3rd INTERNATIONAL

# MULTIDISCIPLINARY CANCER RESEARCH CONGRESS

ISTANBUL, TURKIYE

## 07-10 SEPTEMBER 2023


# ABSTRACT BOOK

CANCER 2023
ISTANBUL

cancer2023.mokad.org

# 3rd International Multidisciplinary Cancer Research Congress

**Published by**
**MOKAD Congress Secretariat**

**Editor:**
**Prof. Dr. Engin Ulukaya, Istinye University**

**3rd International Multidisciplinary Cancer Research Congress Secretariat**



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## Submission and Application for Awards

Öykü Gönül Geyik

Yaren Arasan

## About Registration & Financial Issues

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## About Accommodation

Yelda Birinci Kudu

## Sponsorship & General Issues

Berkcan Doğan

Remzi Okan Akar

**Dear Cancer Researchers,**

A new MOKAD Congress is on the way. Unlike the other traditional MOKAD meetings, this time most of the program is composed of oral presentations (10 minutes) and short talks (15 minutes) by young researchers. Some internationally recognized speakers will give lectures online. However, those lectures will be available to only the participants of the congress.

The main theme of the congress is the clinician-basic scientist interaction on the basis of personalized oncology. You will hear from expert speakers who are at the forefront of personalized oncological research. They will share their insights and visions on how the future of Cancer Research will be. You will make the connections between basic cancer research and medical or surgical oncology. Don't miss out on this opportunity to learn about the cutting-edge science that is shaping the future of cancer treatment!

MOKAD (Molecular Cancer Research Society in Türkiye) believes that the successful development of any scientific area or, even a country, relies on multi- and interdisciplinary approaches. Therefore, the interaction of young researchers with experienced professionals (basic scientists or clinicians) will take place at the "talk to expert" sessions.

Twenty graduated students will again be provided with scholarship in this congress. As everybody knows that MOKAD always organizes reasonably priced congresses. So, this applies to this congress, too. Classic MOKAD awards will again be given to the successful applicants.

We happily state that we are honored to invite you to the congress that will be held at the University of Istinye, Istanbul. Istanbul is vibrant city with everything from ancient history to culture, tourism, cuisine, diverse night life etc.

Hope to meet up at the congress.

All the best,

Chair, Engin Ulukaya
Istinye University
Head of Molecular Cancer Research Center

## CONGRESS PROGRAMME

| 7th September | |
|---|---|
| *13.30-14.00* | **OPENING CEREMONY** |
| *14.00-14.40* | **OPENING KEYNOTE LECTURE**<br>**CHAIR: Engin ULUKAYA**<br>Besim ÖĞRETMEN (USA)<br>**Lipid Metabolism and Signaling in the Regulation of Cancer Metastasis and Therapy** |
| *14.45-15.15* | **KEYNOTE LECTURE**<br>**CHAIR: Ayşegül ÇEBİ**<br>Chiara RAGGI (ITALY - ONLINE)<br>**Metabolic Aspects of Cholangiocarcinoma Stem-Compartment** |
| *15.15-15.45* | **COFFEE BREAK** |
| *15.45-16.15* | **INVITED LECTURE**<br>**CHAIR: Semra DEMOKAN**<br>Evgeny DENISOV (RUSSIA - ONLINE)<br>**Single Cell and Spatial Transcriptomics in Dissecting Mechanism of Cancer Development and Progression** |
| *16.15-16.45* | **INVITED LECTURE**<br>**CHAIR: Öykü Gönül GEYİK**<br>Ranan Gülhan AKTAŞ (USA - ONLINE)<br>**New Era in Life Science: Organoids & Spheroids** |
| *17.00-17.30* | **KEYNOTE LECTURE**<br>**CHAIR: Abdullah YALÇIN**<br>Candan HIZEL PERRY (CANADA - ONLINE)<br>**Polygenic Risk Score (PRS) for Cancer - Can It Deliver on The Promise of Precision Oncology?** |
| *17.30-18.30* | **COCKTAIL PROLONGE** |

## 8th September

| | MAIN HALL | | HALL 2 |
|---|---|---|---|
| *09.00-10.00* | **SHORT TALKS**<br>**CHAIR: Serpil OĞUZTÜZÜN**<br>**1- Esra Nalbat:** Breaking Barriers in Cancer Treatment: Unleashing the Power of Drug Repurposing<br>**2- Sezen Güntekin Ergün:** An overview of tumor agnostic therapies through the Hippo signaling pathway<br>**3- Pınar Siyah:** Cracking the Code of Drug Discovery: Technology's Impact on Novel Therapeutics<br>**4- Mehmet Sarımahmut:** Antioxidant and Anti-growth Properties of Selected Anatolian Plant Species: Exploring the Potential of *Heracleum humile, Doronicum reticulatum, Centaurea drabifolia,* and *Senecio olympicus* | 09.00-11.00 | **ORAL PRESENTATIONS**<br>**CHAIR: Yelda BİRİNCİ**<br>**GASTROINTESTINAL CANCER**<br>**1- Evin İşcan:** TAp73β regulates the Wnt/β-catenin signaling pathway in a zebrafish xenograft model in Hepatocellular Carcinoma<br>**2- Demet Kaçaroğlu:** Naive and TLR4 Stimulated Adipose Derived Mesenchymal Stem Cells Inhibit EMT and Metastasis of Pancreatic Ductal Adenocarcinoma Cells<br>**3- Ergin Turantepe:** Flavopiridol Inhibits Cell Proliferation and Migration in Pancreatic Ductal Adenocarcinoma Cells<br>**4- Seçil Demirkol Canlı:** Evaluation of CSRP1 expression as a prognostic marker in colorectal cancer<br>**5- Ahmet Acar:** Generating single-barcode harbouring cell lines from chemotherapy resistant Caco-2 cell line to study drug resistance<br>**6- Hasan Kurter:** HDAC Inhibitor Induces Mitochondrial Membrane Potential Disruption and Reverses the Epithelial-Mesenchymal Transition in Colorectal Cancer<br>**7- Çağrı Öner:** The Impact Of 1.25-Dihydroxyvitamin D3 On Mitophagy and Apoptotic Pathways in Hepatocellular Carcinoma<br>**8- Semih Şeker:** Molecular and Bioinformatic Investigation of Proteomic Differences Between Cancerous and Normal Tissue Samples of Colorectal Cancer Patients<br>**9- Tolga Sever:** Combination Therapy of Beta-Hydroxybutyrate and Oxaliplatin Augments the Treatment Efficacy in Colorectal Cancer Organoids |
| *10.00-11.00* | **KEYNOTE LECTURE**<br>**CHAIR: İlhan YAYLIM**<br>Serdar DURDAĞI<br>**Developing a Groundbreaking Algorithm for Target-Driven Based Approach in the Design of New Effective Anticancer Therapeutics** | | |
| | **INVITED SPEAKER**<br>**CHAIR: Serdar DURDAĞI**<br>Soykan AĞAR<br>**The Future of *de novo* Drug Design & Repurposing: *In silico* Computational/Simulational Methods** | | |

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| | | | **10- Muzaffer Dukel:** A Novel Palladium (II) Complex Selectively Induces Cell Death and Cell Cycle Arrest In Metastatic Colon Cells<br>**11- Zeynep Büşra Bolat:** Combination Therapy of dual PI3K/mTOR Inhibitor and Curcumin shows anticancer effect on Colorectal Cancer |
| *11.00-11.30*<br>**COFFEE BREAK and CONSULT THE EXPERT**<br>(Engin ULUKAYA- Spheroids and Organoids) | | | |
| *11.30-13.30* | **SHORT TALKS**<br>**CHAIR: Egemen DERE**<br>**1- Filiz Yarımcan:** Determination of intestinal microbiota in pediatric patients who underwent allogeneic stem cell transplantation before and after transplantation<br>**2- Ece Gumusoglu-Acar:** The Expression Analysis of Specific Genes in Ovarian Cancer<br>**3- Deniz Cansen Kahraman:** Anti-Cancer and Anti-Stemness Action of Nonsteroidal Anti-Inflammatory Agents: A Special Focus on Hepatocellular Carcinoma | *11.30 – 13.30* | **ORAL PRESENTATIONS**<br>**CHAIR: Didem KARAKAŞ**<br>**BREAST CANCER SESSION**<br><br>**1- Tuğba Atıcı:** Apoptotic Effects of Paclitaxel/Aloe-Emodin Combination in MCF-7 and MDA-MB-231 Breast Cancer Cell Lines<br><br>**2- Müge Öçal Demirtaş:** miR-30b-3p Regulates Apoptosis in CD8+ T Lymphocytes in Triple Negative Breast Tumor Bearing Mice<br><br>**3- Boran Can Dinçer:** MicroRNA-145's Regulatory Role in Breast Cancer Progression<br><br>**4- Ece Oylumlu:** Indoximod Restricts Tumor Growth by Targeting Breast Cancer Viability<br><br>**5- Muhammet Ocak:** Determination of In Vitro and In Vivo Effects of Taxifolin and Epirubicin on Epithelial-Mesenchymal Transition in Mouse Breast Cancer Cells<br>**6- Hüseyin İzgördü:** The Impacts of Ceramidase Inhibition with D-E-Mapp Sln Formulation upon Cell Death Mechanism in Breast Cancer as an in-vitro and in-vivo models.<br>**7- Gamze Yıldız:** Determination of Antioxidant Properties and Contents of Helichrysum Arenarium L. Extracts, |

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| | | | Investigation of Anti-growth Effects Against Human Breast Cancer Cell Lines |
| *13.30-14.30* | **LUNCH** | | |
| *14.30-15.30* | **INVITED LECTURES**<br>**CHAIR: Huri DEDEAKAYOĞULLARI**<br>Serdar ALTINAY<br>**NGS in Sporadic Medullary Thyroid Cancers: With artificial intelligence and biological pathway enrichment method**<br><br>Hatice Mehtap KUTLU<br>**The Role of Ceramide Metabolism and Signaling in the Regulation of Cancer Therapy** | *14.30 – 16.30* | **ORAL PRESENTATIONS**<br>**CHAIR: Hande Süer MICKLER**<br>**BREAST & GYNECOLOGICAL CANCER SESSION**<br>**1- Serpil Telci:** Synergistic Anticancer Effects of Auraptene and Tamoxifen on MCF-7 and Ishikawa Cell Cultures in Breast and Endometrial Cancer Cell Lines.<br>**2- Erva Özkan:** The Anticancer Potential of Brassinin in Estrogen Receptor-Positive Breast Cancer Cells Through the Activation of Apoptosis And Downregulation Of Matrix Metalloproteinase-2<br>**3- Hacer Kaya Çakır:** Investigation of anticancer activity of mocetinostat (Hdaci) on MDA-MB-231 breast cancer cell line<br>**4- Gizem Bulut:** Investigation of Anti-Cancer Effects of a Palladium Complex (Pd(bpma)(barb).Cl • H2O) in Ovarian Cancer Cell Lines<br>**5- Gamze Namalır:** Long Non-Coding Rna Urothelial Carcinoma-Associated 1 Regulates Proliferation and Migration in Doxorubicin Resistance of Estrogen Receptor Positive Breast Cancer Cells<br>**6- Leyla Tutar:** DNA methylation plays a key role in Triple Negative Breast Cancer?<br>**7- Ceren Tuncay:** Comparison of the Effect of Abemaciclib in MCF-7 Cells in 2D and 3D Systems<br>**8- Ebrucan Bulut:** The role of Trop-2 expression in determining the effectiveness of Sacituzumab Govitecan in the treatment of triple-negative breast cancer patients |
| *15.40-16.20* | **SATELLITE SYMPOSIA (Medsantek)** | | |

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| *16.30-17.00* | **COFFEE BREAK and POSTER SESSION** |

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| *17.00 – 18.10*<br><br>*18.45 –19.15* | **ORAL PRESENTATIONS**<br>**CHAIR: Eray Metin GÜLER**<br>**1- Egemen Dere:** Investigation of the effect of toluene on nitric oxide production and protective properties of resveratrol<br>**2- Salih Gencer:** Ceramide Binds Smad7 to Regulate Solid Tumor Metastasis<br>**3- Sedef Ziyanok:** Anti-growth, Antioxidant, and Hepatoprotective Properties of *Spirulina platensis* Extract<br>**4- Özde Gökbayrak:** Neuroblastoma Targeted Anti-Cancer Drug Delivery<br>**5- Sıla Sığırlı:** Acetylsalicylic acid treatment reduces cancer promoting properties of pancreatic stellate cells<br>**6- Nejdet Memiş:** Investigation of Anti-Cancer Potential of Omeprazole in Prostate Cancer Cells<br>**7- Berkcan Doğan:** Evaluation of Circulating Tumor Cell (CTC) Specific Markers and CTC Status in Metastatic Colorectal Cancer Patients by Immunomagnetic Cell-selection Method | *17.00 – 19.30* | **ORAL PRESENTATIONS**<br>**DRUG RESISTANCE & SENSITIVITY**<br>**CHAIR: Nazlıhan AZTOPAL**<br>**1- Muhlis Akman:** TFEB Drives Chemo-Immuno-Resistance in Lung Cancer<br>**2- Umut Haskök:** Drug Repositioning to Specifically Target Multiple Myeloma Subtypes Based in Silico Investigation of Differential Gene Expression Profiling<br>**3- İrem Durmaz Şahin:** Exploring New Frontiers in HGSOC Treatment: Targeting Drug Resistance with miRNA Mimics<br>**4- Melisa Tecik:** Sphingosine 1-Phosphate Signaling in Midostaurin Resistance in FLT3-ITD Positive Acute Myeloid Leukemia<br>**5- Büşra Bilen:** Effect of PKR Kinase Activation on the sensitivity of choriocarcinoma cells to chemotherapy agent<br>**6- Özlem Ulucan:** Predicting side-effects of chemotherapeutic agents through analysis of drug-induced transcriptomic response<br><br>**RESPIRATORY SYSTEM CANCERS**<br>**CHAIR: Tuba GÜNEL**<br><br>**1- Cemal Çağrı Çetin:** PTEN R234W Variant: A Novel Case Presentation in Non Small Cell Lung Cancer Patients<br><br>**2- Aydın Keskin:** Expressions of GSTO1, GSTP1, GSTM1, GSTS1 isoenzymes in NSCLC tissues<br><br>**3- Alper Gümüş:** Investigation of CD40, CD40L Gene Variants and sCD40, sCD40L Serum Levels in Laryngeal Cancer |

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| | **INVITED LECTURE**<br>**CHAIR: Mehtap KUTLU**<br>Bülent ÖZPOLAT (USA - ONLINE)<br>**MicroRNA-based Therapeutics for Cancer** | | **4- Nadin Bedikyan:** Investigation of methylation and expression levels of the GNG7 gene in oral squamous cell carcinoma<br><br>**5- Tuğba Gül İnci:** Evaluation of Sulfasalazine Drug Repurposing Potantial and Sulfasalazine Encapsulated PLGA Nanoparticles in Non-Small Cell Lung Cancer<br><br>**6- Zeynep Demir:** Investigation of Apoptotic Potential of Catechol in Drug-Resistant Lung Cancer and Healthy Fibroblast Cells<br><br>**7- Şule İnci:** Anticancer effect of Tricholoma atrosquamosum Sacc. against human lung adenocarcinoma cell line<br><br>**8- Kubilay İnci:** Biological Effects of CRISPR/Cas9-mediated Knockout of RAB27A in SCLC |

| **9th September** | |
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| *09.00-10.30* | **MAIN HALL**<br>**ORAL PRESENTATIONS**<br>**CHAIR: Süreyya BOZKURT**<br>**1- Elif Bayram:** Dual Targeting of Glycolysis and Glutaminolysis as a Strategy to Inhibit Tumor Cell Proliferation<br>**2- Elif Kahraman Topgül:** The Effect of Boron Compounds on Androgen Signaling in Prostate Cancer<br>**3- Ezgi Yağmur Tükel:** Increasing Treatment Efficacy by Drug Repositioning in Acute Lymphoblastic Leukemia<br>**4- Ceyda Nur Zaim:** Regulatory Network of miR-27a-5p in Prostate Cancer<br>**5- Ceren Çelik:** Gastric Cancer Spheroids: A Three-Dimensional Model to Study the Effect of Metabolic Alterations in the One-Carbon Pathway<br>**6- Zehra Tavşan:** Investigation of the Relationship Between Bivalent Promotor Regions and Epithelial-Mesenchymal Cancer Cells Plasticity<br>**7- Serdar Karakurt:** Unveiling Potential: Scorpio fuscus Venom for Targeted Colorectal Carcinoma Therapy |
| | **INVITED LECTURES**<br>**CHAIR: Serap ÇELİKLER**<br>Sadettin KILIÇKAP (TURKIYE - ONLINE) |

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| *10.30-11.30* | **'Bench to bedside': Precision Medicine in Oncology**<br><br>Eser ÖZYÜREK<br>**Where We Fall Short in Surgical Management?** |
| *11.30-12.00* | **COFFEE BREAK and CONSULT THE EXPERT**<br>(Eser ÖZYÜREK- Expectations from Clinics) |
| *12.00-12.30* | **INVITED LECTURE**<br>**CHAIR: Caner GEYİK**<br>Özlem ER<br>**Tumor Genomic Profiling and Treatment Decision in Gastrointestinal Cancers** |
| *12.30-13.00* | **INVITED LECTURE**<br>**CHAIR: Özlem ER**<br>Safiye AKTAŞ (TURKIYE - ONLINE)<br>**Precision Medicine in Pediatric Tumors: Next Generation Sequencing Experience** |
| *13.00-14.00* | **LUNCH** |
| *14.00-15.30* | **SHORT TALKS**<br>**Chair: Süreyya BOZKURT**<br>**1- Öykü Gönül Geyik:** How to target tumor heterogeneity to overcome drug resistance?<br>**2- Ece Akhan Güzelcan:** The Role And Importance Of Biobanks Dedicated To Cancer Research<br>**3- Adouda Adjiri:** Cancer Resistance to therapy: Is there a way out?<br>**4- Dilara Kamer Çolak:** Recent Advances In Researching Cancer Types Using 3D Bioprinting |
| *15.30-22.00* | **SOCIAL PROGRAM** |

| **10th September** | |
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| *09.00-11.00* | **MAIN HALL**<br>**SPECIAL SESSION: GBM SECRETS**<br>**CHAIR: Gamze TANRIÖVER**<br>***To The Memory of Prof. Dr. Necdet DEMİR*** |

