and its tumor suppressor activity in cancer. However, the detailed mechanism associated with how Parkin as a tumor suppressor protein, plays a role in the development or progress of both Parkinson's disease and cancers remain a black whole in scientific world. In this study, an effort was placed on changes in phosphorylation levels in total protein extracts upon Parkin expressions were determined using ProQ-Diamond protein stain.

Methods: The changes in protein phosphorylation following the Parkin expression in neuroblastoma cells have been studied by using conventional two-dimensional (2DE) gel electrophoresis. Pro-Q Diamond Phosphoprotein Stain was used to visualizing phosphoproteins pattern on the gels while SYPRO Ruby Stain was performed to visualize total protein on the same gel for the sake of ascertain the relative phosphorylation state of proteins. The samples were represented by three independent biological replicates of 2DE gels. In the further step MALDI-TOF/TOF analysis was performed by identification of the proteins in 2DE gels. The identified proteins were subjected to bioinformatics analysis to elucidate the reactomes and relevant pathways.

Results: In this study, ProQ diamond staining showed the presence of 39 phosphoprotein spots. By comparing the spot intensities using the 2-fold regulation criteria, the proteins with an altered level of phosphorylation were detected. Four proteins were observed tendency of increase in phosphorylation levels following the Parkin expression.

Conclusion: A list of differentially regulated phosphoproteins that were not previously known to interact or associate with Parkin was determined. These findings will shed some light onto phosphoproteome in response to the Parkin expression.

P19
Characterization of Nucleosome Dynamic Recognition by Select Chromatin Factors

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Aim: The eukaryotic genome's intricate organization is orchestrated by chromatin, an intricate amalgamation of proteins and nucleic acids. At the heart of this chromatin structure lies the nucleosome core particle – a minute unit composed of four histones and around 145 base pairs of DNA. While historically viewed as a restrictive entity, the nucleosome is far from inert; it acts as a central regulatory nexus, interacting dynamically with transcription factors, chromatin remodelers, and other regulatory apparatuses. A feature in this paradigm is the arginine anchor motif, frequently employed to tether the nucleosomal domain referred to as the acidic patch. In our study, we embarked on an exploration that harnessed molecular modeling and high-performance computing prowess and nucleosome recognition exhibited by five distinct chromatin factors, each endowed with diverse functional roles yet analogous chromatin binding strategies.

Method: Our methodology entailed procuring atomic coordinates from the Protein Data Bank (PDB) for the quintet of nucleosome-associated chromatin factors: 1-53BP1, 2-SAGA DUB, 3-PRC1, 4-RCC1, and 5-Sir3. The ensuing structural entities were enveloped in cubic water boxes, wherein a structures was simulated with NaCl and Mg ions. The chemical landscape was described by the CHARMM36m force field coupled with the OPC water model. Each complex underwent a 100 ns trajectory in GROMACS, with subsequent analysis employing custom Python scripts, PyMOL, and VMD.

Results: The outcome is a revelation of diverse binding modes employed by the five chromatin factors on the nucleosome core particle's two facets. Notably, these disparities are interlinked with the local stabilities of the chromatin factors, predominantly influenced by flexible loop domains.

Conclusion: In summation, our endeavor employed an in silico strategy, seamlessly intertwined with meticulous computational analyses. The fruits of our labor unveil substantial strides in biophysical understanding, spotlighting the intricate choreography of chromatin-binding proteins in concert with the nucleosome core particle.

P20
Physiological and Proteomic Insight into Vanillin-Probiotics Interactions

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Aim: Probiotic bacteria are microorganisms that benefit the host when taken in adequate amounts and are found in colonies in the intestines. Lacticaseibacillus rhamnosus GG (LGG) is one of the most studied probiotic bacteria that supports the intestinal flora and contributes to the immune system. Vanillin is a phenolic compound that contributes significantly to human health with its beneficial properties such as antioxidant, antitumor, and antimicrobial, as well as being used as a flavoring agent in the food industry. Probiotics and vanillin are found together in the gastrointestinal tract and form a synbiotic interaction. Vanillin may affect the probiotic properties of probiotic bacteria. Thus, this study aimed to investigate the effects of vanillin on physiological properties, as well as exoproteome of LGG.