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| | **1- Emel Sokullu (Invited Lecture) :** 3D Culture Systems for Tumor Microenvironment<br>**2- Gizem Dönmez Yalçın (Invited Lecture) :** The Molecular Link Between Excitotoxicity and Glioblastoma<br>**3- Zehra Güneş:** Investigation of The Effects of Chloroquine on Proteasomal System In Glioblastoma Stem Cells (Oral Presentation)<br>**4- Nazlı Keskin Toklu:** Editing the TP53 Gene Locus in U87 Human Glioblastoma Cell Line by Using CRISPR/Cas9 System (Oral Presentation)<br>**5- Mustafa Kotmakçı:** Enhanced cytokine stimulation in in vitro and in vivo glioblastoma models: Lipid nanoparticles for stimulator of interferon genes agonists delivery (Oral Presentation)<br>**6- Sema Tuğçe Aydın:** Investigating the Effects of Temozolomide on The Viability of Glioblastoma Stem Cells Responsible for Recurrence Using a 3D Culture Model (Oral Presentation) |
| *11.00-11.30* | **COFFEE BREAK** |
| *11.30-12.00* | **CLOSING KEYNOTE LECTURE**<br>CHAIR: Emel Sokullu<br>Abdullah KAHRAMAN (SWITZERLAND - ONLINE)<br>**Comprehensive Genomic Profiling for Precision Oncology** |
| *12.00-13.00* | **AWARD CEREMONY & CLOSING** |

*Keynote Speaker - 01*

## Roles and Mechanisms of Sphingolipid Metabolism in the Regulation of Tumor Growth and Therapeutics: Implications in Personalized Cancer Chemotherapy and Tumor Immunology

Besim Ogretmen

Hollings Cancer Center, Department of Biochemistry and Molecular Biology, Medical University of South Carolina, Charleston, SC, USA

Sphingolipids, including the two central bioactive lipids ceramide and sphingosine 1-phosphate (S1P), have opposing roles regulating cancer cell death and survival, respectively, and there have been exciting developments in understanding how sphingolipid metabolism and signaling regulate these processes in response to anti-cancer therapy, including immunotherapy. Recent studies have provided mechanistic details of the roles of sphingolipids and their downstream targets in the regulation of tumor growth and response to chemotherapy, radiotherapy and/or immunotherapy using innovative molecular, genetic, and pharmacological tools to target sphingolipid signaling nodes in cancer cells. For example, structure–function-based studies have provided innovative opportunities to develop mechanism-based anti-cancer therapeutic strategies to restore anti-proliferative ceramide signaling and/or inhibit pro-survival S1P–S1P receptor (S1PR) signaling. This seminar will summarize how ceramide-induced cellular stress, including aging, mediates cancer cell death through various mechanisms involving the induction of apoptosis, necroptosis and/or mitophagy. Moreover, the metabolism of ceramide for S1P biosynthesis, which is mediated by sphingosine kinase 1 (SPHK1) and SPHK2, and its role in influencing cancer cell growth, drug resistance and tumor metastasis through S1PR-dependent or receptor-independent signaling will be highlighted. Moreover, mechanistic details of aging-mediated changes in mitochondrial bioenergetics and lipid metabolism that affect T cell function will be discussed. For example, ceramide, induced by aging stress, mediates mitophagy, and cell death; however, the aging-related roles of ceramide metabolism in regulating T cell function remain unknown. Here, we will discuss that activated T cells isolated from aging mice have elevated C14-/C16-ceramide accumulation in mitochondria, generated by ceramide synthase 6, leading to mitophagy/mitochondrial dysfunction. Mechanistically, aging-dependent mitochondrial ceramide inhibited protein kinase A, leading to mitophagy in activated T cells. This aging/ceramide-dependent mitophagy attenuated the anti-tumor functions of T cells in vitro and in vivo. Also, inhibition of ceramide metabolism or PKA activation by genetic and pharmacologic means prevented mitophagy and restored the central memory phenotype in aging T cells. Thus, these studies help explain the mechanisms behind stress-related dysregulation of T cells' anti-tumor activity that can be restored by inhibiting ceramide-dependent mitophagy. In addition, biological implications of alterations in ceramide-mediated mitophagy in cancer chemotherapy and immunotherapy regarding personalized therapy will be discussed.

Funding: These studies are supported by research grants from the National Institutes of Health, and South Carolina SmartState Endowment (USA).

Potential Financial Conflict of Interests: Dr. Ogretmen is a Co-Founder of a small biotech company, Lipo-Immuno Tech, LLC, which has financial interests in therapeutics that are mentioned in these studies. Thus, there are potential financial conflict of interests that should be considered accordingly.

*Keynote Speaker – 02*

Metabolic Aspects of Cholangiocarcinoma Stem-Compartment

Chiara Raggi

Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy

Metabolic reprogramming is a hallmark of cancer and allows tumor cells to meet the increased energy demands required for rapid proliferation and metastasis. Several recent studies have explored the metabolic plasticity of cancer cells with the aim to identify new druggable targets, and therapeutic strategies aimed to limit the access to nutrients. Cholangiocarcinoma (CCA) is a deadly tumor without an effective therapy represents thus representing an unmet medical need. Cancer stem/initiating cells (CSCs) are highly drug- resistant but little is known regarding metabolic profiles of CSC and their functional role.

Our studies aim to explore the contribution of mitochondria-related metabolism with particular attention to the role of glucose and lipid metabolic pathways in the maintenance of stemness state.

We have recently demonstrated that the stem-subset of CCA cells, enriched by 3D sphere culture (SPH) revealed a more efficient respiratory phenotype than parental cells. Indeed, alteration of the integrity of the mitochondrial respiratory chain with metformin or downregulation of PGC1α (SR-18292) in the stem-subset of CCA cells severely impair tumor progression, demonstrating a crucial role of OXPHOS in CCA aggressiveness. These data indicate that, besides a general increase in glucose dependency, CCA displays a marked metabolic plasticity, and different pathways may be activated in various cell subtypes within the tumor mass, due to the different availability of nutrients. OXPHOS metabolism is crucial to sustain CCA stemness and the acquisition of a phenotype prone to metastatic dissemination.

Moreover, in CCA-SPH, expression levels of several key genes involved in fatty acid synthesis (i.e. FASN) and transport were upregulated. Notably FASN expression levels correlate with OS in iCCA patients. In vitro FASN depletion by orlistat or siRNA decreased sphere forming capability and expression of stem-like markers. Notably, in CCA xenograft model, growth of SPH derived tumors treated with orlistat was significantly lower than control.

The overall objective of our studies is to determine CSC-specific metabolic and mitochondrial-associated pathways regulating CCA initiation, progression and drug-response. The results of these studies are likely to expand the spectrum of therapeutic targets in CCA.

*Keynote Speaker - 03*

Polygenic Risk Score (PRS) for Cancer - Can It Deliver on The Promise of Precision Oncology?

Candan Hızel Peery

Université de Montréal, Montreal, QC, Canada

Public health strategies aimed at disease prevention or early detection and intervention have the potential to advance human health worldwide. However, their success depends on the identification of risk factors that underline disease burden in the general population. Acordingly, large-scale genotyping and phenotyping efforts, including biobanks, have revolutionized our understanding of the genetic architecture of human traits and diseases. Years of ever-larger genome-wide association studies (GWAS) have identified dozens or even hundreds of common single nucleotide polymorphisms (SNPs) associated with many complex diseases, including common cancers, e.g., breast cancer and prostate cancers. Combining genetic and clinical data and translating findings from GWAS to clinical utility in terms of informative risk prediction profile of complex traits is an important ambition of precision medicine that aims to improve human health. Against this backdrop, polygenic risk scores (PRS) that aggregate the effects of many genetic variants across the human genome into a single score can provide useful information for personalized risk stratification and disease risk assessment, especially when combined with non-genetic risk factors. This presentation aims to provide various perspectives on the application of PRS for different types of cancer, particularly, breast and prostate cancer.

*Keynote Speaker – 04*

Comprehensive Genomic Profiling for Precision Oncology

Abdullah Kahraman

Institute for Chemistry and Bioanalytics, School for Life Sciences, University of Applied Sciences and Arts Northwestern Switzerland, Muttenz, Switzerland

Comprehensive genomic profiling assays have become a key cornerstone for today's clinical decision making in precision oncology. In this context, NGS sequencing results are often discussed at molecular tumor boards, where amongst other oncologists, pathologists, bioinformaticians, and geneticists discuss these results to decide on the best treatment options for a patient. However, with the increasing size and complexity of NGS cancer panels, NGS results have become challenging to interpret, especially if presented merely in the form of a written report. Manual analysis of several mutations from a comprehensive NGS cancer panel is time-consuming and often incomplete. Here I will describe the complexity on the example of the FoundationOneCDx assay and present the Molecular Tumor Profiling Pilot (MTPpilot) software, which provides automated annotation, linking and interactive visualization to support the interpretation of NGS results at molecular tumor boards.

*Keynote Speaker - 05*

Developing a Groundbreaking Algorithm for Target-Driven Based Approach in the Design of New Effective Anticancer Therapeutics

Serdar Durdağı

Department of Pharmaceutical Chemistry, School of Pharmacy, Bahcesehir University, İstanbul, Türkiye

*Invited Speaker - 01*

Single Cell and Spatial Transcriptomics in Dissecting Mechanisms of Cancer Development and Progression

Evgeny Denisov[1,2], Tatiana Gerashchenko[1,2], Maxim Menyailo[1,2], Elena Kolegova[1], Marina Patysheva[1], Anna Khozyainova[1], Elizaveta Prostakishina[1], Anastasia Frolova[1], Vyacheslav Korobeinikov[1], Marina Patysheva[1], Pavel Iamshchikov[1], Anton Fedorov[1], Mrinal Vashisth[1]

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[2]Laboratory of Single Cell Biology, Peoples' Friendship University of Russia (RUDN University), 117198, Moscow, Russia

Introduction and Aim: Single-cell technologies are becoming revolutionary tools for studying the physiology of normal and pathologically altered tissues. Among them, single-cell and spatial transcriptomics are the most widely used providing information about not only differentially-expressed genes, but also cell types, differentiation trajectories, cell-cell interactions, and genetic and epigenetic alterations. In our studies, we use single-cell and spatial transcriptomics to decipher the heterogeneity of circulating tumor and hybrid cells in breast cancer, to reveal molecular mechanisms of early-onset tongue cancer, and to assess how the immune system is involved in chemotherapy efficacy.

Materials and Methods: Blood samples of breast cancer patients and FFPE samples of tongue tumors were used for single-cell RNA sequencing (10x Genomics 3' Chromium) and spatial transcriptomics (10x Genomics Visium), respectively. RNA libraries were sequenced using Genolab M instrument (Genemind).

Results: Circulating tumor and hybrid cells are highly heterogeneous in breast cancer patients and are represented by transcriptionally distinct populations that include both aneuploid cells and diploid cells. Cancer-associated signaling pathways are abundant only in one aneuploid population, which may represent an aggressive subset of circulating tumor cells. Early-onset tongue cancer enriches with immunosuppressive gene signature, oxidative stress, and the MAPK molecular pathway. Chemotherapy stimulates a series of immune changes enhancing adaptive immune response in breast cancer patient blood and accelerating accumulation of M2 macrophages in breast tumor tissue.

Conclusion: Single-cell and spatial transcriptomics are powerful tools for deciphering tumor biology and providing significant clinical value for cancer diagnosis.

Keywords: single cell analysis, cancer, chemotherapy, metastasis

*Invited Speaker - 02*

New Era in Life Sciences: Organoids & Spheroids

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We are witnessing history! In recent years, creating three-dimensional models of tissues and organs in the lab has been a huge milestone in life sciences. Now, it is possible to take the cells from a person's body and create a miniature of an organ or tissue in the lab. Those 3D models, named organoids, opened the doors of many scientific projects from disease modeling to personalized treatment. We have started to be more hopeful about developing an organ in a dish, bringing better solutions in drug discovery, and finding the best-personalized treatment for our patients. In December 2022, FDA Modernization Act 2.0 removed the mandate for animal testing to assess the safety and efficacy of a drug. U.S. FDA approval of the first drug to enter clinical trials based on efficacy data derived only from these advanced cell models has become another milestone in history. Organoids are becoming more superior models to in vivo animal models, primary cell culture studies, and in vitro cell lines. From big pharma companies to research institutions, we are hearing more about the establishment of labs focusing on 3D cell culture models.

Spheroids are representing another 3D cell culture model forming from clusters of cells. They are being used in drug discovery and screening tests worldwide since they tell us much about cancer cells 'real' behavior in the 3D world.

All those recent developments show that organoids and spheroids will provide spectacular advances in cancer research as they mimic the 3D world in the human body. As a scientist working with cell culture models and liver cancer for over 20 years, I will talk about those rising stars in oncology. I will describe those models, discuss their advantages, and share some recent exciting developments. I will also present the results from different labs related to our patent pending products that simplify, speed up, and accelerate those 3D cell culture studies.

*Invited Speaker - 03*

NGS in Sporadic Medullary Thyroid Cancers: With artifcial intelligence and biological pathway enrichment method

Serdar Altınay

Medullary thyroid carcinomas (MTCs) occur 75% sporadic and 25% hereditary. In this study, it was aimed to determine the histopathological parameters of metastatic and non-metastatic sporadic MTCs and molecular changes by next generation sequencing (NGS) in a university hospital. 13 patients included in the study were analyzed by whole-exome sequencing (WES) for a total of 62 genes, including lung-thyroid gene panel and other cancer-related genes. Mutations that could be drivers or passangers were investigated with biological pathway enrichment analysis and artificial intelligence modeling. In patients with nodal metastases, only stage and capsule invasion showed a statistically significant relationship among histopathological parameters, while no correlation was found for RET mutation. The RET mutation rate was 30.8% (4/13) and all RET mutations were missense mutations. While there was a KDR gene mutation in nodal involvement among patients with RET mutation, no KDR gene mutation was observed in patients without nodal involvement. MLH1, GNAS, HRAS gene mutations in nodal involvement among patients without RET mutation, while these gene mutations were not observed in patients without nodal involvement. With artificial intelligence modeling, mutations with the potential to be important were found on the HRAS, MAP3K1 and EIF1AX genes. In conclusion, we identified different gene mutations that could predict lymph node metastasis in the presence or absence of RET mutation. We identified mutations that may be involved in tumor progression and have prognostic significance, such as HRAS, MAP3K1 and EIF1AX. We show that KDR mutation can predict nodal involvement. We believe that additional studies with a larger number of patients should be conducted so that the findings can be included in the guideline treatments to be prepared by the ATA (American Thyroid Association).

Key points

Artificial intelligence showed mutations that could be driver or passanger for the relevant patient in the presented data, and as a result, mutations with the potential to be important on HRAS, MAP3K1 and EIF1AX genes were found.

The presence of MAP3K1 mutation in patients with lymph node metastasis is important in terms of showing that this mutation can predict lymph node metastasis.

It was thought that KDR mutation might predict nodal involvement in RET mutation-positive patients.

Among RET mutation-negative patients, MLH1, HRAS and GNAS mutations were observed only in patients with nodal involvement, suggesting that these mutations may predict lymph node metastasis.

*Invited Speaker - 04*

## The Role of Ceramide Metabolism and Signaling in the Regulation of Cancer Therapy

H. Mehtap KUTLU

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Ceramide and sphingosine known as the main bioactive lipids. Sphingolipids are structural molecules of cell membranes and have regulatory roles in various biological processes such as invasion, metastasis, migration, proliferation and growth by controlling communication in cancer cells.

It is known that ceramide synthesis or accumulation in the cell, which occurs due to cellular stress, mediates cancer cell death through different mechanisms. These mechanisms include apoptosis, necroptosis, autophagy, and endoplasmic reticulum stress. These ceramide-mediated cell death pathways are regulated differently depending on cell or tissue type, subcellular localization of ceramide, or molecular ceramide targets.

Contrary to ceramide, sphingosine 1 phosphate (S1P), formed from ceramide through ceramidase enzymes, stimulates cell proliferation and suppresses apoptosis. As a result, the shift of the ceramide/S1P balance in the cells to the ceramide direction causes the death of the cells. Therefore, suppression of ceramidase activity in sphingolipid metabolism is thought to be an important pathway in cancer treatment.

How ceramide-based cellular stress mediates cancer and promotes apoptosis, necroptosis or mitophagy by different mechanisms is important. At the same time, sphingolipid metabolism enzymes are also important targets in order to develop cancer therapeutics.

Changes in the expression or activity of sphingolipid pathway enzymes are key in cancer treatment.

The level of sphingolipids is highly regulated by metabolic enzymes. Changes in the expression or activity of these enzymes have a key role in triggering the death or survival of cancer cells.

*Invited Speaker - 05*

MicroRNA-based Therapeutics for Cancer

Bulent Ozpolat

After 20 years of the discovery of microRNA that has revolutionized the world of science and opened up new opportunities in cancer treatment, microRNA-therapeutics finally enter human clinical trials. MicroRNAs (miRNAs) are non-protein-coding RNA molecules 20–25 nucleotides in length that can suppress the expression of genes involved in numerous physiological processes in cells. miRNA dysregulation in cancer cells plays a crucial role in cell proliferation, invasion, metastasis, and angiogenesis, drug resistance tumor growth and progression in a broad range of cancers including breast cancer. Thus, strategies involving either restoring the expression of tumor suppressor miRNAs or inhibiting overexpressed oncogenic miRNAs hold potential for targeted cancer therapies. Although the use of miRNA therapy in cancer treatment is promising, its effective and safe applications in patients is highly challenging. Breast cancer the most common cancer in women and the second leading cause of cancer related deaths. Triple negative breast cancer (TNBC) is highly aggressive, metastatic and the deadliest and incurable type of breast cancer. Significant heterogeneity with 6 genetically defined subtype has prevented development of targeted therapeutics for TNBC. The chemotherapy remains as a mainstay treatment, however only 30% of the patients achieve remission and most patients develop resistance and relapse. To develop highly effective targeted therapeutics and prolong patient survival novel molecular targets needed to be identified. To specifically target oncogenes such as EF2K and KRAS we identified developed microRNA-based nanotherapeutics. We demonstrated that these microRNAs TNBC and pancreatic tumors in mice, inhibit EF2K gene and KRAS suppresses tumor growth with no toxic or side effects in mice, suggesting that this technology may be used clinical translation to patients for Phase 1 clinical trials. Overall, the talk will focus the current state of targeted therapies and development of successful novel RNA-based nanotherapeutics which is considered a novel era of targeted therapeutics in treatment of human cancers and diseases.

*Invited Speaker - 06*

Tumor Genomic Profiling and Treatment Decision in Gastrointestinal Cancers

Özlem Er

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Tumor genomic profiling, also known as molecular profiling, is a process where the genetic makeup of a tumor is analyzed to identify specific genetic alterations, mutations, or abnormalities within cancer cells. This information can help oncologists make more personalized treatment decisions for patients with gastrointestinal cancers and other types of cancer.

In the context of gastrointestinal cancers (which include cancers of the esophagus, stomach, liver, pancreas, colon, rectum, and other digestive organs), tumor genomic profiling can have several important implications:

Targeted Therapies: Genomic profiling can identify specific genetic mutations or alterations that are driving the growth of the cancer.

Immunotherapy: Some genomic alterations may make tumors more susceptible to immunotherapy drugs like checkpoint inhibitors (e.g., pembrolizumab, nivolumab). Genomic profiling can help identify patients who may benefit from immunotherapy.

Prognosis: Certain genetic mutations can provide information about the prognosis and aggressiveness of the cancer, which can guide treatment decisions and help patients and doctors understand the likely course of the disease.

Clinical Trials: Genomic profiling may reveal opportunities for patients to participate in clinical trials testing new targeted therapies or experimental treatments based on their tumor's specific genetic profile.

Personalized Treatment Plans: With the information obtained from genomic profiling, oncologists can develop more personalized treatment plans that take into account the unique characteristics of the patient's cancer. This can lead to more effective and less toxic treatment options.

In summary, tumor genomic profiling is an important tool in the field of oncology, helping oncologists tailor treatment strategies to the individual characteristics of a patient's cancer, ultimately aiming to improve treatment outcomes and minimize side effects.

*Invited Speaker - 07*

## Precision Medicine in Pediatric Tumors: Next Generation Sequencing Experience

Safiye Aktas

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Molecular methods are gaining importance with increasing momentum in the diagnosis and treatment of cancer. Next-generation sequencing (NGS) method has provided the opportunity to examine the status of multiple genes in a short time. It is studied somatically in solid tumour tissue. In addition, if necessary, germline studies can be performed on peripheral blood mononuclear cells. In this speak, we will discuss mutations of relapsed or refractory paediatric tumour patients that were examined by next generation sequencing (NGS) for targeted therapy related specific gene mutations. Most of the cases are neuroblastoma (NB) patients who underwent NGS for 60 gen cancer DNA panel and fusion panel. The cases were among 1965 neuroblastic tumours diagnosed, risk stratified, treated according to INSR protocols in Turkey. Among these cases, the patients that recurred even after multi model therapies, are requested NGS to evaluate for targeted therapy decision. We will also discuss the importance of the pan cancer panel investigated with NGS in paediatric tumours that are rare, have differential diagnosis problems, and have an aggressive course. NGS pan cancer panel cases applied in paediatric cancers with diagnostic difficulties were included.

We study single nucleated variations after DNA isolation using Pillar Onco/Reveal Multi cancer v4 with CNV Panel with 60 genes (ALK, BRAF, ERB2, PIK3CA, EGFR, KRAS, MET, etc.) on İllumina Miniseq platform and Pillar RNA fusion panel. The mutations were statistically evaluated with clinic pathologic data. Library preparation, sequencing, quality control evaluation, alignment, laboratory validation, identification of variants, variant annotation visualization, and prioritization/filtering were applied. In cases that microsatellite instability and tumour mutation burden are required, we study 500 gene pan cancer panel. Illumina MiniSeq device was used for next generation sequencing. Pan Cancer Panel, 405 gene Celemics kit was used. Peripheral blood MN cells were studied in cases where paraffin tumour tissue samples were required. Samples were run according to the manufacturer's instructions. Quality and quantity were evaluated after DNA isolation. After the desired amount of DNA was obtained, the library preparation stage for the NGS device was started. For this purpose, NGS kit based on Target Capture was used. We worked with Genomize company for bioinformatic analysis Raw reads (FASTQ), Alignment/Gene mapping (BAM), Variant calling (VCF) Single nucleotide changes, Deletions, Insertions were investigated. Microsatellite instability levels were evaluated. Tumour mutation burden was evaluated.

Most common mutation in NB was ERBB2 (I655V), (39.65%). We detected ALK mutations which has indication for crizotinib or alectinib. Out of our investigated patients 29.31% had ALK mutations F1174L, R1275Q in common and rare mutations in tyrosine kinase domain were also detected (H1124R, G1125S, A1126T, V1135A, L1152R, I1171T, S1189F, E1197K D1203G, T1211P, R1214C, P1215L, V1229L, E1241G, H1244R, and F1271L). Fusion mutations of NTRK3, ROS1, RET, FGFR3, ALK were observed in 19.64% of the cases. Pan cancer panel was studied in 9 rare paediatric cancer cases out of 180 cases included in molecular analysis. Presence of DICER-1 mutation in kidney tumour anaplastic sarcoma case contributed to the diagnosis. The contribution of DNA repair gene-associated mutations and additional mutations in DICER1 to carcinogenesis has been described when compared with cystic areas of the kidney without malignant tumours. Detection of DICER1 mutation in a case of ovarian juvenile granulosa cell tumour contributed clinically in terms of genetic predisposition. The importance of mutations in the differential diagnosis of aneurysmal bone cyst and telangiectatic osteosarcoma has been questioned.

Our patient cohort showed ALK mutations F1174L (sensitive to alectinib), R1275Q mutation (sensitive to crizotinib) in NB cases. Five of these patients received targeted therapies and had longer survival. Role of ERBB2 mutations, BRAF mutations, and ABL1 mutations should be explored. Pan cancer panel analysis (including SNV, CNV, MSI and TMB) with next generation sequencing has been found useful in paediatric cancers. It has made a significant contribution to reaching diagnosis, determining new treatment targets, understanding molecular pathogenesis, and predicting familial cancer susceptibility and genetic-related syndromes. The importance of tumour mutational burden in immunotherapy decision has not yet been proven.

Keywords: Paediatric tumours, precision medicine, next generation sequencing

*Invited Speaker - 08*

The molecular link between excitotoxicity and glioblastoma

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Introduction and Aim: Glioblastoma multiform is a primary brain tumor derived from glial cells. Glutamate accumulation in brain leads to excitotoxicity which leads to the death of neurons to create space for the growing glial tumor in brain. Excitotoxicity is an underlying molecular mechanism of all brain diseases. Glutamate Transporter 1 (GLT-1), Glutamine Synthatase (GS) and Glutamate Dehydrogenase (GDH) are major glutamate metabolism modulators that help the glutamate cycle function in brain. In our previous study, we showed that the mRNA expression of GLT-1 was significantly lower in primary brain tumors when compared to control brain tissues. GLT-1 expression was inversely correlated with the tumor grade, implicating its potential role in tumor progression (Donmez Yalcin et al. 2020; Akkulak et al. 2021; Dagdelen et al 2021). Sirtuins are metabolic enzymes that deacetylate or ADP-ribosylate enzymes and found to be related to age-related diseases.

In this study, we aimed to analyze the GLT-1 degradation pathway in glioblastoma and how it is regulated by SIRT4.

Materials and Methods: Molecular biology techniques such as western blotting, qPCR, immunoprecpitation and cell culture were used in the study.

Results: We showed that GLT-1 is dynamically regulated by SIRT4 in glioblastoma cell line. GLT-1 is ubiquitinated and degraded in the absence of SIRT4, which was found to play a role in the formation of GLT-1 oligomers leading to its functional form.

Conclusion: SIRT4 activators or inhibitors targeting GLY-1 ubiquitination might be studied to develop therapies against excitotoxicity. We keep on investigating the underlying molecular mechanisms which may help therapies against glioblastoma targeting excitotoxicity.

*Short Talk - 01*

## Breaking Barriers in Cancer Treatment: Unleashing the Power of Drug Repurposing

Esra Nalbat

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Cancer is a major global cause of death, with a projected 29 million diagnoses by 2040. Despite advancements in technology, the introduction of new drugs into clinical practice is experiencing significant delays. Additional clinical assessment is essential for determining dosage, ensuring safety, and evaluating effectiveness. Developing new cancer therapies traditionally requires a lengthy process including regulatory requirements, approval procedures, and commercial considerations. Thus, a promising alternative approach called “drug repurposing” has emerged to tackle this challenge. It involves discovering new therapeutic uses for known compounds or identifying novel targets for existing drugs. Leveraging existing knowledge provides improved efficiency, reduced time and financial investment, and a lower risk of failure.

Viewing diseases as networks of interconnected molecular pathways have paved the way for exploring the combined effects of multiple drugs. Combining repurposed drugs with chemotherapeutic agents has shown intriguing results, particularly in cases where standard anti-cancer monotherapy has limitations in terms of safety and tolerability. Drug combinations target various oncologic pathways simultaneously, leading to enhanced therapeutic efficacy and decreased likelihood of drug resistance development. The synergy achieved by combining drugs with different targets or signaling pathways allows for lower drug concentrations, further optimizing treatment outcomes.

This study discusses the significance of drug repurposing in cancer treatment and highlights recent advancements. We provide a comprehensive perspective on the role of drug repurposing in addressing the challenges of cancer therapy. Ultimately, drug repurposing holds great promise for improving patient outcomes and accelerating the success rate of drug discovery and development in oncology.

Keywords: Drug discovery and development, drug repurposing, combinatory therapy, synergism, cancer

*Short Talk - 02*

## An Overview of Tumor Agnostic Therapies Through the Hippo Signaling Pathway

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Tumor agnostic therapy means drug therapy that can be used in the treatment of all cancers, regardless of the type of tissue in which the cancer develops. This treatment is used when the tumor has a specific molecular alteration targeted by the drug or when the drug is predicted to work. If the cancer type has the specific molecular alteration that the drug targets, tumor agnostic therapy will work. The Hippo signaling pathway is evolutionarily conserved and is generally described as a pathway that regulates and controls growth. In most studies on the Hippo signaling pathway, it has been reported that the main task of this pathway is the control of organ development, stem cell function, regeneration and tumor suppression. However, some studies report that this pathway may also play a role in tumor initiation and progression in different types of cancer. Targeting the YAP1 protein, one of the most important components of this pathway, has recently been shown as an important treatment option. Today, drug repurposing through FDA-approved molecules and virtual activity scanning of libraries containing non-FDA-approved commercial molecules/compounds are frequently preferred methods. There are very few studies in the literature within the scope of the Hippo signaling pathway and tumor agnostic approach. As it had been reported that YAP1 expression can be used as a tumor agnostic predictive biomarker, in this presentation, a novel compound targeting the Hippo signaling pathway YAP1 protein in different cancer types and how these studies will progress in the future will be discussed.

*Short Talk - 03*

## Anti-Cancer and Anti-Stemness Action of Nonsteroidal Anti-Inflammatory Agents: A Special Focus on Hepatocellular Carcinoma

Deniz Cansen Kahraman

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Inflammation is strongly linked to cancer and plays a crucial role in the development and progression of tumors. Chronic inflammation is characterized by the infiltration of certain immune cells, tissue damage, fibrosis, elevated angiogenesis and is also linked to genomic damage, suppression of programmed cell death (apoptosis). Although nonsteroidal anti-inflammatory drugs (NSAIDs) have been used for many years in the treatment of acute and chronic conditions characterized by pain and inflammation, recent clinical investigations have shed light on the therapeutic potential of NSAIDs in cancer treatment. Numerous preclinical and clinical studies have documented that NSAIDs can have anti-cancer activities in many cancer types. Moreover, combining chemotherapeutic drugs with NSAIDs can greatly improve prognosis of patients and may be beneficial as adjuvants to conventional therapeutic approaches.

Liver cancer, in particular hepatocellular carcinoma (HCC) is one of the leading causes of cancer-related mortality worldwide and poses a significant challenge in terms of therapeutic options due to diagnosis at advanced stage, tumor heterogeneity and activation of resistance mechanisms. The vast majority of HCCs develop as a result of chronic inflammation where inflammation-mediated events such as production and release of cytokines, reactive oxygen species and the activation of inflammatory pathways takes place. This study focuses on current literature emphasizing the potential of NSAIDs as anti-cancer agents, and their mechanism of action with a special focus on HCC. Moreover, we discuss recent findings from our laboratory on novel and well-known NSAIDs against HCC cells and liver cancer stem cells.

Keywords: Nonsteroidal anti-inflammatory drugs (NSAIDs), hepatocellular carcinoma (HCC), liver cancer stem cells

*Short Talk – 04*

## The Role and Importance of Biobanks Dedicated to Cancer Research

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The development of efficient therapies against cancer necessitates better understanding of cancer molecular mechanisms and quicker translation of laboratory findings to clinical implications. To facilitate an effective cancer research; tumor tissue samples, blood and other body fluids are greatly required to be collected and stored accordingly. Biobanks, therefore, are at the cornerstone of cancer research since they allow obtaining a sufficient number of high quality samples, archiving them, allow re-usability of the sample without re-sampling over the years. The importance of biobanks in cancer research continuously growing since they provide high quality annotated bio-sample which can be used for biomarker discovery and validity, drug screening, identifying molecular mechanisms. Biobanks are also very critical for personalized medicine because biobanks and omics technology are very closely related. Biobanks assures harmonization to provide interoperability, so that, larger research projects with sufficient number of samples (even for rare cancers) collected from different biobanks can be conducted and duplications in research is reduced. The multidisciplinary work between different steakholders requires the adoption of common standards for each biobanking step. Biobanks are very important for facilitating logistics and infrastructure where biological samples and associated information are collected, processed, stored with quality assurance. Since biobanks works with human samples; ethical, legal and social (ELSI) issues are major topics to be considered. To sum up, the aim of this short talk is to understand the biobanking role in cancer research, discuss biobanking processes and challenges, indicate implications to overcome challenges and to long term sustainability in oncology research.

Keywords: Biobank, Bio-sample, Bio-repository, Cancer research, personalized medicine

*Short Talk – 05*

Cancer Resistance to therapy: Is there a way out?

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Introduction and Aim: Cancer hallmarks are indisputable and the key question here is; how malignant-cells acquire and coordinate together numerous modifications for a unique purpose of sustained growth? If the philosophy of cancerous behavior is not grasped; it will be difficult to find the real therapeutic target(s) needed to stop cancer growth. In clinics today, cancer resistance to different treatment modalities has been linked to a subpopulation of cells named cancer stem-cells (CSCs).

Materials and Methods: This work is based on the analysis of cancer hallmarks looking for insights as to the nature of cancer stem-cells. Two questions remain to be answered: (1) Are biomarkers currently used to identify CSCs proper to cancer stem-cells themselves or shared with other cell types? (2) How CSCs could rise?

Results: To efficiently target cancer stem-cells; we need to understand: (i) What shapes their molecular identity; (ii) This means we need to find biomarkers proper to cancer stem-cells that are not shared with other cell types; (iii) This leads to understand the molecular event that gives rise to cancer stem-cells. The protein model for cancer genesis may lead us into that direction.

Conclusion: There should be a clear-cut difference between cancer and non-cancer cells. Genetic instability is a hallmark of cancer therefore using various combinations of DNA mutation-based biomarkers as targets for cancer therapy has not resulted in cancer cure as patients hope for. The protein model offers a fresh perspective on the nature of CSCs and could pave the way for innovative cancer treatment and prevention.

Keywords: cancer hallmarks; cancer resistance; cancer stem-cells; cancer biomarkers; protein model for cancer genesis.

*Short Talk – 06*

How to target tumor heterogeneity to overcome drug resistance?

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With the development of targeted and personalized medicine, the genetic heterogeneity observed in cancer cells has gained importance especially in terms of therapy resistance and disease recurrence. Tumors are formed as a result of mutations in a single gene in a single progenitor cell. Subsequent mutations and waves of clonal expansion in the offspring of this cell lead to the development of daughter cells with a cancer-specific growth advantage. Genetic instability, which allows for sequential selection of more aggressive subspecies and is most easily acquired cytogenetically, leads to highly individualized human malignancies, karyotypic and biological. Thus, each patient's cancer may require specific individual treatment, and even this treatment may not result in the emergence of a treatment-resistant variant subtype.

Tumors may spontaneously become drug resistant or develop resistance to chemotherapy during treatment. Acquired resistance is one of the biggest obstacles to anticancer therapies. Certain mutations that occur in clones within a heterogeneous population activates drug resistance mechanisms by leading to various results such as inhibited drug transport, target change, and metabolic changes. It has been determined that resistance to chemotherapy causes treatment failure in more than 90% of patients with metastatic cancer, and it is thought that resistant micrometastatic tumor cells may reduce the effectiveness of chemotherapy in adjuvant therapy. In the future, it is anticipated that the power to predict tumor and patient response to cytotoxic drugs and to modulate this response with targeted therapies will allow the selection of the best combined therapy for each patient.

Keywords: tumor heterogeneity, drug resistance, anticancer therapy, targeted therapy

*Short Talk – 07*

Recent advances ın researchıng cancer types usıng 3d bıoprıntıng

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Introduction and Aim: Cancer is a type of disease of which the incidence is increasing significantly day by day. Pre- and post-clinical research for cancer prevention and treatment continues rapidly. Limitations in in vivo and in vitro methods and ethical concerns have led researchers to find alternative methods for cancer modelling. The printing of various human tissues using three-dimensional (3D) bioprinters has also been an important step for cancer research. In this study, it is aimed to summarise new approaches in the use of 3D bioprinting in cancer by scanning the current literature.

Materials and Methods: Using the keywords "3D bioprinting" and "cancer", studies in which 3D Bioprinting was used in cancer research between 2016 and 2023 in a variety of databases (PUBMED, JSTOR, IEEE Xplore, Science Direct, DOAJ) were scanned.

Results: To date, there are current studies in which 3D bioprinting has been used in mimicking the microenvironment and modelling of most cancer types such as melanoma, glioblastoma, breast, lung, colorectal, etc. and in drug trials for the disease.

Conclusion: In order to understand and treat cancer by using 3D bioprinting, the priority is to maximise the similarity of the designed tissue with the target tissue. Therefore, it is recommended to focus on mimicking the targeted cancer model with maximum similarity.

*Short Talk – 08*

Determination of intestinal microbiota in pediatric patients who underwent allogenic stem cell transplantation before and after transplantation

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Introduction and Aim: Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a curative treatment option (1,2). Preparation regimens and high-dose antibiotic use before allo-HSCT cause dysbiosis in the intestinal microbiota. In this study, it was aimed to determine the intestinal microbiota before and after allo-HSCT in 10 pediatric patients diagnosed with acute leukemia, thalassemia, aplastic anemia and primary immunodeficiencies and to investigate its relationship with the development of acute gastrointestinal-GVHD.

Material and Method: Gut microbiome were sequenced by 16S metagenomic analysis from the fecal DNA of patients.

Results: In children with malign and non-malign hematological diseases, Bifidobacterium and Bacteroides were found to be dominant in the pre-transplant microbiome. In patients with Primary Immunodeficiencies, in addition to Bifidobacterium, Actinobacteriota were found to be high. While dysbiosis was found in 2/3 of the patients in the hematological malignant patient group before allo-HSCT, dysbiosis was not found in the patients with Primary Immunodeficiencies. The fact that the patients had dysbiosis without any treatment in the study shows that the pathogenesis of the disease may be related to dysbiosis and at least, the dysbiosis status in these patients is not dependent on the preparatory regimens.

In the post-transplant microbiota analyzes, Bifidobacterium genus was seen predominantly. Actinobacterium and Firmicutes were observed the most, respectively. Acute gastrointestinal-GVHD did not develop in 2 patients with dysbiosis in the intestinal microbiota, while GVHD developed in 2 patients without dysbiosis.

Conclusion: It was concluded that studies with a larger patient population are needed in order to associate the gut microbiota with gastrointestinal-GVHD in patients undergoing allo-HSCT.

Keywords: Graft Versus Host Disease, Microbiota, Dysbiosis

*Short Talk – 09*

# THE EXPRESSION ANALYSIS OF SPECIFIC GENES IN OVARIAN CANCER

Ece Gumusoglu-Acar[1], Berkcan Dogan[2,3], Mehmet Ulas Bılır[4], Tugce Senturk-Kırmızıtas[5], Samet Topuz[6], Tuba Gunel[7]

Introduction and aim: Due to a lack of diagnostic and prognostic biomarkers, ovarian cancer (OC), the most lethal gynecologic malignancy, is frequently diagnosed at an advanced stage. Therefore, identification of OC specific biological markers is a vital step for diagnosis and treatment response. Our goal is to examine functional gene sets which are possibly markers for ovarian cancer and their expression profiles in OC patients. We also aim to determine the potential of the genes which could be possible therapeutic targets for OC patients.