Methods: Effects of vanillin on probiotic properties such as auto-aggregation, co-aggregation, and antioxidant capacity were investigated. Furthermore, the exoprotome of LGG grown with vanillin was separated by 2-dimensional gel electrophoresis. The resulting gel images were stained and the gel images analyzed. Then, protein spots that differed compared to the control group were detected using SameSpots 2D gel analysis software.

Results: Vanillin positively modulated auto-aggregation and co-aggregation properties of LGG. Furthermore, antioxidant capacity of LGG was increased when the probiotic bacteria were grown in the presence of vanillin. Then, vanillin-treated LGG and controls were analyzed by comparative proteomics. Growth at the presence of vanillin altered relative abundancy of 1 protein spot in the exoproteome of LGG. Spot showed 1.6-fold decreased relative abundance compared to the control.

Conclusion: In conclusion, these results suggest that the interaction of LGG with vanillin may show more probiotic properties.

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P21
Quantification of HR-MS Annotated MRJP Proteotypes Utilizing Native-SEC-UV Methodology

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Aim: Royal jelly (RJ) is a nutritious substance secreted from the hypopharyngeal and mandibular glands of bees. Health-promoting properties of RJ are due to its polymorphism proteins named MRJPs. Within this study, it was aimed to develop an accurate method for absolute MRJP quantification. Furthermore, a novel purification workflow for each MRJP was also purposed.

Method: The supernatant of RJ proteome extract was loaded to CFT mix-mode resin. Eluted fractions were transferred to SEC for polishing step. Tandemly purified MRJPs were analyzed at SDS-PAGE and HR-MS. For MS analysis, after bottom-up preparation implementing FASP, MRJP digests were subjected to UHPLC fractionation and Orbitrap Exploris 240-MS annotation conducting FS-ddMS2 acquisition. Data was processed at Proteome Discoverer and sequences were retrieved from UniProtKB. Pure and annotated MRJPs were used as references for the native mode LC-SEC-UV quantification. For this, MRJPs were extracted and Thyroglobulin was spiked for normalization. UHPLC-UV system was operated under 0.7 mL/min PBS isocratic flow and Premier Xbridge SEC column was used for chromatographic resolution.

Results: Molecular interactions provided by CFT and combining SEC polishing enabled us to separate each MRJP into single fraction. Utilizing SDS-PAGE and subsequently HRMS analysis confirmed that Apisin is characterized at between 290-320 kDa (mainly 350 kDa homo-pentamer of MRJP-1), meanwhile, MRJP-3, MRJP-2, and MRJP-1 were identified at between 60-70, 49-51, and, 50-55 kDa respectively. According to LC-SEC-UV analysis, storage conditions, and packaging dramatically affects the protein profiles. Apisin results laid between 1.2 and 148 mg/g. MRJP-2, MRJP-1, and MRJP-3 concentrations were between 1.1-59.5, 0.6-48.6, and 0.4-37.9 mg/g respectively. Apisin was determined as the most fragile variant and it was found that lyophilization induces MRJP3 fragmentation.

Conclusion: These results proved that analyzing RJ products using LC-SEC-UV method and adapting methodology into the MAM workflows can reliably indicate the quality of the final product.

P22
Targeted Micro-proteomic Workflow for Label-Free Quantification of Honey Defensine-1

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Aim: Honey is progressively valued thanks to its pronounced potent biological properties including antibacterial, antibiofilm, as well as wound healing activity. However, the underlying mechanism and compounds responsible for the strong antibacterial activity of honeys have not been fully elucidated. Well-characterized major components; hydrogen peroxide, and cationic antibacterial peptide honeybee defensin-1 were identified as antibacterial substances responsible for the antibacterial activity. In this study, we aimed to evaluate the content of defensin-1 in honeys of different botanical origins (pine, blossom, citrus, chestnut honey, and manuka and Revamil as medical honey) with the developed bottom-up targeted proteomic approach.