Materials and methods: By using qRT-PCR, the expression profiles of seven genes (FOS, FOSL2, JUN, MMP-2, MMP-9, TIMP-2, and VEGFA) were identified. The tumor-free control group consisted of total abdominal hysterectomy (n=1) and bilateral salpingo-oophorectomy (n=9) patients who underwent gynecologic procedures. High-grade serous OC epithelial samples (n=10) were used for the experiment group.

Results: According to the qRT-PCR data, there is an increased expression of FOS (p=0.0089), MMP-9 (p=0.0029), VEGFA (p=0.0434) and decreased expression of FOSL2 (p=0.0271), JUN (p=0.0041), TIMP-2 (p=0.0062).

Conclusion: In conclusion, the data may produce new insights regarding OC pathogenesis and treatment. The candidate genes may improve individualized diagnosis and therapy for OC in the future.

Keywords: Ovarian cancer, gene expression, biomarker

*Short Talk – 10*

## Antioxidant and Anti-growth Properties of Selected Anatolian Plant Species: Exploring the Potential of Heracleum humile, Doronicum reticulatum, Centaurea drabifolia, and Senecio olympicus

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Introduction and Aim: Plants are increasingly acknowledged as potential sources of novel compounds for public health challenges like cancer. Yet, only a small proportion of higher plants have undergone biological investigation. The Anatolian region, notable for its vast floristic diversity, hosts over 12,000 vascular plant species with an endemism rate surpassing 30% [1]. This study investigated the anti-growth and antioxidant properties of four plant species native to the Anatolian region: Heracleum humile, Doronicum reticulatum, Centaurea drabifolia, and Senecio olympicus.

Materials and Methods: Plant materials were collected, authenticated, and extracted using a Soxhlet apparatus. The growth inhibitory activities of the extracts were evaluated in human breast cancer cell lines MCF-7 and MDA-MB-231, and the nonmalignant immortalized human breast cell line MCF-10A, using the sulforhodamine B (SRB) assay. Antioxidant capacities were assessed via DPPH and CUPRAC assays.

Results: The study revealed that the extract of C. drabifolia showed strong cytotoxic properties. In contrast, D. reticulatum exhibited selective toxicity and demonstrated the most significant antioxidant activity among the species assessed.

Conclusion: These findings contribute to our understanding of the therapeutic potential of these indigenous plant species in addressing various public health issues, including cancer.

Keywords: cytotoxicity, antioxidant, Heracleum humile, Doronicum reticulatum, Centaurea drabifolia, Senecio olympicus.

*Short Talk – 11*

## Cracking the Code of Drug Discovery: Technology's Impact on Novel Therapeutics

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The pursuit of novel therapeutic agents to combat a wide spectrum of diseases remains an enduring imperative in pharmaceutical research.

Historically, drug discovery relied on costly and time-consuming experimental methods. However, the integration of computational chemistry, molecular simulations, and data-driven approaches has revolutionized the landscape, significantly expediting the identification of potential drug candidates, shaped by advanced methodologies including molecular docking, computer-aided drug discovery, molecular mechanics energy calculations, virtual screening, shape screening, and data mining, now integral components of pharmaceutical research. These techniques constitute an indispensable toolset, fostering innovation and precision in the quest for therapeutic solutions. Researchers can now predict molecular interactions and evaluate drug candidates with unprecedented precision. The integration of artificial intelligence (AI) is transformative, with machine learning algorithms navigating vast datasets to pinpoint drug targets and anticipate interactions. The dynamic nature of drug discovery is underscored by perpetual innovation and technology's role in drug development, streamlining the identification and optimization of small molecules to combat diseases. Looking ahead, the anticipation of delivering targeted treatments for various diseases is vested in future small molecule drug candidates. Supported by computational modeling, molecular simulations, and AI-driven insights, these candidates have the potential to redefine therapeutic approaches, offering target-specific solutions with minimized side effects.

In conclusion, drug discovery is undergoing a profound redefinition, shaped by the synergy among computational chemistry, molecular simulations, data mining, and cutting-edge technologies. This abstract offers a glimpse into the promising future of pharmaceutical research, where innovation and technology converge to address pressing healthcare challenges.

Keywords: Computer-aided drug discovery, computational biology, artificial intelligence, target-specificity, molecular simulations, small molecules.

*Oral Presentation – 01*

# TAp73β regulates the Wnt/β-catenin signaling pathway in a zebrafish xenograft model in Hepatocellular Carcinoma

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Introduction and Aim: Hepatocellular carcinoma (HCC) is the most common type of liver cancer. Preclinical studies are essential in the identification of new targets for HCC treatments. However, most of the data obtained from in vitro studies and in vitro models have several limitations in predicting cancer's biology. Recently, zebrafish xenograft model has become popular because of its cost-effectiveness, in vivo dynamic visualization of tumor growth. This study aimed to investigate the interaction between TAp73β and Wnt/β-catenin signaling pathway in a HCC zebrafish xenograft model.

Materials and Methods: First, we tested the effect of TAp73β expression on β-catenin activation in HCC cell lines by western blot, immunofluorescence and luciferase studies. Then we examined the β-catenin expression and localization under the TAp73β expression in a zebrafish xenograft model. Finally, we performed a β-catenin rescue assay by ectopic expression of Axin-1, and we examined the effect of TAp73β-induced Wnt/β-catenin signaling pathway activation on the metastatic abilities of HCC cells using the zebrafish xenograft model.

Results: Our results showed that TAp73β significantly increased the expression of phospho-b-catenin (Ser675) and its nuclear localization in HCC cells. We also showed that TAp73β activated the Wnt/β-catenin pathway in HCC cells. In addition, overexpression of TAp73β overexpression increased the nuclear localization of active p-β-catenin (Ser675) in the zebrafish xenograft model. Moreover, overexpression of Axin-1 caused the degradation of β-catenin and inhibited TAp73β-induced metastasis

Conclusion: Consequently, our results indicate that TAp73β increases HCC cell metastasis through β-catenin activation in a zebrafish xenograft model.

Keywords: Hepatocellular carcinoma, p73, metastasis, Wnt/β-catenin pathway, zebrafish xenograft

*Oral Presentation – 02*

# Naive and TLR4 Stimulated Adipose Derived Mesenchymal Stem Cells Inhibit EMT and Metastasis of Pancreatic Ductal Adenocarcinoma Cells

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Introduction: In order to intervene in the dense desmoplastic tumor microenvironment, it is important to first elucidate the interactions between cells. To investigate the potential antitumorigenic effects of adipose-derived MSC(ADMSC) on the pancreatic ductal epithelial cell Panc-1, we aimed to investigate the effects of both proinflammatory and anti-inflammatory ADMSC phenotypes on EMT and metastasis.

Material and method: ADMSCs were treated with TLR4 agonist and antagonist. Pro-inflammatory and anti-inflammatory characters were determined according to their responses to cytokines. An indirect co-culture model was established using 0.4 μm inserts and Panc-1 and ADMSCs were cultured at a ratio of 1:10. Next, gene expression levels of CDH1, VIM, ZEB1 and CLDN1 were evaluated for EMT analysis. Analysis of vimentin and E cadherin proteins was also evaluated by immunofluorescence staining. Metastatic potential was also analyzed by gene expressions of MMP2, KDR, PLAU, MMP9, TIMP1, IGF2R and COL1A1.

Results: At the end of the 96h, naive and proinflammatory ADMSCs increased the expression of CDH1 and CLDN1 of Panc-1 cells and decreased the expression of VIM gene. In metastasis related genes, it significantly decreased the expression of MMP2, KDR, MMP9, TIMP1, IGF2R and COL1A1 genes, except for the PLAU gene. ADMSCs with anti-inflammatory character, showed opposite effects.

Conclusion: Both naive ADMSCs and proinflammatory ADMSCs were showed antitumor effects on Panc-1 cells. Anti-inflammatory ADMSCs were showed tumor promoting effects. Understanding the role of MSCs in the tumor microenvironment will be a guiding factor for the development of microenvironment-targeted therapeutic approaches in the future.

Keywords: Pancreatic cancer, Mesenchymal stem cell, TLR4, Tumor microenvironment.

This study was supported by Süleyman Demirel University Scientific Research Projects Coordination Unit with project number TDK-2022-8583. Ethical approval was obtained from the Lokman Hekim University Non-Invasive Ethics Committee with decision number 2021/136 (Code: 2012131).

*Oral Presentation – 03*

Investigating The Effects of Temozolomide on The Viability of Glioblastoma Stem Cells Responsible for Recurrence Using a 3D Culture Model

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Introduction and Aim: Glioblastoma (GB) is the most lethal brain tumor. The inevitable recurrence after initial treatment has been the most challenging for GB. The presence of GB stem cells (GSCs), which cause the recurrent GB untreatable and the main cause of recurrent, is currently lacking effective therapeutic options. In this regard, we aim to evaluate the behaviors of GSCs in a more realistic approach by utilizing a 3D scaffold for the brain environment and develop a more effective treatment by targeting GSCs.

Materials and Methods: We developed a 3D scaffold using bacterial cellulose that incorporates hyaluronic acid and collagen for 3D culture studies. The structure of the scaffold was demonstrated through FTIR and SEM analysis. The attachment of GSCs to the scaffold was demonstrated using Ki67, Nestin, and DAPI staining techniques. GSCs were seeded using a suitable medium cocktail for 2D and 3D scaffold cultures. The main aim of this experimental design was to compare the Temozolomide (TMZ) dosage in 2D and 3D cultures; thus, cell viability was determined using Live&Dead analysis.

Results: The data demonstrate that when 2D and 3D GSC cultures are treated with TMZ, the dosage required for 3D cell culture is four times higher than that for 2D cell culture. This result significantly indicates the impact of the cell environment on their viability.

Conclusion: Creating 3D environments that closely mimic reality can bridge the gap between clinical and in vitro experiments, improving treatment efficacy.

Keywords: glioblastoma stem cells, 3D culture, Drug exposure, Tumor microenvironment

Supported by TUBITAK 120N087.

*Oral Presentation – 04*

# Drug Repositioning to Specifically Target Multıple Myeloma Subtypes Based *in silico* Investigation of Differentıal Gene Expression Profiling

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Introduction and Aim: Multiple myeloma (MM) is a malignancy that develops in a kind of white blood cell known as a plasma cell. The aim of this study is to propose a new treatment for MM and its genetically distinct subtypes which clinical treatment success rate is still low. This project used bioinformatics analysis to reposition existing licensed drugs in accordance with differential gene expression profiles discovered from patient-specific datasets by using NCBI-GEO in order to find potential pharmacological roles for MM.

Materials and Methods: Gene Expression Omnibus 2R (GEO2R) was used to analyze top differential genes in patients. Subsequently, for each comparison, a STRING database was created and transferred to Cytoscape for representing protein-protein interactions. Drugs capable of inhibiting hub genes identified for each comparison were recorded using DrugBank and PubChem. Finally, in silico toxicity analysis was performed to determine whether the drug is appropriate for usage by using SwissADME.

Results: The most up-regulated genes were determined for MM and its subtypes. The protein-protein interaction maps of the most up-regulated 150 genes were created and reduced to 10 hub genes. A total of 227 candidate drugs were identified for targeting these hub genes. Drugs that target the selected gene, but are FDA approved under MM or any cancer types are not included in the study. According to the mentioned parameters and toxicity analyses, the candidates were reduced to 16 drugs.

Conclusion: Finally, the candidate drugs currently in use with non-cancer purposes are promised for each MM subtype.

Keywords: DEG, drug repositioning, multiple myeloma, toxicity

*Oral Presentation – 05*

Exploring New Frontiers in HGSOC Treatment: Targeting Drug Resistance with miRNA Mimics

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Introduction and Aim: High-grade serous ovarian cancer (HGSOC) is the predominant histological subtype of epithelial ovarian cancer. Despite the success of first-line chemotherapy, and approval of PARP inhibitors, significant portion of patients develop resistance leading to poor outcomes. Therefore, identifying regulators of drug resistance and new treatment strategies is essential.

MicroRNAs (miRNAs) have been shown to play significant roles in cancer progression by acting as either tumour suppressors or oncogenes. However, the specific targets of miRNAs in ovarian cancer remain largely unknown. Therefore, this study aimed to explore the role of miRNAs in drug response, and resistance in HGSOC.

Materials and Methods: 40 FFPE samples of patients with HGSOC were used, and miRNA expression was analysed using microarray and qRT-PCR assays. Resistant HGSOC cells were generated through a stepwise dose-escalation method. miRNA-mimics were transfected into the resistant cells, and their interactions with conventional therapies were examined.

Results: The study found that let7b-5p and 188-5p were significantly downregulated in both the resistant FFPE samples and *in vitro* resistance models. Based on the results of combinational drug and miRNA-mimic treatment, upregulation of miR188-5p using miRNA mimic resensitize Olaparib resistance cells and increase apoptotic cell death.

Conclusion: This study sheds light on the role of miRNAs in drug resistance, and progression in HGSOC. The findings of this study may contribute to the development of alternative treatment options for HGSOC, improving patient outcomes.

Keywords: HGSOC, miRNA, drug-resistance

*Oral Presentation – 06*

Flavopiridol Inhibits Cell Proliferation and Migration in Pancreatic Ductal Adenocarcinoma Cells

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Introduction and Aim: Flavopiridol, a semi-synthetic flavonoid analog of an alkaloid called Rohitukine, was shown to have anti-inflammatory and antioxidant effects. Although it is known to be a pan-CDK inhibitor, its detail cellular mechanisms related to the anti-tumorigenic potential are still unclear. Here, we aimed to investigate the therapeutic potential of flavopiridol in pancreatic ductal adenocarcinoma (PDAC) cells through cell proliferation and migration.

Materials and Methods: MiaPaca-2 and Panc-1, as human PDAC cell lines, and HPDE, as a non-tumorigenic human pancreatic ductal epithelial cell line, were used in cell proliferation and migration analysis. MTS assay were performed in MiaPaCa-2, Panc-1, and HPDE cells treated with 5, 10, 25, 50, 100, 200, 400, 500, 600, 800, 1000 nM doses of flavopiridol for 24h, 48h and 72h. To confirm the cyclin inhibition and investigate the anti-metastatic potential, Cyclin D1 mRNA expression and migration were analyzed by RT-PCR and wound healing assay, respectively, in Panc-1 cells.

Results: Based on our MTS results, flavopiridol significantly inhibits the PDAC cell proliferation, but not HPDE cells, especially in 100 nM and 200 nM doses for 48h. Flavopiridol mediated Cyclin D1 inhibition was almost completely proven by RT-PCR in Panc-1 cells with 100 nM and 200 nM doses for 48h. Additionally, flavopiriol decreased the cell migration in Panc-1 cells with enhanced dose and time dependent manner.

Conclusion: Flavopiridol is a powerful anti-tumorigenic, anti-metastatic and anti-proliferative agent for PDAC cells. However, its specific detail mechanisms of action need to be investigated in pancreatic cancer.

This study was supported by Suleyman Demirel University, Scientific Research Projects Coordination Unit with project number TDK-2021-8312 and supported as a PhD fellow under TUBITAK Priority Areas (2211-C) Domestic Scholarship Program and supported by 100/2000 YÖK PhD fellow in the thematic field of “Molecular Oncology”.

*Oral Presentation – 07*

# Sphingosine 1-Phosphate Signaling in Midostaurin Resistance in FLT3-ITD Positive Acute Myeloid Leukemia

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Introduction and Aim: ITD mutation in the FLT3 gene, found in 20-25% of AML cases, leads to continuous activation of FLT3, promoting cell proliferation and inhibiting apoptosis. Midostaurin, an FDA-approved drug targeting FLT3-ITD, is used for AML treatment. Resistance to treatment poses a challenge in the clinic. This study aims to explore a novel approach by targeting SK-1, an anti-apoptotic sphingolipid with roles in multi-drug resistance, in combination with midostaurin.

Materials and Methods: Basal levels of SK-1 was evaluated in midostaurin resistant and sensitive FLT3-ITD+ AML cells. The antiproliferative effects of SK-1 inhibition combined with midostaurin on resistant and sensitive cells were investigated. Combination indexes were analyzed to determine whether SK-1 inhibition enhances the efficacy of midostaurin to overcome resistance. The apoptotic effects of the combinations were evaluated through caspase-3 and PARP activation.

Results: The resistant cells exhibited elevated levels of SK-1 compared to the sensitive cells. Compared to midostaurin treatment, midostaurin and SK-1 inhibitor combination significantly decreased cell viability in midostaurin resistant cell lines. Synergistic effects of the combination treatment were observed in both midostaurin resistant and sensitive cells. Apoptosis induced via cleaved PARP and cleaved caspase-3 levels in combination group compared to control and midostaurin treated group.

Conclusion: Our findings show that SK-1 plays an important role in drug resistant in FLT3-ITD+ AML cells. Targeting SK-1 successfully increases the efficacy of midostaurin in midostaurin resistant cells and induces apoptosis in both resistant and sensitive cells making it a potential therapeutic target to overcome acquired midostaurin resistance.

Keywords: FLT3-ITD, FLT3 inhibitor, sphingosine kinase 1, drug resistance, apoptosis

*Oral Presentation – 08*

Evaluation of CSRP1 expression as a prognostic marker in colorectal cancer

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Introduction and Aim: Colon cancer is a leading cause of cancer-related death worldwide. Despite developments in the clinical management of this disease, currently, around 60% of patients with colon cancer are expected to survive 5-years. In this study we aimed to evaluate cysteine-rich protein 1 (CSRP1) which encodes a member of the cysteine-rich protein family as a prognostic marker in colorectal cancer.

Materials and Methods: Publicly available bulk and single-cell transcriptomic data (scRNA-seq) of colon tumors were downloaded from GEO database and GDC data portal. Raw data processing was performed using RMA and DESeq2 methods for microarray and bulk RNA sequencing data, respectively. scRNA-seq data was processed via Seurat R package.

Results: Bioinformatic analyses showed that high expression of CSRP1 was associated with poor overall and recurrence-free survival. Multivariate analysis revealed that CSRP1 expression was a significant poor prognostic predictor independent of MSI status, TNM stage and KRAS-BRAF mutation status. CSRP1 expression had significant positive correlation with the expression of mesenchymal markers ($p<0.001$). When the consensus molecular subtypes of colon cancer (CMS) was considered (1), CMS4 type tumors had the highest CSRP1 expression. Analysis of a scRNAseq dataset (GSE178318) of colon tumors revealed that CSRP1 was expressed mainly by epithelial cells and CAFs (unpublished data).

Conclusion: CSRP1 expression was associated with a mesenchymal and aggressive molecular profile in colorectal cancer. The prognostic value of this putative biomarker needs to be validated in independent cohorts.

Keywords: Colorectal Cancer (CRC), CSRP1, prognosis, transcriptomics, bioinformatics

*Oral Presentation – 09*

Dual Targeting of Glycolysis and Glutaminolysis as a Strategy to Inhibit Tumor Cell Proliferation

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Introduction & Aim: Activating mutations of the oncogenic-KRAS is common in several types of tumors, including pancreatic ductal adenocarcinoma (PDAC). Increased glycolysis and glutaminolysis are two prevalent metabolic phenotypes that can be controlled by activated KRAS signaling. Given that a recent study showed that KRAS induction sensitizes pancreatic ductal epithelial cells to dual inhibition of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 (PFKFB3) and glutaminase (GLS1), key enzymes of glycolysis and glutaminolysis, respectively, we aimed to examine whether co-targeting of PFKFB3 and GLS1 exhibits an antiproliferative effect on tumor cells lines of various origin with or without mutant KRAS.

Material & Methods: PFKFB3 and GLS1 activities were inhibited pharmacologically using AZ-PFKFB3-26 and CB-839, respectively. Cell proliferation was determined by crystal violet staining. Western blot was used to analyze PFKFB3 and GLS1 proteins. Fructose-2,6-bisphosphate (F2,6BP) levels were analyzed using an enzyme-coupled assay.

Results: Simultaneous inhibition of GLS1 and PFKFB3 exhibited a much greater anti-proliferative effect on HCT116 and Mia PaCa-2, both of which harbor KRAS mutations, and H1299, which has a wild-type KRAS, than either inhibitor alone. While short-term (8 hours) GLS1 inhibition did not affect PFKFB3 protein levels, PFKFB3 inhibition decreased GLS1 protein levels, suggesting a functional interaction between PFKFB3 and GLS1. Further, although GLS1 inhibition did not affect PFKFB3 protein levels, it reduced F2,6BP levels, suggesting that GLS1 activity may be required for a fully active PFKFB3.

Conclusion: Co-inhibition of PFKFB3 and GLS1 may prove effective in preventing tumor cell proliferation, regardless of KRAS mutation status.

Keywords: Pancreatic adenocarcinoma, KRAS, PFKFB3, GLS1

*Oral Presentation – 10*

Effect of PKR Kinase Activation on the sensitivity of choriocarcinoma cells to chemotherapy agent

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Introduction and Aim: Choriocarcinoma is a gestational disease that originates from trophoblasts, is a malignant tumor with proliferation of abnormal placental trophoblast cells. We aim to determine how proliferation, apoptotic properties will change as a result of PKR activation. we investigated whether PKR activation can be an alternative combination strategy in cancer treatment, by determining the sensitivity of choriocarcinoma cells to the chemotherapeutic agent doxorubicin during PKR activation.

Materials and Methods: In choriocarcinoma cells , poly I:C LPS were treated to activate PKR, cell viability was determined using a cell counter to determine the effect of doxorubicin. Viability, apoptosis pathways, necrosis were examined by looking at the effect of doxorubicin in cells in which we created PKR activation by poly I:C/ LPS treated swan 71 cells by performing flow cytometry experiment. Western blot analysis was applied to determine the protein-level expression of p-PKR in Swan 71 cells.

Results: Doxorubicin was found to dose-dependently inhibit cell viability from swan 71 cells. It was found that sensitivity to doxorubicin in swan 71 cells treated with Poly I:C /LPS showed resistance to PKR activation compared to controls. Upon further investigation we determined that doxorubicin killed cells by apoptosis. PKR activated cells were moderately less sensitive to doxorubicin compared to controls. Interestingly, we observed that PKR phosphorylation greatly inhibited in the cells treated with doxorubicin upon Poly I:C, LPS treatment.

Conclusion: It has been observed that PKR activation increase resistance to the chemotherapy drug doxorubicin in choriocarcinoma cells.

Keywords: PKR activation, choriocarcinoma, apoptosis

*Oral Presentation – 11*

Investigation of the Effects of Chloroquine on Proteasomal System in Glioblastoma Stem Cells

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Introduction and Aim: Glioblastoma (GBM) is a highly lethal and aggressive malignant disease of the brain. The success rate of standard therapy is quite low because of the recurrence. The main reason for the recurrence of the neoplasm is the remaining glioblastoma stem cells (GSCs) in the resected area. It is predicted that a treatment plan based on the stem cells will increase the efficacy of GBM treatment. Chloroquine (CQ) is an autophagy inhibitor drug and many studies have shown that it has beneficial effects in various malignancies. Since one of the most important regulators of cell homeostasis is the closely linked proteasomal system and autophagy pathways, we aimed to investigate the effects of CQ on the proteasomal system of GSCs.

Materials and Methods: GSCs were cultured with appropriate medium cocktails. Cells were treated with CQ. Proteasomal activity assay and western blotting were performed to analyze the proteasome subunits expression levels.

Results: CQ treated GSCs expressed lower levels of proteasomal α4, α6, β1, β2, and β5 subunits. In addition, the cells showed some decrease in K48 polyubiquitination. In parallel with these findings, the cells showed a decrease in their proteasomal activity, as expected.

Conclusion: The proteasomal system and autophagy pathway enhance tumor cell survival under treatment conditions. The results show that CQ attenuates the activity of the proteasomal system within the cell. A potential GBM therapeutic approach involving CQ may hold promise for patients in the future.

Keywords: Glioblastoma, Glioblastoma Stem Cells, Chloroquine, Proteasome, Proteasomal Activity

*Oral Presentation – 12*

Indoximod Restricts Tumor Growth by Targeting Breast Cancer Viability

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Introduction and Aim:Breast cancer is the second most common cancer in women having potential to metastasize through the lymph and blood circulation.IDO(Indoleamine 2-3 dioxygenase) is an enzyme highly expressed in neoplastic cells and tumor leukocytes.Enhanced IDO activation in different cancer cells causes T cells to lose their proper function.Nowadays,IDO inhibition has received much attention in the field of cancer immunotherapy.Indoximod,an IDO inhibitor involved in the regulation of immune responses.In this study,we hypothesized that Indoximod restricts tumor growth by targeting cell viability in metastatic breast cancer.Herein,we aimed to interpret the possible effects of IDO inhibition on tumor cells and changes in immune responses.

Material and Methods:*In vitro* and *in vivo* experiments were designed using 4T1 metastatic breast cancer cell line.4T1 cells were supplemented with TNF-α for mimicking tumor microenvironment.Annexin V staining was performed to investigate apoptosis.For *in vivo* experiments, Control(n=5),4T1(n=10) and 4T1+Indoximod(n=10) groups were generated.$1x10^6$ cells were orthotopically injected into the mammary tissues of Balb/C female mice.One week later,Indoximod was administered intraperitoneally twice daily.Tumor sizes were measured twice a week.29 days after tumor injection, blood was collected from the eye socket of the animals. Peripheral blood smear analysis was performed to evaluate the immune responses. Moreover, primary and metastatic tissues were excised and analyzed.

Results: Indoximod significantly reduced 4T1 cell viability by inducing apoptosis. Tumor sizes were reduced after Indoximod injection in experimental model.We observed less metastatic regions in group 4T1+Indoximod compared to group 4T1.Moreover,enhanced immune responses in group 4T1 were detected,while immune responses were reduced in group 4T1+Indoximod.

Conclusion: Indoximod induces apoptosis thereby restricting tumor growth and metastasis.

Keywords: Breast cancer, 4T1, Indoximod, Apoptosis, Metastasis

*Oral Presentation – 13*

# Apoptotic Effects of Paclitaxel/Aloe-Emodin Combination in MCF-7 and MDA-MB-231 Breast Cancer Cell Lines

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Introduction and Aim: Cancer is a group of diseases characterized by uncontrolled cell proliferation and has different clinical manifestations and treatments. Breast cancer is the second leading cause of cancer-related deaths worldwide, following lung cancer. Due to the high cost and concerning side effects of conventional cancer treatments, there has been a growing interest in natural and herbal alternatives. Many cancer treatment studies are focused on drug discovery from herbal compounds. In this study, we aimed to investigate the apoptotic effects of aloe-emodin and paclitaxel on estrogen receptor-positive (MCF-7) and estrogen receptor-negative (MDA-MB-231) human breast cancer cell lines.

Materials and Methods: We assessed the effects of combined treatment with aloe-emodin, a herbal anthraquinone derivative, and paclitaxel on cell death through apoptotic pathways in MCF-7 and MDA-MB-231 cells. This was accomplished by analyzing annexin V binding, total caspase activity, and cell cycle distribution.

Results: The combination treatment of paclitaxel and aloe-emodin significantly induced apoptosis in MCF-7 and MDA-MB-231 cell lines, leading to enhanced cell death and cell cycle arrest.

Conclusion: Combining plant-derived compounds with cytotoxic agents to achieve more effective results in cancer treatment is a widely explored approach. However, there is a lack of sufficient studies on the combined use of aloe-emodin and paclitaxel in the literature. Hence, our study holds significant importance in this context.

Keywords: Aloe-emodin, Apoptotic effect, Breast cancer, Paclitaxel

*Oral Presentation – 14*

miR-30b-3p regulates apoptosis in CD8+ T lymphocytes in triple negative breast tumor bearing mice

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Introduction and Aim: Breast cancer had been regarded as immune-cold cancer type until the last decade. Recent studies have revealed breast cancer exhibits different immunological states based on subtypes. Especially, triple negative breast cancer (TNBC) stands out immunologically. Despite the recognized role of miRNAs as key regulators in immune cells, studies on breast cancer remain limited. This study aimed to investigate miRNA profiles in CD8+ T cells during the formation and remission of TNBC in vivo model.

Materials and Methods: TNBC model was established with 4T1 cells and Balb/c mice. Spleen-derived CD8+ T cells were isolated from control, primary tumor and remission groups. miRNA profiling was performed with microarray. miRWalk3.0 target prediction and WebGestalt-KEGG pathway enrichment analysis tools were used. Validations of miRNAs and mRNAs were performed by qPCR. Detection of apoptosis was done by flow cytometry.

Results: Microarray analysis showed that mmu-miR-30b-3p emerged as the most significant differentially expressed common miRNA among the comparisons. qPCR analysis confirmed its upregulation in primary tumor group, while expression of miR-30b-3p was low in remission group. Bioinformatic analysis revealed miR-30b-3p regulates apoptosis, particularly by targeting anti-apoptotic genes BCL-2 and BCL-XL. Validation experiments showed that decreased expression of BCL-2 and BCL-XL in CD8+ T cells from primary tumor group resulted in increased apoptosis. Conversely, remission group showed elevated expression of target genes, suggesting suppression of apoptosis in CD8+ T cells.

Conclusion: mmu-miR-30b-3p may contribute to immune evasion of tumor cells in TNBC through apoptosis and could serve as an immunological biomarker for CD8+ T cell responses against tumor cells.

Keywords: Triple-negative breast cancer, Adaptive immune system, miRNA

*Oral Presentation – 15*

PTEN R234W Variant: A Novel Case Presentation in Non Small Cell Lung Cancer Patients

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Introduction and Aim: PTEN is a strong tumor suppressor gene, with mutations being found in around %5 of NSCLC cases. The aim of this study is to investigate mutations and prognosis of NSCLC harboring PTEN mutations.

Materials and Methods: A total of 24 patients with NCSLC who had not yet undergone chemotherapy were recruited for the study between 2021-2023. We conducted liquid biopsy analysis using the Onco/Reveal-cfDNA-Multicancer Panel by NGS. To predict variants deleterious effects, we used SIFT(v5.2.2) and PolyPhen-2(v2.2.2) in silico tools via Pivat software. I-Mutant2.0 software evaluated aminoacid substitution's impact on protein stabilization.

Results: In our study, the frequency of PTEN-R234W was observed in %16,7(4/24) of the cases, and histologically, it was detected in %25(4/16) of the cases diagnosed with SCC. It has been associated with hereditary diseases. In our study, no hereditary disease was identified in the epicrisis of stage 3A cases 8th and 17th carrying this variant. Additionally, the epicrisis of case 18th(stage-4A) and 22nd(stage-4B), both with this mutation, could not be accessed. According to the PolyPhen2 and SIFT analysis programs, the potential effect of this variant on protein function was determined to be probably damaging(0.971) and tolerated(0.12), respectively. Due to the I-Mutant analysis, it was observed that this SNP has a destabilizing effect on protein stabilization.

Conclusion: The R234W variant is the first SCC report associated with the oncogenesis process. This variant has been reported as a VUS in the Clinvar databases. Therefore, further studies are needed to determine its clinical significance.

Keywords: non-small cell lung cancer, in silico tools, clinical significance, liquid biopsy

This study was supported by Suleyman Demirel University, Scientific Research Projects Coordination Unit with project number TDK-2021-8346 and by 100/2000 YÖK PhD fellow in the thematic field of “Molecular Oncology”.

*Oral Presentation – 16*

Expressions of GSTO1, GSTP1, GSTM1, GSTS1 isoenzymes in NSCLC tissues

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Introduction and Aim: Glutathione S-Transferases (GSTs) are Phase II enzymes which are involved in metabolism of various xenobiotics including the drugs. GSTP1 and GSTM1 isoenzymes have the role of protecting the lung tissue by catalyzing the conjugation of carcinogens with glutathione. In this study, we investigated the immunohistochemical staining characteristics of GSTOmega (GSTO1), GSTpi (GSTP1), GSTmu (GSTM1), p38, bcl-2 and caspase-3 in adenocarcinoma (n=20) and squamous cell carcinoma (n=20) lung tumor tissues from 40 patients.

Materials and Methods: Non-small cell lung cancer (NSCLC) tissues of patients were compared according to their immunohistochemical staining intensity from the patients. Relationships between GSTO1, GSTP1, GSTM1, p38, bcl-2 and caspase-3 expressions in carcinoma tissue were examined by the Mann Whitney-U test, and the clinicopathological data were examined by the Spearman correlation rank test.

Results: The results showed that GSTO1, GSTP1, GSTM1, p38, bcl-2 and caspase-3 expressions were significantly higher in lung tumor group than benign lung group ($p<0.01$). However, *no statistically significant differences* in the level of GSTSigma1 protein expression between tumor and benign lung groups ($p>0.05$). p38, caspase-3, bcl-2 and GSTSigma expressions were positively correlated in the tumor group ($p<0.01$). The higher expressions of GSTP1, GSTM1 and caspase-3, p38 in tumor group could be important in lung cancer progression and development.

Conclusions: As a result, the difference of the GSTO1, GSTP1, GSTM1 isoenzymes expressions between the groups show that they play an important role in the diagnosis of NSCL carcinoma.

Keywords: GST, Non-Small Lung Carcinoma, Apoptosis

*Oral Presentation – 17*

Generating single-barcode harbouring cell lines from chemotherapy resistant Caco-2 cell line to study drug resistance.

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Introduction and Aim: Resistance to cancer therapeutics can inevitably lead to treatment failure through the selection of drug resistant clones. Quantifying the drug resistance has begun to be possible with the advent of single-cell barcoding approach whereby frequencies of selected clones harbouring unique cellular barcodes can be determined. It was aimed to establish single barcode harbouring chemotherapy-resistant Caco-2 cells to characterize barcode frequencies.

Materials and Methods: Lentiviral barcode library was used to integrate cellular barcodes into initial Caco-2 cell line before the establishment of their chemotherapy-resistant derivatives. To identify the mechanism of resistance, whether pre-existing or de novo drug resistance was in place, amplicon-based NGS approach was carried out. Drug resistant barcoded Caco-2 cells was faced to single cell dilution assay to establish a new cell line from harbouring a single barcode. Barcode characterization and validation of single barcode in a newly generated drug resistant Caco-2 cell lines was validated.

Results: The results demonstrate a cellular barcoding technology incorporated with single-cell dilution approach to establish single-cell derived colonies under the chemotherapeutic selection pressure in Caco-2 cells. The barcoding approach show the frequencies of barcode enrichment under drug resistant derivatives of Caco-2 cells. Moreover, unique-barcode harbouring drug-resistant Caco-2 single cell-derived cell lines exhibited the suitability of this experimental model system to study drug resistance at the single-cell resolution.

Conclusion: The power of monitoring drug resistance at the single cell level with the advent of recently developed cellular barcoding technology provides capacity to exploit the tumour’s vulnerability.

Keywords: drug resistance, barcoding

*Oral Presentation – 18*

The Effect of Boron Compounds on Androgen Signaling in Prostate Cancer

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Introduction and Aim: Progression of prostate cancer is largely dependent on androgen receptor (AR) and AR related signaling pathways. Second-generation non-steroidal anti-androgens (enzalutamide) used for androgen ablation therapy have some side effects and moreover they often lead to the formation of highly aggressive castration-resistant tumors. Therefore, it is extremely important to develop new therapeutic approaches that will prevent the formation of resistant tumors. Studies have reported that boron compounds are promising agents in the treatment of prostate cancer. In this context, our study aimed to investigate the efficiency of boric acid and patented boron compounds in the regulation of AR signaling pathway and target genes.
Material and Methods: For this purpose, the effect of boron compounds on AR and its target genes such as PSA, NKX3.1 and NFκB was investigated by MTT, immunoblot, immunoprecipitation and qRT-PCR.
Results: In our results, it was determined that patented boron compounds have a higher cytotoxic effect and more importantly they act as a chemosensitizer by increasing the efficacy of enzalutamide. Furthermore, it was found that M7m reduced the level and activation of AR and its target genes most effectively. However, we observed that M7m inhibits AR nuclear translocation and also increases its degradation. Eventually, it was observed that M7m leads to a decrease in mRNA levels of the target genes.
Conclusion: It was concluded that blocking the AR signaling pathway, which plays a critical role in the development of castration-resistant prostate cancer, via boron compounds may offer an important therapy option.

Keywords: Prostate Cancer, Boron Compounds, Enzalutamide, AR Signaling

*Oral Presentation – 19*

Investigation of CD40, CD40L Gene Variants and sCD40, sCD40L Serum Levels in Laryngeal Cancer

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Introduction and Aim: Genetic factors can influence how our immune system functions and potentially affect the development of cancer. Laryngeal cancer is a prevalent form of head and neck cancer. Our research aimed to explore the influence of alterations in the CD40 (rs1883832) and CD40L (rs1126535) genes, as well as the levels of their corresponding proteins in the bloodstream, on the progression of laryngeal cancer.
Materials and Methods: We performed PCR-RFLP to genotype SNPs in 96 patients diagnosed with laryngeal cancer and 127 healthy individuals. Additionally, we measured the circulating levels of sCD40 and sCD40L using ELISA.
Results: A significant difference was noticed in genotype between those with laryngeal cancer and healthy individuals for the CD40 gene (rs1883832). It has been found that the C allele is the dominant gene variant and individuals with the CC variant are at a greater risk of developing laryngeal cancer. The study found that although there was a difference in genotype between the patients and the control group, this did not correspond to a difference in sCD40 levels. The patient group was found to have a significant correlation between the levels of sCD40 and sCD40L, with a correlation coefficient of 0.52 and a p-value of less than 0.01.
Conclusion: Based on our findings, the CD40 (rs1883832) polymorphism we identified in patients with laryngeal cancer could serve as a marker for determining an individual's risk of developing this type of cancer.
Keywords: Laryngeal Cancer, CD40, CD40L, sCD40, sCD40L

*Oral Presentation – 20*

# MicroRNA-145's Regulatory Role in Breast Cancer Progression

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Introduction and Aim: MicroRNAs (miRNAs) have emerged as focal points in cancer research, particularly tumor suppressor miRNAs like miR-145, which are suggested to play a vital role in cancer diagnosis and prognosis. This study aims to explore the potential of miR-145 expression in breast cancer as a biomarker for early diagnosis, prognosis prediction, and staging. The findings could underscore miR-145's clinical significance as a biomarker, potentially advancing novel approaches for early diagnosis and treatment of breast cancer.