Method: Honey proteins were isolated by TCA precipitation, subjected to tryptic digest, and analyzed using the micro-proteomic LC-MRM-MS method. The honeybee defensin-1 sequence was retrieved from the UniProt and imported to Skyline to identify convenient MRM signature peptide transitions. *In silico* digestion yielded six peptides representing Defensine-1. These were used to acquire MRM data from pooled samples of all the protein extracts. The results were used to optimize the transitions and scheduled retention times. Three of unique peptides were selected for label-free quantification that yielded intense peaks and was fully tryptic, with no variable modifications or missed cleavages.

Results: This was the first targeted proteomic approach in literature for absolute defensine-1 quantification in honey and results demonstrated that pine honey contains high levels of bee defensin-1. It was found that concentrations ranged between 1.8 to 8.0 ug/g pine honey and its levels increased in correlation with the HDE content. Whereas for other botanical origins, defensin-1 levels were relatively lower with the range of 0.2 – 4.5 ug/g honey.

Conclusion: Our findings revealed that pine honey may be the potential for being medical-grade honey with high levels of antimicrobial peptide ingredients and further research is needed to investigate the therapeutic effects of bee defensin-1.

P23
Design,Solid Phase Peptide Synthesis, and Characterization of Antimicrobial Peptides

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Aim: Antimicrobial peptides (AMPs) have shown a crucial role in the innate immune system as a host defence mechanism against devastating pathogens such as bacteria, parasites, fungi, and viruses. Most AMPs consisting of short segments of positively charged amino acids are cationic and amphiphilic. The design and synthesis of antimicrobial peptides are intriguing subjects in the field of life sciences. The main application areas of AMPs are drug delivery systems, biomaterials, pharmaceuticals, and vaccines. Solid phase peptide synthesis (SPPS) is a practical and robust method, developed for the synthesis of peptides. In this synthesis method, an insoluble polymeric resin is used as a solid support to grow peptides on it. For the validation of peptide synthesis, mass spectrometry, and chromatography methods are mainly used in the characterization of antimicrobial peptides.

Method: 2C- PW- KC (CCKPLKQYWWRPCI) peptide was synthesized by microwave peptide synthesis system CEM Liberty Blue 2.0 using Fmoc solid-phase method. The peptide chain was elongated in consecutive cycles of deprotection and coupling. After completing synthesis, peptides were cleaved from the resin, and the protecting groups were removed in a one-step procedure using cleavage cocktails.

Result: The crude peptide was purified by preparative reverse-phase high-performance liquid chromatography (PREP-RP-HPLC). The peptide was then analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), and the measured molecular weight was consistent with the theoretical molecular weight, which proved that the designed peptides were successfully synthesized and characterized.

Conclusion: Preliminary data showed that the novel 2C-PW-KC peptide was successfully synthesized, and its structure was confirmed by MALDI-MS. Its antimicrobial activity will be evaluated in further studies. After the activity evaluation, small amino acid variations will also be applied on the peptide structure to optimize and improve antimicrobial activity.

P24
Profiling Metabolites in Regional Monofloral Turkish Honeys Using GC-MS

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Aim: Bees are vital for maintaining the ecosystem and equilibrium of nature. In addition, they provide honey as essential food source for humans, besides, royal jelly, propolis, pollen, etc. Honey contains a variety of sugars like fructose and glucose, as well as, proteins, amino acids, vitamins, and other essential nutrients. Despite numerous benefits of honey, there are limited studies in the literature about the molecular composition of honey. Furthermore, similar studies on Turkish Honeys are also very limited and less qualified, and research usually focuses on only flavonoids and volatile compounds. Several studies focus on the analysis of honey metabolites based on the origin of flowers , while others involve the analysis of observed metabolomic differences depending on whether the honey is mature or immature. However, a comprehensive study on the metabololite profile of regional monofloral honeys of Turkey has not been literally conducted yet. The aim of the study is profiling maximum number of metabolites in regional monofloral honeys of Turkey. Furthermore, the systematic categorization will be accomplished through the execution of bioinformatic analysis of the collected data.

Method: After extraction of metabolites from the samples, GC-MS analyzes were conducted and the data were scanned with the Fiehn Metabolomics Library. Bioinformatic studies were also performed using statistical approaches.

Results: Monofloral Honeys were collected from 9 different regions of Turkey and their metabolite profiles were acquired by GC-MS. Significant differences were observed for different regional honey samples in terms of metabolite types and number of identified metabolites. Then, the obtained data were categorized according to honey type and regional differences.

Conclusion: Molecular compositions of Turkish regional monofloral honeys were successfully elucidated by GC-MS. And classification studies were carried out according to molecular compositions by taking into account regions, monofloral origins, geographical conditions and climate.

P25
Platelet Proteome Alterations in Intensive Case Unit Patients with COVID-19

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The underlying mechanisms and molecular pathways in response to COVID-19 infection are yet to be discovered. Consistent alterations in platelet related clinical parameters in COVID-19 positive patients suggest their involvement in disease mechanisms and their potential to give insights about disease severity and mortality. Here, we performed a shotgun label-free proteomics-based approach for the investigation of protein alterations in platelet samples from COVID-19 patients in intensive care units to investigate platelet proteome response. LC-MS/MS analysis revealed that 235 proteins were differentially expressed ($p < 0.05$, fold change ≥ 1.5) between control (n=8) and Covid-19-positive (n=6) groups. Among them, 162 proteins were upregulated, and 73 proteins were downregulated in COVID-19 group. Moreover, principal component analysis showed a clear separation between COVID-19 and control groups. Bioinformatics analysis suggested that the most important pathways were 'platelet degranulation', 'platelet activation', 'signaling and aggregation', 'integrin signaling', 'immune system', neutrophil degranulation', and 'signaling by interleukins'. Some of these pathways have been previously shown to have a strong impact in the disease pathology. Thus, we conclude that mass-spectrometry is a useful tool for COVID-19 research to provide valuable information about disease mechanisms and the results supports the use of proteomics approaches to development of blood biomarkers and potential therapeutic targets to facilitate diagnosis and furthermore improve the prognosis of COVID-19.

P26
Enrichment and Identification of Bacterial Membrane Proteins by TurboID-Mediated Biotinylation

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Aim: Bacterial membrane proteins are pivotal players in cellular processes, exerting a profound impact on the overall function and survival of bacteria. These proteins are intricately associated with or embedded within the cell's membrane structure. However, the enrichment and identification of bacterial membrane proteins pose significant challenges due to factors like their inherent hydrophobic nature, relatively low abundance, and considerable heterogeneity. Despite all scientific developments, achieving comprehensive and ideal enrichment remains an ongoing endeavour. In this study, our objective was to address these challenges using TurboID, an evaluated biotin ligase, was used to label membrane proteins of E. coli (K12). This approach is designed to enhance the accuracy and efficiency of analysing these membrane proteins, shedding light on their roles within the bacterial cell and potentially advancing our understanding of bacterial physiology.

Method: The His-tagged TurboID was expressed in E. coli BL21 (DE3*) and purified using Ni-NTA column. E. coli was cultured in LB and harvested at OD600 of 0.6-0.8. The cells were washed with PBS and TurboID was mixed with the cells along with the constituents of biotinylation reaction. After 30 min of incubation, cell-free extracts were prepared and the biotinylated proteins were enriched and identified using streptavidin beads and nHPLC LC-MS/MS, respectively.

Findings: Western blot analysis using HRP-conjugated streptavidin demonstrated biotinylation in both cell-free extracts and enriched fractions of biotinylated bacteria, compare with negative control. The subsequent process of identifying enriched proteins unveiled a total of 178 membrane proteins.