Materials and Methods: This study analyzed miR-145 expression profiles using blood samples from 300 individuals, including 200 breast cancer patients and 100 healthy controls. RNA extraction followed a standardized method. The extracted RNA was reverse transcribed into complementary DNA (cDNA) using Reverse Transcriptase and miR-145 specific stem-loop miRNA primer. Finally, Real-Time qPCR was used to analyze miRNA-145 expression changes at cancer patient stages.

Results: Significant miR-145 expression differences were observed between groups. Statistical analyses revealed noteworthy distinctions in mean miR-145 expression levels between healthy controls, stage 1, stage 2, stage 3, and stage 4 patient groups ($p<0.001$ for each). The control group exhibited higher miR-145 expression compared to patient groups ($p<0.001$ for each). Fold change rates (fold change $2^{-\Delta\Delta CT}$) decreased in patient groups (stage 1, stage 2, stage 3, and stage 4) compared to the control group.

Conclusion: These findings support miR-145's tumor suppressor role as documented in the literature. Therefore, an association between breast cancer stage advancement and downregulated miR-145 expression can be inferred.

Keywords: miR-145, breast cancer, fold&change, tumor suppressor

*Oral Presentation – 21*

Investigation of methylation and expression levels of the *guanine nucleotide-binding protein gamma-7 (GNG7)* gene in oral squamous cell carcinoma

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Introduction and Aim: Oral squamous cell carcinoma (OSCC) is responsible for more than 91% of all malignancies in oral cavity. Epigenetic alterations and other environmental factors can cause changes in gene expression of OSCC pathogenesis. DNA methylation is just one of many epigenetic alterations. Herein, in our study we focused on predictive biomarker potential of guanine nucleotide-binding protein γ-7 (*GNG7*) gene methylation. The clinical significance of *GNG7* methylation and the association with oral carcinogenesis is still remain unknown.

Materials and Methods: DNA and RNA samples of tissues and body fluids obtained from OSCC patients and healthy individuals were used to examine the methylation and expression levels of the *GNG7* gene by using QMSP/QRT-PCR methods, respectively. All results were compared with clinicopathological and demographical parameters.

Results: *GNG7* gene hypermethylation was observed in 18% of patients. It was observed decreased expression levels in 48% and increased expression levels in 32% OSCC patients. There was a statistical significance was found between the classification of retromolar trigone with tongue, floor of the mouth and decreased expression levels. Decreased expression levels of *GNG7* gene in tumor, matched-normal tissues were downregulated in patients than healthy individuals and *GNG7* gene expression levels in patients and healthy individuals' serum were seen abundant significance.

Conclusion: The *GNG7* gene promoter hypermethylation depends on loss of expression in OSCC patients. The result of loss of expression due to the existence of tumor hypermethylation compared to healthy people suggests that there is a specific subgroup of OSCC patients for the *GNG7* gene in Turkish population.

Keywords: Oral squamous cell carcinoma; *GNG7* gene; Epigenetics; Expression; Methylation

Acknowledgement: This study was supported by Scientific Research Projects Coordination Unit of Istanbul University (Project number: IU-BAP-TYL-2019-35412).

*Oral Presentation – 22*

Increasing Treatment Efficacy by Drug Repositioning in Acute Lymphoblastic Leukemia

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Introduction and Aim: Acute lymphoblastic leukemia (ALL) is a malignant hematological cancer that is caused by different genetic alterations. The most common subtype of ALL is the Philadelphia positive ALL (Ph+ALL), which carries the BCR/ABL translocation, the most aggressive and high-risk subtype due to imatinib resistance among ALL subtypes. This research aims to analyze the cytotoxic activity and drug combination efficiency of three drugs Maytansine, Desipramine, Glipizide which are determined by meta-analysis of bioinformatics approach, on sensitive and Imatinib resistant Ph(+) and also Ph(-) ALL cell lines.

Materials and Methods: SUP-B15 and Jurkat cell lines were used as a Ph(+) and Ph(-) ALL cell lines respectively. Initially, SUP-B15 cell line was treated with Imatinib with increased concentration generate drug resistant cell line (SUP-B15/R). Additionally, Jurkat cells were treated with Maytansine. SUP-B15 and SUP-B15/R treated with desipramine and Glipizide to determine their cytotoxic effects on cell lines by MTT Assay. Finally, cytotoxic drugs were also applied in combination with Imatinib to detect possible synergistic effect.

Results: SUP-B15/R cells achieved 8-fold Imatinib resistance. IC50 and IC20 values of each agent were determined. Combination therapy of Imatinib with desipramine and Imatinib with Glipizide showed increased inhibitory effect on cell proliferation compared to the treatment by drugs alone.

Conclusion: The use of repositioned drugs, whose cytotoxic effects have been determined, in both Ph(-) ALL and Ph(+) ALL patients may pave the way to increase the survival rate by increasing the efficacy of the treatment, including overcoming imatinib resistance.

Keywords: ALL, drug resistance, imatinib, repurposing, philadelphia chromosome

*Oral Presentation – 23*

# HDAC Inhibitor Induces Mitochondrial Membrane Potential Disruption and Reverses the Epithelial-Mesenchymal Transition in Colorectal Cancer

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Introduction and Aim: Colorectal cancer (CRC), which arises from genetic and epigenetic alterations, is a major contributor to cancer-related mortalities worldwide. Despite many chemotherapy options, the outcomes underline the need for a better understanding of the underlying tumorigenic mechanisms. Histone deacetylases (HDACs), known for their role in gene expression regulation and malignant behaviors, are targeted by HDAC inhibitors, whose anticancer mechanisms vary based on cancer type. This study examines the effects of combined Quisinostat and 5-Fluorouracil (5FU) therapy on CRC cell death and epithelial-mesenchymal transition.

Methods: HCT116 cells were treated with Quisinostat, 5FU, and a combination of both. Cell viability and apoptotic changes were assessed using resazurin reagent and a Mitochondrial Membrane Potential kit, respectively. Epithelial-Mesenchymal Transition changes were evaluated through immunofluorescence staining using E-cadherin and vimentin as markers.

Results: IC50 for 5FU was found to be 84 µM as per the viability analysis. For Quisinostat, an IC50 could not be determined, prompting the use of the highest concentration (20 nM) that did not show statistical significance, in the combined treatment. The combination treatment caused a significant rise in Mitochondrial Membrane Potential disruption and E-cadherin levels, compared to 5FU alone.

Conclusion: The results imply that Quisinostat-5-FU combination could potentially enhance drug sensitivity in CRC, thus offering a promising new treatment approach. Combinatorial therapy disrupts the Mitochondrial Membrane Potential, possibly leading to increased cancer cell death, and induces an increase in epithelial characteristics, hinting at a reversal of Epithelial-Mesenchymal Transition, which may limit cancer metastasis. These promising findings necessitate further exploration and validation.

Keywords: Colorectal cancer, Histone deacetylases, Drug sensitivity, Apoptosis, Epithelial-mesenchymal transition

*Oral Presentation – 24*

# Evaluation of Sulfasalazine Drug Repurposing Potantial and Sulfasalazine Encapsulated PLGA Nanoparticles in Non-Small Cell Lung Cancer

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Introduction and Aim: Drug development for Non-Small Cell Lung Cancer (NSCLC) is challenging and new therapeutic options are needed due to high mortality rate. Drug repurposing could speed up the drug discovery process by reducing the pharmacokinetic uncertainty and offering a solution to the global burden of cancer as well as NSCLC. In addition, nanoparticles are used to enhance pharmacokinetic properties of drugs. The aim of this study is to evaluate the potential of repurposing sulfasalazine (SSZ) as a therapeutic agent for NSCLC, to synthesize PLGA encapsulated SSZ nanoparticles (SSZ-PLGA NPs) and to study its effect on A549 cell line via MTT assay.
Materials and Methods: SSZ-PLGA NPs were synthesized by using single emulsion solvent evaporation method and characterized by determining encapsulation efficiency, drug loading percentage, particle sizes, zeta potentials and release profiles. Cell viabilities were determined by applying the MTT assay on both A549 and HUVEC cell lines for both SSZ and SSZ-PLGA NPs. HUVEC cells are used as control cells.
Results: Size and zeta potentials of SSZ-PLGA NPs ranged between 220nm to 360nm and -17,7mV to -9,21mV, respectively. MTT analysis revealed the effectiveness of SSZ on A549 cell line. $IC_{50}$ is determined as 1,197mM.
Conclusion: This study presents the synthesized SSZ-PLGA NPs which can be a promising candidate according to cell viability and *in vitro* release profile studies. Primary results revealed anticancer effect of SSZ on NSCLC. The effect of SSZ alone and SSZ-PLGA NPs will further be studied in molecular level.
Keywords: Drug repurposing, Sulfasalazine, NSCLC, PLGA nanoparticles

Acknowledgement: This research has been supported by Yıldız Technical University Scientific Research Projects Coordination Department. Project Number: FDK-2020-4086. The authors would like to acknowledge that this oral presentation abstract is submitted in partial fulfilment of the requirements for PhD degree at Yildiz Technical University.

*Oral Presentation – 25*

Biological Effects of CRISPR/Cas9-mediated Knockout of RAB27A in SCLC

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Introduction and Aim: Small cell lung cancer (SCLC) is characterized by rapid growth and early metastasis. Identifying new molecular targets are important in the pathogenesis of SCLC in order to develop new treatment strategies. RAB27A is the critical protein for intracellular exosome trafficking and is a driver of tumour progression. However, demonstrating the potential impact of suppressing RAB27A in SCLC as therapeutic approach is an important deficiency.

Materials and Methods: *RAB27A* gene knockout SCLC cell lines were generated using a CRISPR/cas9 system. qRT-PCR, Western blotting and Sanger sequencing were performed to confirm *RAB27A* knockout in SCLC cells. TEM and EXOCET assays were used to detect the alteration of exosomes. Proliferation and colony formation were detected by MTT and microscopy. Subsequently, we intrapulmonally injected N417 and H524 SCLC cells (control and RAB27A knockout for each cell) into SCID mice. The effects of RAB27A knockout on mouse tumor model were analysed using 18F-FDG PET/CT scans.

Results: Knocking out RAB27A significantly decreased the expression of CD9, CD63, Tsg101, exosome secretion and exosomal protein in SCLC*($p<0.0001$)*. We found that RAB27A knockout dramatically reduced proliferation and colony formation in SCLC cells *($p< 0.001$, $p<0.0001$).* Furthermore, *RAB27A* knockout decreased proliferation and especially metastasis in mouse model (*$p<0,0001$*).

Conclusion: These studies clearly demonstrated that RAB27A plays an important role in the pathogenesis of SCLC, and targeting the *RAB27A* gene in SCLC cell lines significantly reduces the activity of the exosomal pathway. *RAB27A,* therefore, can be a promising cancer therapeutic strategy.

Keywords: RAB27A, exosome, SCLC, CRISPR/Cas9, Carcinogenesis

*Oral Presentation – 26*

Determination of *In Vitro* and *In Vivo* Effects of Taxifolin and Epirubicin on Epithelial-Mesenchymal Transition in Mouse Breast Cancer Cells

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Introduction and Aim: The aim of our study was to investigate potential effects of Taxifolin (Tax) on enhancing the effectiveness of Epirubicin (EPI) in treating breast cancer (BC), specifically in 4T1 cells and an allograft BALB/c model.

Materials and Methods: To examine the effects of Tax and EPI, both individually and in combination, we performed cell viability assays (MTT) and cytotoxicity assays (LDH) in 4T1 cells. In addition, we implanted 4T1 cells into female BALB/c mice to conduct in vivo studies and evaluate the therapeutic efficacy of Tax and EPI alone or in combination. Tumor volumes and histological analysis were also assessed in mice. To further understand mechanisms involved, we examined mRNA and protein levels of EMT-related genes, as well as active Caspase-3/7 levels, using qRT-PCR, western blot, and enzyme-linked immunosorbent assays, respectively.

Results: In vitro results demonstrated that the co-administration of Tax and EPI reduced cell viability and cytotoxicity in 4T1 cell lines. In vivo, co-administration of Tax and EPI suppressed tumor growth in BALB/c mice with 4T1 BC. Additionally, this combination treatment significantly increased the levels of active Caspase-3/7 and downregulated mRNA and protein levels of N-cadherin, β-catenin, Vimentin, Snail, and Slug, but upregulated E-cadherin gene. It significantly decreased mRNA levels of Zeb1 and Zeb2 genes.

Conclusion: We concluded that the co-administration of Tax and EPI is efficient in inhibiting BC growth compared to EPI alone. Therefore, our results suggest that Tax has the potential to be a promising agent in clinical treatment of highly aggressive BC patients.

Keywords: Epirubicin, Taxifolin, Breast cancer, 4T1 cells, Epithelial mesenchymal transition

*Oral Presentation – 27*

# Molecular and Bioinformatic Investigation of Proteomic Differences Between Cancerous and Normal Tissue Samples of Colorectal Cancer Patients*

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Introduction and Aim: New treatment strategies targeting the factors that play a critical role in the transition from normal cells to malignant cells are urgently needed. Therefore, we believe that the discovery of novel targets may generate new treatment options and cancer biomarkers. The aim of this study was to identify the proteins whose expression changes between colorectal cancer and normal tissues using the proteomics tools.

Materials and Methods: In order to determine the differentially expressed proteins between the tissue samples, the label-free nLC-MS/MS method was used for the proteomic analysis. The statistically significant proteins were then uploaded and analyzed using the Metascape portal to determine the affected biological process. The experimentally determined proteins were also compared with proteomic data generated by CPTAC via the UALCAN platform.

Results: A total of 77 proteins were identified as statistically significant using proteomic analyzes between cancerous and normal tissues. Using the Metascape web portal, the biological processes involved were determined. The expression profiles of the 77 proteins were compared with CPTAC data using the UALCAN portal. It was found that 45 of the 77 proteins clearly matched the CPTAC results.

Conclusions: The results of this proteomic study performed with clinical samples from patients are very valuable, and if the results are validated with different methods such as ELISA with serum samples from patients, it may lead to the discovery of new biomarkers for colorectal cancer diagnosis.

Keywords: Colorectal Cancer, Proteomics, Label-free nLC-MS/MS, Metascape, CPTAC

Acknowledgement: The study was funded by the Scientific and Technological Research Council of Türkiye (TÜBİTAK) with Project no 121S170.

*Oral Presentation – 28*

The Impacts of Ceramidase Inhibition with D-E-Mapp Sln Formulation upon Cell Death Mechanism in Breast Cancer as an in-vitro and in-vivo models.

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Introduction and Aim: Sphingolipids regulate various biological processes such as growth, proliferation, migration, invasion and metastasis by controlling signaling functions within the cancer cell signaling network. Breast cancer is the most common type of cancer among women. While advances in the early diagnosis and treatment of breast cancer in recent years have led to a significant reduction in mortality, these advances have been insufficient to completely cure the disease. The aim of this study was to investigate the cytotoxicity of D-e-MAPP, a ceramidase inhibitor, and D-e-MAPP SLN formulation on 4T1 cells *in vitro* and the changes in 4T1-induced breast tumor tissue in BALB/c mice *in vivo* by immunohistochemistry.

Materials and Methods: Cytotoxicity was tested by MTT test. For *in vivo* experiments mice were injected with 4T1 breast cancer cells to form breast tumors. They were then treated with D-e-MAPP and D-e-MAPP SLN formulations.

Results: The results showed that D-e-MAPP and its SLN form exerted cytotoxicity at low doses in 4T1 cells. *In vivo* results indicated that positive staining of ER, PR and CerB2 oncogene antigens were determined in control group of tissues. While, the p53 staining in the D-e-MAPP and D-e-MAPP SLN treated groups was positively arisen compared to control tissues as well as staining of ER, PR, and CerB2 was slightly decreased.

Conclusion: We believe that the results of the research on the application of D-e-MAPP and its SLN form in breast cancer treatment will contribute to the next studies for designing target therapy agents and approaches.

Keywords: Breast cancer, Sphingolipid, Solid lipid nanoparticles

Acknowledgments: This study was supported by ESTU BAP Commission with the project number 20DRP029, by the Technological Research Council of Turkey 1002-Rapid Support Program number 118Z943, by the Scientific and Technological Research Council of Turkey (BİDEB) 2211/C and by the Council of Higher Education 100/2000.

*Oral Presentation – 29*

# Combination Therapy of Beta-Hydroxybutyrate and Oxaliplatin Augments the Treatment Efficacy in Colorectal Cancer Organoids

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Introduction and Aim: Treatments focused on targeting cancer metabolism are a promising option in the battle against colorectal cancer. Beta-hydroxybutyrate (BOHB), serves as a supplier of acetyl-CoA for the Trichloroacetic acid (TCA) cycle and it potentially redirects energy metabolism the cycle. Recently revealed oxaliplatin ‘s mechanism of action was proceeding on reactive oxygen species (ROS) caused apoptotic cell death. This investigation delves into BOHB's potential to enhance the cytotoxic impact of oxaliplatin by shifting energy metabolism into TCA cycle resulting in electron transport chain which is primary sources of ROS.

Materials and Methods: This study was performed on advanced in vitro organoid technology. The combined efficacy of BOHB and oxaliplatin was assessed using a cell viability assay. Western Blot analysis was used to indicate the levels of pivotal proteins involved in energy metabolism, apoptotic pathways, DNA damage, and histone acetylation markers. Flow cytometry was utilized to quantify ROS levels.

Results: BOHB with oxaliplatin elevated the cytotoxic effect on colorectal cancer organoids. Administration of BOHB and/or melatonin yielded noticeable reductions in Lactate Dehydrogenase A and increased Mitochondrial Carrier Protein 2 levels, signifying aerobic glycolysis suppression and augmentation in oxidative phosphorylation rate. This metabolic shift triggered apoptotic cell death through oxaliplatin, attributed to elevated ROS levels. As a positive control, melatonin countered this impact by safeguarding cancer cells against heightened oxidative stress conditions.

Conclusion: These novel combinations hold promise in improving treatment outcomes for individuals afflicted by colorectal cancer. (Tolga Sever was supported by TUBITAK 2211A Domestic Doctoral, 2214 Overseas Doctoral Research and Council of Higher Education 100/2000 Scholarship Programs.)

Keywords: Colorectal cancer, Organoid, Beta-hydroxybutyrate, Oxaliplatin, Reactive oxygen species, Metabolic targeted therapy.