Conclusion: In this study, the process of biotinylating bacterial membrane proteins was shown to be straightforward and practical by directly combining purified TurboID with intact cells. Through the utilization of this method, a newfound capability emerges to identify potential drug target membrane proteins, a simple yet significant advancement in the quest to develop treatments for infectious diseases.

P27
Efficacy Comparison of Lysis Buffers for Microfluidic Chip-based Proteomics

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Aim: Organ-on-a-chip systems facilitate the cultivation of human cells within microfluidic chips, allowing for the application of mechanobiological forces to replicate physiological conditions. In the existing literature, protein extraction from these microfluidic chips has predominantly employed RIPA lysis buffer. However, recent findings have indicated that RIPA buffer may not be optimal for comprehensive protein extraction. Consequently, our study seeks to identify the most efficient lysis buffer for protein extraction, ensuring high-quality identifications, and enabling the collection of cells from the microfluidic chip.

Method: Human umbilical vein endothelial cells (HUVECs) were seeded in custom-designed microfluidic chips. The cells were harvested using either SDS buffer (n=3) or RIPA buffer (n=6). Subsequently, the samples were sonicated, boiled, and centrifuged. The resulting supernatants were transferred to new tubes. For the three samples harvested with RIPA buffer, the pellets were treated with Urea buffer. After another round of centrifugation, the resulting supernatants were combined with the previous supernatants from their respective samples. All samples underwent the widely adopted filter aided sample preparation (FASP) method and were subsequently analyzed using an untargeted label-free nLC-MS/MS approach.

Results: Protein extraction efficiency was determined using a protein quantitation assay. The identification and quantification of proteins in each group of cells, isolated using different lysis buffers from the microfluidic chips, were performed through nLC-MS/MS analysis. Biostatistical and bioinformatic analyses were conducted to identify the most effective lysis buffer among SDS, RIPA, and RIPA+Urea buffers.

Conclusion: The comparison of extraction buffers, based on protein yield and their identifications, revealed an optimal protein extraction protocol for harvesting cells from the small volumes of the microfluidic organ chip models in subsequent experiments.

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P28
New Approach for Combating Solid Tumors with Bispecific Anti-EGFR/CD51 Antibody

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Treatment with antibodies is a story that started with Paul Ehrlich's findings a century ago. Even though it was revolutionary, monoclonal antibodies are outdated, especially regarding treating complex diseases such as cancer. Revolutions in synthetic biology also lead to the development of new types of antibodies, including bispecific antibodies, which have promising results for medical usage. Bispecific antibodies, the combination of two specific antibodies that can act on different therapeutic targets, can block dual signaling pathways and immune checkpoints, recruit immune cells, and force the association of protein complexes. Therefore, they are more advantageous than combinational therapies to create a robust response in the body to cancer. In line with this, we combined the Cetuximab (anti-EGFR) and the Abituzumab (anti-CD51) in a single plasmid using Crossmab technology and a knob-into-hole approach to produce functional, high-yield bispecific antibodies for cancer treatment. We expect more enhanced inhibition of cell proliferation (as a result of anti-EGFR epitope) and metastasis (as a result of anti-CD51 epitope) compared to usage of antibodies single or combinatorial. In the long term, testing the anticancer effects of purified antibodies on in vitro models is among the targets after the functionality tests. We expect this approach to contribute to the R&D studies regarding second-generation drugs in overcoming drug resistance, decreasing the treatment period, and cost-effectiveness.

P29
Progress in Enhancing Antitumor Efficacy through Anti-EGFR/HER2 Bifunctional Antibody

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Cancer is a major global challenge and monoclonal antibodies have emerged as inevitable instruments for combatting of cancer types. By using synthetic biology methodologies, we enhance antibody functionalities, offering versatile and potent approaches for addressing tumor landscapes. A particularly promising method in tackling systemic maladies lies in the development of bispecific antibodies. These pioneering therapeutic agents possess the remarkable capacity to target two distinct antigens with a single antibody, engineered to ensure harmonious subchain interactions. Malignant tissues exhibit neoplastic and metastatic configurations, orchestrated by the human epidermal growth factor receptor 2 (HER2) and epidermal growth factor receptor (EGFR). Our primary goal is to craft bispecific antibody molecules that synergistically present anti-proliferative effects by concurrently homing in on HER2 and EGFR. Through the creation of anti-EGFR/HER2 bispecific antibodies, our aim is to block oncogenic signaling pathways, offering a viable therapeutic paradigm for cancer treatment. Our ongoing research emphasizes the vast potential of dual-function antibody therapies across a spectrum of solid tumors. This study was supported by TUBITAK Project Number 119C054 and 217S118.

P30
Delving into the Proteins of Turkish Monofloral Honeys Proteomic Study

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Aim: Honey is produced in various regions worldwide and is considered a significant natural remedy and a source of energy due to its functional properties and nutritional value. One of the notable components of honey is proteins. However, when compared to other constituents, the protein content in honey is relatively low. This study aims to determine the protein profiles of different monofloral honey types produced in different regions of Turkey. Such research has not been conducted on Turkish Honeys before. Proteomic analysis of the honey is an emerging tool in food authentication that has yet to be fully optimized. The implementation of omics approaches in this context will make substantial contributions to the classification, quality assessment, and verification of the authenticity of honey.

Method: Proteins of the monofloral honey samples were extracted using chemical precipitation with trichloroacetic acid. The total protein content in honey samples was assessed using the bicinchoninic acid assay. Sample preparation procedures included protein extraction and digestion with trypsin. Analysis of the resulting peptide mixtures was conducted using a nano-LC connected ESI-QTOF-MS/MS system.

Result: Firstly, the protein content of the included monofloral honey samples was quantified. Subsequently, proteomic analyses were conducted, revealing the presence of both bee-derived and plant-derived proteins. The obtained data were then correlated with the regional characteristics of the honeys through bioinformatic studies.

Discussion: To date, research efforts on Turkish honeys have predominantly centered around analyzing honey for sugar content and assessing the presence of 5-Hydroxymethylfurfural. However, a conspicuous gap exists in the literature regarding the quantification of honey's protein content. This study is aimed at employing a proteomic approach to profile the various monofloral honey types collected from different regions of Turkey. Such information holds significant importance in unveiling the regional distinctions among honeys produced in Turkey.

P31
Composition Characterization of Various Viperidae Snake Venoms Using MS-based Proteomics

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Viperidae snake species are distributed in a wide geographical region in Turkey. Many snake envenomation cases are mainly associated with species from Viperidae family, including *M. lebetina, M. xanthin, V. mmodytes, and V. berus*. Specific proteome and glycoproteome composition profiles provide comprehensive information to study the venom's biological function and taxonomical classification. In this context, we used proteomics, glycoproteomics and glycomics strategies to characterize proteins present in the proteome and glycoproteome of five venoms belonging to the Viperidae family. The finding showed a distinct composition for each venom, particularly the glycoproteome profile. The overall mass spectrometry profiles identified 144 different proteins, 36 glycoproteins and 78 distinct *N*-glycan structures varying in composition across the five venoms. The glycoprotein composition data obtained from glycoproteomics aligns consistently with the findings from glycomics. Many the identified proteins across the five venoms belong to glycosylated protein families, snake venom serine protease (SVSP), snake venom metalloprotease (SVMP), C-type lectins (CTL). The clustering and principal component analyses (PCA) illustrated the composition-based similarities and differences between venom proteome, glycoproteome and glycan profiles. Specifically, the *N*-glycan profiles of *M. xanthina (Mx)* and *V. a. ammodytes (Vaa)* venoms were identical and difficult to differentiate; in contrast, their proteome profiles were distinct. Clustering analysis enabled classification of venom species into different groups presenting their taxonomical classification.