*Oral Presentation – 30*

# Editing the *TP53* Gene Locus in U87 Human Glioblastoma Cell Line by Using CRISPR/Cas9 System

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Introduction and Aim: The p53 tumor suppressor is mutant in nearly half of cancer cases. Although the roles of p53 mutations are well-studied, for some cancer types such as glioblastoma, there is still need for comprehensive studies. In this study, we aimed to engineer the *TP53* gene locus in U87 cells to generate a cell line lacking p53 expression for further mutant p53 studies in glioblastoma.

Materials and Methods: We targeted the *TP53* gene locus by using CRISPR/Cas9 system in U87 cells. After evaluation of the gene editing by performing genotyping, we screened the single-cell clones derived from the gene edited cell pool and determined the homozygous knockout clones. We confirmed the edit by Sanger Sequencing and analyzed the p53 protein levels by western blot. To compare WT and p53 knockout U87 cell behaviors, we performed trypan blue exclusion, wound healing and colony formation assays.

Results: We managed to edit the *TP53* gene locus in U87 cells and confirmed the loss of p53 protein expression. Also, we did not observe any off-target effect. By considering proliferation, migration and colony formation abilities of WT and p53 knockout cells, the newly generated cell line was not only genotypically but also phenotypically bearing the p53 knockout profile.

Conclusion: The newly generated cell line can be used as a glioblastoma cell line model for mutant p53 overexpression studies.

Keywords: p53, Glioblastoma, CRISPR/Cas9, U87

Acknowledgement: This study is funded by TÜBİTAK 3501 Career Development Program (Project Number: 120Z817).

*Oral Presentation – 31*

# Gastric Cancer Spheroids: A Three-Dimensional Model to Study the Effect of Metabolic Alterations in the One-Carbon Pathway

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Introduction and Aim: Cancer cells alter their metabolism compared to healthy cells to survive, proliferate, and metastasize. This leads to high demand for 1C units to successfully complete these processes which are supplied by the one-carbon pathway. Analysis of 1C metabolism and its relation to anti-cancer drug responses requires cell culture models that closely mimic the tumor organization and functionality as observed in vivo such as 3D multicellular tumor spheroids (MCTS). Here, we developed high-throughput gastric MCTS, and characterized their growth and 1C metabolism-related protein levels compared to 2D cultures.

Materials and Methods: We developed MCTS from gastric cancer cell lines, SNU484 and NCI-N87, in 96-well plates by using the liquid overlay technique. We characterized spheroid morphology and growth by imaging, MTT assay, and flow cytometric analysis. Their metabolites were analyzed by NMR, Real-Time qPCR, and western blotting.

Results: We formed compact spheroids with both cell lines with high cell viability during 6 days of culture according to flow and MTT data. NMR data shows high formate levels in both cell lines. qPCR and western blots show changes in the expression levels of 1C metabolism-related proteins such as SFXN1, SHMT1/2, and MTHFD1/2 in spheroids compared to monolayers. Bioinformatic analysis of comprehensive patient datasets revealed that these genes are associated with a poor response to chemotherapy in gastric cancer.

Conclusion: Targeted metabolomics, gene, and protein levels have the potential to indicate altered one-carbon metabolism in our gastric MCTS models which can be useful to investigate drug resistance in gastric cancer.

Keywords: multicellular tumor spheroids, gastric cancer, one-carbon metabolism, drug resistance, formate overflow

*Oral Presentation – 32*

Regulatory Network of miR-27a-5p in Prostate Cancer

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Introduction and Aim: miR-27a-5p was shown to be significantly downregulated in prostate cancer (PCa) and its forced-overexpression lead to a decrease in the viability of LNCaP cells. Therefore, miR-27a can be considered as a therapeutic target in PCa. The regulatory network of miR-27a-5p and its target genes remain to be clarified. Here, we aimed to reveal the tumor suppressor role of miR-27a-5p by directly targeting CCR5, STAT3, and Bcl-2, all of which are components of chemokine signaling and known to promote PCa progression.

Materials and Methods: CCR5, STAT3, and Bcl-2 were determined as the potential target genes of miR-27a-5p via bioinformatics. Expression levels of miR-27a-5p and its predicted target genes were determined by qRT-PCR in tumor samples of nude PCa mice models, generated with either PC3 or LNCaP cells.

Results: It was found that miR-27a-5p is significantly downregulated, and CCR5 and Bcl-2 gene expression levels were found to be significantly upregulated in all PCa tumors. STAT3 expression was detected only in LNCaP tumors.

Conclusion: We showed that miR-27a-5p expression is inversely correlated with the expression of CCR5 and Bcl-2 genes. These results support the hypothesis of miR-27a-5p directly targeting chemokine signaling components and highlight the therapeutic potential of miR-27a-5p in PCa. To further confirm this regulatory network and tumor suppressive function of miR-27a-5p, in vitro experiments, including gene expression profiles upon mimic/anti-miR transfection will be performed, and the effects on cellular processes will be assessed.

Keywords: miRNA, prostate cancer, chemokine signaling, gene expression

*Oral Presentation – 33*

Investigation of Apoptotic Potential of Catechol in Drug-Resistant Lung Cancer and Healthy Fibroblast Cells

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Introduction and Aim: Many herbal compounds are applied for phytotherapy purposes in lung cancer (H1299), the incidence of which is increasing globally. However, it is a fact that this compound, which will be used to treat lung cancer, also affects healthy cells. Activation of the apoptotic pathway was investigated to demonstrate the anticancer effect of catechol on drug-resistant lung cancer cells. In addition, the activation of the apoptotic pathway was studied with healthy fibroblast (Bj) cells and the results were compared.

Materials and Methods: The 24-hour cytotoxic effect of catechol on cells was demonstrated by the Cell Titer-Blue® cell viability test. In order to determine the apoptotic potential, the caspase 3/7 activity of the cells exposed to catechol was measured and the potential of catechol to stimulate the apoptotic pathway was determined. Caspase 3/7 enzyme activity was determined using the Promega 'ApoTox-GloTM Triplex Assay' kit.

Results: In our study, the IC50 values of the cytotoxic effect of catechol on drug-resistant lung cancer and healthy fibroblast cells were found to be 90 and 207 µg/ml, respectively. Caspase-3/7 activity in drug-resistant lung cancer and Bj cells increased 2.3-fold and 1.6-fold, respectively, compared to control after 24 hours of catechol incubation.

Conclusion: In conclusion, drug-resistant lung cancer cells have higher caspase 3 activity than healthy cells, indicating that catechol has a higher apoptotic potential in drug-resistant cancer cells than healthy cells.

Keywords: Catechol, Drug-resistant, Apoptosis

Acknowledgement and/or disclaimers if any: The authors wish to thanks to Akdeniz University BAP-Scientific Research Projects Coordination Unit (FYL-2022-5902) for financial support of this work.

*Oral Presentation – 34*

Epigenetic Changes/ DNA methylation plays a key role in Triple Negative Breast Cancer?

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Introduction and Aim: Breast cancer is among the most common cancers in women in the world and is the second leading cause of cancer-related deaths. Triple negative breast cancer (TNBC) is a subgroup of breast cancer in which estrogen (ER), progesterone (PR) and HER2 receptors are not expressed. It is aimed to demonstrate the usability of DNA Methylation profiles, which is one of the epigenetic changes of SFRP1 (Secret Frizzled Related Protein-1) in a selected tumor suppressor gene, as biomarkers in TNBC patients.

Materials and Methods: In the literature studies on the SFRP1 gene, it was observed that there was no extensive methylation study in the TNBC subtype. DNA isolation was performed from the tissue (paraffinized) of 110 patients diagnosed with TNBC. After bisulfite modification, methylation states were determined using MSP-PCR. Finally, it was visualized by running on agarose gel. The methylation results and demographic characteristics of the patients were evaluated using the SPSS program.

Results Conclusion: As a result of the studies, it was observed that the SFRP1 gene was methylated in almost all of the patients. Gene methylation are thought to be effective in gene silencing in TNBC patients.

Conclusion: The emergence of results in parallel with the literature studies is important for the continuity and elaboration of the study.

Keywords: Epigenetic, Methylation, TNBC

*Oral Presentation – 35*

## Synergistic Anticancer Effects of Auraptene and Tamoxifen on MCF-7 and Ishikawa Cell Cultures in Breast and Endometrial Cancer Cell Lines

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Introduction and Aim: Since breast cancers express high estrogen receptors the use of selective estrogen receptor modulator Tamoxifen is common in the treatment. Tamoxifen increases the risk of endometrial cancer. We planned to examine the possibility of cytotoxicity of the monoterpene coumarin compound Auraptene, which has been shown to have an antiproliferative effect in breast cancer cells, in estrogen-dependent endometrial cancer modeling by Ishikawa cells. No study was found in the literature showing the cytotoxicity of Aurapten against Ishikawa cells and endometrial cancer. We studied the potential of Auraptene combined with Tamoxifen in suppressing the endometrial carcinogenesis induced by Tamoxifen and attaining a synergistic effect in breast cancer therapy.

Materials and Methods: Single and combined doses of Auraptene and Tamoxifen were administered to human breast and endometrial cancer cell lines MCF-7 and Ishikawa cell cultures passaged from cell culture laboratory stocks. Cell viability and proliferation were quantified by WST-8 assay with a Multiscan ELISA microreader. Apoptosis assays with the Annexin V/PI staining method and cell cycle tests were analysed with flow cytometry. Cells and nuclear morphology were visualized by laser scanning confocal microscope.

Results: Auraptene elicited cytotoxic effects in Ishikawa and MCF-7 cells by increasing apoptosis and inducing their arrest in the G0/G1 phase of the cell cycle ($p<0.001$). Tamoxifen incited the proliferation in Ishikawa cells however this stimulation was curbed when combined with Auraptene.

Conclusion: Auraptene can be recommended as a synergistic therapeutic adjuvant in breast cancer to enable Tamoxifen to use more safely by reducing the risk of endometrial cancer and effectively at lower doses.

Keywords: Auraptene, Tamoxifen, breast cancer, flow cytometry, cytotoxicity.

*Oral Presentation – 36*

Determination of Antioxidant Properties and Contents of Helichrysum Arenarium L. Extracts, Investigation of Anti-growth Effects Against Human Breast Cancer Cell Lines

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Introduction and Aim: H. arenarium (dwarf everlast or immortelle) is used in the treatment of various diseases. In addition, scientific studies show that H. arenarium is a rich source of antioxidants. Natural antioxidants are known to have many positive biological activities. This study aims to determine the most efficient solvent in the extraction of H. arenarium using solvents with different polarities, to reveal its antioxidant properties, to determine the its active ingredients and growth inhibitory effects against MCF-7 and MDA-MB-231 human breast cancer cells.

Materials and Methods: Ultrasonic extractions of H. arenarium were performed with four different solvents: hexane, acetone, ethanol and glycerol-water. The total phenolic content and antioxidant capacity of the extracts were determined by spectrophotometric methods, and the quantitative analysis of the phenolic components was carried out by HPLC-DAD. The extracts were lyophilized and dissolved with DMSO. Then, the MCF-7 and MDA-MB-231 cell lines were treated at a final concentration of 0.1-1000 µg/mL and their viability was assessed by using sulforhodamine B viability assay.

Results / Conclusion: The highest total phenolic content and antioxidant capacity among four different solvents was observed in the extract prepared with glycerol-water solvent mixture. When the HPLC-DAD results were examined, the highest amount of phenolic component contained in the extracts was determined as kaempferol-3-β-D-glycoside. All extracts of H. arenarium significantly inhibited the growth of both cell lines in a concentration-dependent manner. The effects of H. arenarium extracts in combination different chemotherapeutic agents or against different cancer cell lines can be studied in the future to better elucidate its anti-growth properties.

Keywords: Breast cancer, Antioxidant, HPLC-DAD, Helichrysum Arenarium L., MCF-7, MDA-MB-231.

*Oral Presentation – 37*

## The Anticancer Potential of Brassinin in Estrogen Receptor-Positive Breast Cancer Cells Through The Activation of Apoptosis and Downregulation of Matrix Metalloproteinase-2

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Introduction and Aim: Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes that are responsible of tissue remodelling. Overexpressed MMPs are known to play a crucial role in cancer invasion and metastasis. Therefore, suppression of these enzymes is particularly important in cancer treatments. Brassinin is an indole derivative of a family of natural compounds known as phytoalexins. They exert antimicrobial and antioxidant activities. However, it has been reported that brassinin exhibits potent antiproliferative effects in several cancers. In the present study, the cytotoxic and apoptotic effects of brassinin were investigated in estrogen receptor-positive breast cancer cells. Additionally, the suppressive impact of the compound on MMP2 activity was examined.

Materials and Methods: The cytotoxic effects of brassinin on breast cancer cells was determined by MTT and cell cycle assays. The apoptotic activity of the compound was investigated by annexin V binding assay. The activity of MMP2 was detected by ELISA.

Results: The results revealed that brassinin decreases cell viability significantly at 200 μM and causes cell cycle arrest at S phase in all applied concentrations. Furthermore, it has been observed that brassinin significantly increases apoptotic cell population and suppresses MMP2 activity.

Conclusion: In conclusion, the present study demonstrated that brassinin exhibits significant anticancer activity on estrogen receptor-positive breast cancer cells via leading to apoptosis and downregulating the activity of MMP2.

Keywords: Brassinin, breast cancer, MMP2, phytoalexins.

*Oral Presentation – 38*

Unveiling Potential: Scorpio fuscus Venom for Targeted Colorectal Carcinoma Therapy

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Introduction and Aim: Colorectal carcinoma (CRC) is a significant cancer-related cause of death. This study extensively characterizes *Scorpio fuscus* venom, identifies potent peptides/proteins, models their structures, evaluates *in vitro* effects on CRC cells, and investigates *in vivo* impact using a mouse model.

Materials and Methods: Utilizing a tandem approach of 2D gel electrophoresis and high-resolution mass spectrometry, we identified *S.fuscus* venom constituents. The ensuing peptides underwent rigorous scrutiny, including three-dimensional modeling and docking with pivotal proteins in colon cancer and apoptosis pathways. The venom's impact on colorectal carcinoma cell lines(DLD-1, HT-29, CaCo-2, CCD-18Co) was meticulously assessed, spanning cytotoxicity, migratory behavior, colony-forming, and apoptosis, quantified via advanced flow cytometry. Detailed mRNA and protein cascade changes in apoptosis and CRC were illuminated through pathway panels. An orthotopic colon cancer model in male non-scid mice facilitated an in-depth evaluation of the venom's *in vivo* tumor developmental effects.

Results: Proteomic exploration unveiled 18 distinct bioactive peptides in *S.fuscus* venom. In colon cancer cells, dose-dependent cytotoxicity exhibited IC50 values of 14.8 µg/mL(DLD-1) and >250 µg/mL(CCD-18Co). Remarkable inhibition emerged, with 84% metastasis reduction and 49% colony formation decline. Strikingly, BAK1 and TRAF3 mRNA plummeted tenfold, while BIRC2-3-6, CASP8, TNFRSF8-11, and BOK surged tenfold. Intratumoral venom reduced primary tumor volume by 30%, with no metastatic loci, emphasizing potential therapeutic value.

Conclusion: Intriguingly, *Scorpio fuscus* venom's bioactive peptides exhibit dose-dependent cytotoxicity, hinder metastasis, and modulate crucial genes, suggesting potential for targeted CRC therapy.

Keywords: *Scorpio fuscus*, Colorectal carcinoma, Proteomics, Apoptotic pathway, Cytotoxicity

This work was supported by TUBITAK (Project no: 120Z942).

*Oral Presentation – 39*

Enhanced cytokine stimulation in *in vitro* and *in vivo* glioblastoma models: Lipid nanoparticles for *stimulator of interferon genes* agonists delivery

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Introduction and Aim: The most common brain tumour in adults, Glioblastoma (GBM) is still considered incurable. Researchers are in a continuous search for an efficient therapeutic strategy. Natural and synthetic cyclic dinucleotides (CDNs) and non-nucleic acid agonists of the stimulator of interferon genes (STING) demonstrated huge potential for cancer treatment and entered clinical studies. However, these have disadvantages such as low solubility, and adverse effects related to excessive cytokine release upon systemic administration. The aim of this study was to develop diABZI-loaded lipid nanoparticles (LNPs) to be used as an intranasal treatment against GBM.

Materials and Methods: LNPs loaded with a lipophilic non-nucleotide STING agonist, diABZI were prepared by lyophilisation-rehydration-sonication method. Nanoparticles were evaluated in terms of physicochemical characteristics and cytotoxicity on L929 and GL261 cell lines. Cellular uptake was evaluated on GL261 cell line. Cytokine stimulation activity was assessed on THP1 monocytes *in vitro* and in an orthotopic syngeneic mouse tumour model after IV and IN application.

Results: PEGylated cationic LNPs with particle size <250 nm and drug entrapment efficiency over 99% were obtained. No significant cytotoxicity was observed. Higher cellular uptake was observed with cationic nanoparticles. *In vitro* and *in vivo* studies revealed higher cytokine stimulation and improved healing after IN and IV application of diABZI LNPs.

Conclusion: diABZI LNPs had high cytokine stimulating activity *in vitro* and *in vivo* and demonstrated good *in vivo* performance when applied as a single treatment. Further research is needed to examine the kinetic profile of the cytokine stimulation after treatment.

Keywords: diamidobenzimidazole compound 3, lipid nanoparticles, nanomedicine, immunostimulation, nose-to-brain delivery, STING agonists

Acknowledgement: This study is supported by the Health Institutes of Türkiye (TÜSEB, Project #4469)

*Oral Presentation – 40*

Investigation of anticancer activity of mocetinostat (Hdaci) on MDA-MB-231 breast cancer cell line

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Introduction and Aim: HDACi (histone deacetylase İnhibitors) stop the cell cycle, induce apoptosis and inhibit angiogenesis are recognised as important agents in the treatment of cancer. Mocetinostat (MGCD0103) is one of the members of Class I Histone Deacetylase Inhibitors (HDACi) and its mechanism of action has not been defined, yet in cancer researches. The aim of the study is ınvestigation of anticancer activity of mocetinostat on MDA-MB-231 breast cancer cell line.

Materials and Methods: The effects of mocetinostat on MDA-MB-231 breast cancer cells were investigated by cell viability, migration assays and ros sssay technique.

Results: The concentrations of drug that give a half-maximal response ($IC_{50}$) were detected for mocetinostat (5µM) for 48 hr. We observed that cell migration decreased, DNA fragmentation increased compared to the control group. ROS generation in breast cancer cells was increased due to mocetinostat exposure.

Conclusion: Mocetinostat played a role through inducing apoptosis on breast cancer cells in a time.

Keywords: MDA-MB-231, Breast cancer cell, Mocetinostat

*Oral Presentation – 41*

# Investigation of Anti-Cancer Effects of a Palladium Complex (Pd(bpma)(barb).Cl • $H_2O$) in Ovarian Cancer Cell Lines

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Introduction and Aim: Chemotheraphy for ovarian cancer relies on cisplatin and carbolaplatin which are highly prone to cause therapy resistance. The palladium compexes gather great interest since they constitute a more stable and soluble structure compared to platinum isostruchers. The main objective of this study is to investigate anti-cancer activity of a palladium complex (Pd(bpma)(barb).Cl • $H_2O$) in ovarian cancer cell lines and to explain the possible mechanism behind it.