Interestingly, the variety of the proteins across venom species highlight the impact of glycosylation on the diversity of glycosylated protein in venom proteom. This proposed high throughput approach provides accurate and comprehensive profiles of the composition and function of various Viperidae snake venoms

P32
Samples' Journey in The Proteomics Facility: Method Development & Validation in Orbitrap-Mass-Spectrometry-Systems

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Aim: In this study, the development and validation stages of proteomic methods are determined at the KUPAM (Koç University Proteomics Center) center, and the impact of the differences between commercial columns and homemade columns used in liquid chromatography on the analysis results is investigated.

Method: At the KUPAM center, samples containing cell pellets, pull-downs, complexes, or single protein mixtures sent in solution or gel are subjected to enzymatic reactions once the appropriate method, such as in-solution, on-bead, or in-gel digestion, is determined. Prepared samples are analyzed using high-precision and high-resolution Orbitrap mass spectrometry systems. To evaluate the impact of commercial and home-made liquid chromatography (LC) columns on raw data, standard and in-house digested HeLa samples were analyzed in two different Orbitrap systems with the same method and run time, using different types of columns. One of the columns used is the original commercial column for LC systems, while the other is a column produced at the KUPAM center. Thermo Q Exactive and Q Exactive HF analytical systems were employed as Orbitrap mass spectrometers.

Result: The method development and validation parameters have been established on the mass spectrometer, and the contribution of LC columns to raw data has been recorded. Homemade columns yielded higher results at the same concentration and run time. Using a 50 cm C18 reversed-phase homemade column with 200 ng of standard HeLa-digested samples, both systems detected 4,500 proteins.

Conclusion: In this study, the analysis workflow of a proteomic sample and how to ensure the quality of data obtained from the sample are explored. Additionally, the impact of LC column choice, specifically home-made columns, which is one of the data enrichment methods, is demonstrated. Qualitative analyses using homemade columns are recommended for instrument users and facilities due to their cost-effectiveness and superior results.

P33
Regular FLOW-LC-HRMS Systems for Experimental and Clinical Proteomics

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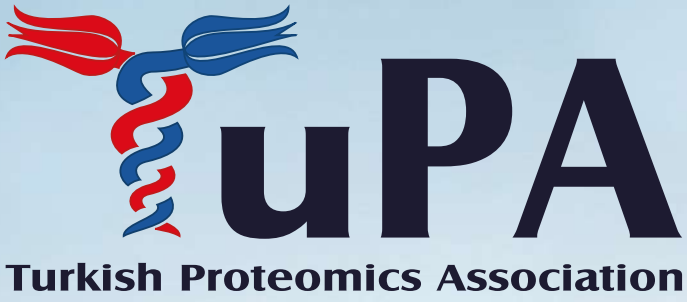
Aim: Proteomics has gained popularity in understanding biological systems. Today nano-LC-based proteomics dominates this area because of its sensitivity to analyzing thousands of proteins. However regular flow-LC systems could be an alternative to nano-LC-HRMS systems with their more reproducible results.

Method: In the present work, we used regular flow-LC systems for bottom-up proteomics studies to analyze the proteome structure of plasma, bacteria, cell culture and plant samples. The samples were prepared in three steps including isolating of proteins, denaturation and digestion. The isolation of the proteins was done according to the nature of the sample matrix. We used LC-qTOF-MS (Agilent 6530) systems for proteomics analysis. The column diameters were ranged from 0.5 to 1 mm. The flow rate was adjusted between 0.07-0.2 mL/min. The raw MS/MS data was processed with DDA DDA-based proteomics approach in the Maxquant platform.

Results: In this study, we analyzed the proteome structure of plasma, cell and bacteria culture with a regular flow LC-HRMS system. we could identify 140 proteins for the plasma sample, 704 proteins for E.coli, approximately 1000 proteins for cell culture and 300 proteins for plants.

Conclusion: All results showed that regular flow-based proteomics could be used for proteomics analysis. This system especially gives more information about the physiology of organisms than 2D-gel-based proteomics, and it is a good alternative for nano-LC systems.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