Materials and Methods: The anti-cancer potency of the palladium complex was investigated in varying doses from 1 nM up to 100µM for 48 hours by using MTT assay in three different high grade serous ovarian cancer cell lines; CaOv-3, Kuramochi, Ovsaho. The mechanism of cell death were analyzed with fluorescent staining and flow cytometry. Scratch assay were used for quantification of migration rate.

Results: Palladium complex was found to be effective on all cell lines espacially on Ovsaho with 13µM $IC_{50}$ which is comparable to that of cisplatin while the migration were inhibited espacially on CaOv-3. The complex causes the apoptotic cell death trigered by DNA damage and oxidative stress.

Conclusion: The palladium complex is a promissing anti-cancer agent for the treatment of a particular sub-class of ovarian cancer.

Keywords: palladium, ovarian cacer, anti-cancer

*Oral Presentation – 42*

## Comparison of the Effect of Abemaciclib in MCF-7 Cells in 2D and 3D Systems

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Introduction and Aim: Breast cancer represents a prominent global oncological challenge. Three-dimensional (3D) models have been proven superior to two-dimensional (2D) systems in recapitulating *in vivo* conditions. We evaluated the anti-tumor efficacy of Abemaciclib, an active constituent of Verzenio, against human breast cancer cells (MCF-7) cultivated in 2D and 3D models for hormone receptor-positive (HR+) advanced breast cancer.

Material and Method: The half-maximal inhibitory concentration ($IC_{50}$) of Abemaciclib on MCF-7 cells in 2D cultures was ascertained via the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay. Subsequently, MCF-7 cells were cultured in 3D Petri Dish® plates, and spheroid diameter/area were quantified over 6 days. The responsiveness of the spheroids to Abemaciclib, according to the determined $IC_{50}$, was analyzed using MTS. CellTracker immunofluorescent dyes illustrated cellular localizations in both systems.

Conclusion: MCF-7 cells formed spheroids within 72 hours in a medium containing 10% FBS, accompanied by a decrease in diameter/area. Comparative analysis of 2D and 3D viability revealed anti-cancer properties at specific concentrations in the 2D system, whereas the 3D system exhibited no corresponding decline in cell viability, and indeed, the number of viable cells increased at the same concentration.

Argument: 3D Petri Dish® models demonstrate suitability for soft tissue modeling, such as breast cancer. Our findings underscore that 3D MCF-7 spheroids exhibit enhanced resistance to Abemaciclib in comparison to 2D models, thereby closely mimicking *in vivo* conditions.

Keywords: 3D Petri Dish, MCF-7, Abemaciclib, MTS Analysis, Cell Tracker Staining

This work was financially supported by the Scientific and Technical Research Council of Turkey (Application No: 1919B012224314).

*Oral Presentation – 43*

Anticancer effect of *Tricholoma atrosquamosum* Sacc. against human lung adenocarcinoma cell line

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Introduction and Aim: Mushrooms have been collected from nature and consumed since ancient times. Due to their rich nutritional content, they are considered functional foods that are beneficial for health. For this reason, they have been used for many years for medicinal purposes. Thanks to the bioactive components they have, they are known to have antimicrobial, antioxidant, anti-inflammatory, immunomodulatory, and anticancer effects. *Tricholoma* sp. are edible and medicinally important mushrooms. Among these species, *Tricholoma atrosquamosum* Sacc., known as the scaly blackgirl fungus, is a mushroom species with medical importance in the geography of our country. This species is found in the Central and Eastern Black Sea Region, Adana, Siirt, and Kahramanmaraş regions in our country where there are coniferous trees. In this study, it was aimed to determine the cytotoxic effect of ethanol extract of *T. atrosquamosum* at different concentrations (62.5-1000µg/mL) against A549 cells in 24 and 48 hours.

Material and Methods: 3-(4,5dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide method was used to determine the cytotoxic effect.

Results: In the results obtained, the $IC_{50}$ value at the 24th hour was 54.449 µg/mL, while the $IC_{50}$ value at the 48th hour was determined as 99.447 µg/mL.

Conclusion: These results show that *T. atrosquamosum* ethanol extract has a cytotoxic effect depending on increasing concentrations.

Keywords: Medicinal mushroom, *Tricholoma atrosquamosum*, Cytotoxic effect.

*Oral Presentation – 44*

TFEB Drives Chemo-Immuno-Resistance In Lung Cancer

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Introduction and Aim: Transcription factor EB (TFEB) is a leucine zipper protein and a major regulator of lysosomal biogenesis and autophagy. These two events confer chemoresistance in solid tumors, by sequestrating chemotherapeutic drugs, and also modulate the immune-recognition. In this study, we investigated if TFEB affects the response to chemotherapy and to Vγ9δ2 T-lymphocytes in non-small cell lung cancer (NSCLC).

Materials and Methods: Changes in the expression of TFEB and ABC transporters and their effect on survival in NSCLC were analyzed by using the TCGA-LUAD dataset. TFEB was silenced in H441 and H2228 cells. Metabolic associated pathways were measured by RT-PCR, immunoblotting, and radiolabeling. Co-cultures between NSCLC cells and γδ T-lymphocytes were set-up to measure their expansion and cell killing. Wild-type (WT) and shTFEB NSCLC xenografts implanted in Hu-$CD34^{+}$ NSG mice were used for in vivo validation.

Results: $TFEB^{high}ABCA1^{high}ABCC1^{low}$ phenotype is associated with overall survival. By reducing the pERK1/2-SREBP2 axis that modulates genes of cholesterol homeostasis, TFEB silencing decreased expression and activity of the cholesterol/IPP transporter ABCA1, the efflux of IPP, and the NSCLC killing by γδ T-lymphocytes. shTFEB NSCLC xenografts implanted in Hu-CD34+ NSG mice, were resistant to cisplatin, but were resensitized by zoledronic acid, which re-activates γδ T-lymphocytes killing and down- regulates ABCB1/ABCC1.

Conclusion: We propose TFEB as a driver of chemo-immuno-resistance in NSCLC. Future experiments including a tumor single-cell transcriptomic profile are clarifying which cell populations and pathways make TFEB a controller of chemo-immuno-resistance in NSCLC. Supported by the AIRC (Grant No. IG21408).

Keywords: TFEB, ABCA1, ABCB1, ABCC1, NSCLC, Zoledronic Acid

*Oral Presentation – 45*

# Long Non-Coding RNA Urothelial Carcinoma-Associated 1 Regulates Proliferation And Migration in Doxorubicin Resistance of Estrogen Receptor Positive Breast Cancer Cells

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Introduction and Aim: Breast cancer is the most frequently diagnosed cancer in women and doxorubicin is common used chemotherapy drug in the treatment of many cancer types including metastatic breast cancer. Cancer cells with a negative response to the treatment of doxorubicin trigger drug resistance. Urothelial carcinoma-associated 1 (UCA1) is long non-coding RNA (lncRNA), known to be overexpressed in breast tumorigenesis, but its role in chemotherapy resistance is largely unknown. The aim of this study was to investigate the role of UCA1 in proliferation and cell motility in doxorubicin resistance MCF-7 cell line.

Materials and Methods: Previously developed doxorubicin resistant MCF-7 cells (MCF-7/Dox) up to 640 nM were used. In order to transfect small interfering RNAs (siRNAs) specifically targeting lncRNA UCA1 purchased from Ambion (USA), LipofectMax (A.B.T Biosciences, Turkey) protocol was used according to the modified manufacturer's instructions. At 48 h after transfection, the cells were used to analyze cell viability and wound healing assay.

Results: According to MTT results, the inhibition concentration ($IC_{50}$) value of doxorubicin in MCF-7/Dox cells was determined as 128.5 µM. UCA1 silencing was confirmed by qRT-PCR. After UCA1 silencing, it was determined that the $IC_{50}$ value of doxorubicin on MCF-7/Dox cells as 88.5 µM. Finally, the cell motility decreased after silencing the UCA1 gene in MCF-7/Dox cell line.

Conclusion: It could be concluded that UCA1 partially reversed doxorubicin resistance by regulation of cell proliferation and motility.

Keywords: MCF-7 cell line, doxorubicin, UCA1

*Oral Presentation – 46*

Predicting side-effects of chemotherapeutic agents through analysis of drug-induced transcriptomic response

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Introduction and Aim: Among the most common side effects induced by chemotherapy are alopecia, edema, and diarrhea, although their severity depends on the drug, dosage of the drug, and frequency of the treatment. While there exists proposed mechanisms by which anticancer agents cause these side effects, the exact underlying mechanisms have not been entirely clarified. Our aim was twofold: to develop a model that uses drug-induced transcriptome response to predict side effects accurately and to explain the mechanism of the side effects of interest.

Materials and Methods: The selection of drugs was carried out based on the side effect information, collected from SIDER database. Induced transcriptome responses for the selected drugs were obtained from LINCS L1000 project. We trained several classifiers using random forest with iterative feature selection. We used the side effects of interest as class labels and a pool of differentially expressed genes as features. We employed several performance metrics to select the optimal model.

Results: Our approach revealed an expressionsignatures involving 40 genes, which accurately predicted side effects of interests with an 89% accuracy. Out of 40 signature genes 27 were associated to at least one of the side effects by previous studies. The resulting gene signature was further investigated, and its relation to the side effects was explored through functional enrichment analysis and protein-protein interaction networks.

Conclusion: In this study, we developed a model based on random forest algorithm to accurately predict the widely occurring side effects of chemotherapy. The approach that we employed here can be generalized to other side effects.

Keywords: side-effects, chemotherapeutic agents, random forest

*Oral Presentation – 47*

# The Impact of 1.25-Dihydroxyvitamin D3 on Mitophagy and Apoptotic Pathways in Hepatocellular Carcinoma

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Introduction and Aim: 1.25-dihydroxyvitamin D3 is the active form of vitamin D which effects the cellular mechanisms directly. Mitophagy is a type of autophagic mechanism that aids in cellular recycling by consuming unhealthy or damaged mitochondria. In this study, we aimed to know how 1,25-dihydroxyvitamin D3 affected the apoptotic and mitophagy pathways in hepatocellular carcinoma (HCC) cells.

Materials and Methods: Total RNA was isolated after 48 hours of treatment with 250 nM 1,25-dihydroxyvitamin D3 on HepG2 cells. By using RT-PCR, isolated total RNAs were used to identify the gene expressions of MFN1, MFN2, Parkin, and PINK1 genes for mitophagy, as well as Cyt C and p53 for apoptosis.

Results: MFN1, p53 and Cyt C gene expressions were downregulated following a 250 nM 1.25-dihydroxyvitamin D3 treatment at the 48$^{th}$ hour in comparison to the control group ($p<0.001$). Despite the decrease in Parkin gene expression, no statistical difference was observed ($p>0.05$). In comparison to the control group, statistically significant increases in MFN2 and PINK1 gene expressions were observed ($p<0.001$).

Conclusion: By promoting mitophagy, 1,25-dihydroxyvitamin D3 helps HepG2 hepatocellular carcinoma cells to prevent apoptosis. Although some researchers suggest that 1,25-dihydroxyvitamin D3 has anti-carcinogenic and preventive characteristics, a growing number suggest that tumor cells can have aggressive behavior following HCC. 1,25-Dihydroxyvitamin D3 was not recommended for the treatment of HCC in our previous research. Based on the obtained data from this study, we continue to support the same hypothesis.

Keywords: 1.25-dihydroxyvitamin D, Apoptosis, Hepatocellular Carcinoma, Mitophagy

*Oral Presentation – 48*

Combination Therapy of dual PI3K/mTOR Inhibitor and Curcumin shows anticancer effect on Colorectal Cancer

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Introduction and Aim: Colorectal cancer is a heterogeneous disease that is highly diagnosed worldwide. Curcumin is an anticancer agent that is effective in colorectal cancer. However, poor chemical stability of curcumin limit its antitumor activity in clinical applications. PI3K/AKT pathway plays an important role in the aggressive nature of cancer including resistance to chemotherapy. NVP-BEZ235 is a dual PI3K/mTOR kinase inhibitor that induces apoptosis and suppresses the growth of cancer. The current study investigates the synergetic anticancer effect of NVP-BEZ235 and curcumin on colorectal cancer.

Materials and Methods: MTS assay was conducted to detect the cytotoxic effects of curcumin and NVP-BEZ235. Cellular uptake was determined by flow cytometry. Colony forming assay was investigated the growth inhibition of HCT116 cells. Cell cycle, Annexin V/PI and gene expression levels were evaluated to determine the anticancer effect.

Results: Synergistic effect was seen in combination treatment of curcumin and NVP-BEZ235 in HCT116 cells. Cytotoxicity of both anticancer agents was observed in a dose and time dependent manner. Colony forming assay reveals that combination therapy of curcumin and NVP-BEZ235 can inhibit the growth of HCT116 cells. Cell cycle arrest at SubG0 phase shows improved anti-cancer characteristics. Annexin V/PI assay showed increase in early and late apoptosis in combination group when compared to control group. Moreover, qPCR results showed significant increase in the apoptosis related gene expression levels.

Conclusion: Taken together, our findings demonstrate that combination therapy of NVP-BEZ235 and curcumin has anticancer potential in HCT116 cells. These anticancer agents together may be a promising therapeutic candidate for colorectal cancer therapy.

Keywords: NVP-BEZ235, curcumin, synergistic effect, anti-cancer effect, colorectal cancer

*Oral Presentation – 49*

# The role of Trop-2 expression in determining the effectiveness of Sacituzumab Govitecan in the treatment of triple-negative breast cancer patients.

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Introduction and Aim: Triple negative breast cancer (TNBC) is the most aggressive of all breast cancer subtypes. In recent years, antibody-drug conjugate based therapies developed for TNBC have shown promising results. Sacituzumab govitecan (SG), is an anti-Trop-2 antibody-drug conjugate with SN-38, which was approved by the FDA in 2020. In our study, we aimed to evaluate the expression of Trop-2/Tacstd2 in TNBC patients to determine who would benefit from SG treatment and, in addition, to evaluate the significance of clinical parameters associated with the Trop-2 expression data obtained in these patients.

Materials and Methods In this study, RNA was isolated from 45 paraffin-embedded tumors and normal tissues, and then cDNA synthesis was performed. Trop-2 expression levels were investigated using the RT-PCR method. Quantitative data obtained were evaluated using normality tests, t-tests, $X^2$-tests and correlation analysis.

Results: The gene expression differences between tumor and normal tissues of the patients were analyzed. A 1,935-fold increase in Trop-2 expression (p=0,033) was observed. When the expression differences obtained were evaluated together with the clinicopathological data of the patients, statistical significances was determined between the Trop-2 expression difference and necrosis (p=0,041), in-situ component (p=0,037) and pathological tumor size (p=0,035).

Conclusion: It is crucial to elucidate the differences in Trop2 expression (increased or decreased expression) in specific cancer types and disease stages in order to unveil the full role of Trop2 in cancer growth and metastasis. Our findings provide crucial clues for the first time regarding the role of Trop-2 as a prognostic biomarker in TNBC.

Keywords: Breast Cancer, TNBC, Sacituzumab Govitecan, SN-38 Trop-2

This study was supported by a grant from the Scientific Research Projects Foundation (BAP) of Bursa Uludag University in Turkey [Project No: THIZ-2022-2021]

*Oral Presentation – 50*

# Investigation of the Relationship Between Bivalent Promotor Regions And Epithelial-Mesenchymal Cancer Cells Plasticity

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Introduction and Aim: Cell plasticity contributes to transition across distinct cell states by epithelial-mesenchymal transition (EMT) and vice versa. It enables to acquire phenotypic and functional features of pathological conditions during tumor progression. The dynamism points to epigenetic regulation such as “Bivalent promoter” regions which are simultaneously marked by both activating H3K4me3 and repressive H3K27me3 modifications. The aim of our study was to investigate bivalency and changes in “bivalent promoter” regions of genes including stem cell markers, differentiation markers and polycomb group members.

Materials and Methods: To mimic cancer cell plasticity, HT-29 cells which undergo spontaneous MET/EMT, were used. Three cell population were generated: parental (pHT-29), epithelial (eHT-29) and mesenchymal (mHT-29) cells. The chromatin domains containing both H3K4me3 and H3K27me3 were immunoprecipitated by sequential chromatin immunoprecipitation and the bivalency in the promoters of stem cell markers (CD44 and CD133), differentiation and epithelial marker (CDX2), and polycomb group members (CBX4, CBX7 and CBX8) as well as expression levels were determined by qPCR.

Results: Bivalent marks in CD133 and CD44 promoters were found at higher levels in mesenchymal cancer cells in line with increased gene expression. Similar bivalency in CDX2 promoter regions were seen in mHT-29 cells with decreased CDX2 mRNA levels. Also, for CBX4, CBX7 and CBX8, bivalency was determined in mesenchymal cells. While CBX7 and CBX4 mRNA decreased, CBX8 mRNA increased in mHT-29 cells.

Conclusion: The effect of bivalent promoters on the plasticity of cancer cells has not been fully explained. The results showed that mesenchymal cancer cells had bivalent promoter marks in line with stem cell phenotype.

Keywords: colon cancer, cancer plasticity, epithelial-mesenchymal transition, mesenchymal-epithelial transition, epigenetic regulation, bivalent promotor.

*Oral Presentation – 51*

Ceramide Binds Smad7 to Regulate Solid Tumor Metastasis

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Introduction and Aim: Although the cancer survival rate has been significantly improved during the past several years, progress in the treatment of cancer metastasis has been limited. Therefore, development of mechanism-based novel therapeutic strategies for targeting cancer metastasis is urgently needed. Thus, in this study we aimed to determine the structural details of how CerS4-generated C18-ceramide binds Smad7 for the regulation of TßR-Smo-mediated cancer cell invasion/migration.

Materials and Methods: Interactive docking prediction of protein-lipid complexes and modeling between ceramide and Smad7 were performed using ZDOCK and Phyre2. Ceramide-Smad7 interaction was analyzed with IP and PLA assay. P-Smo protein abundance was detected by immunohistochemistry using anti-P-Smo (S615)-specific antibody.

Results: Our in vitro lipid-protein binding studies showed that recombinant human Smad7 bound ceramide with Kd= 382 nM, which is within its physiological range. Our molecular modeling/simulation study showed that Q300 of Smad7 might be involved in ceramide binding. Ectopic expression of wt-Smad7 highly associated with ceramide, but not mut-Smad7 in PLA assay. We then examined the phosphorylation of Smo in response to CerS4 knockdown. Data showed that CerS4 knockdown resulted in 4.5-fold increase in P-Smo expression compared to controls without affecting total Smo abundance. These data were also consistent with overexpression of P-Smo measured by IHC in metastatic NSCLC compared to non-metastatic tumors using a TMA.

Conclusion: Overall, these data indicate that ceramide might stabilize Smad7 inhibitory complex by directly binding to Smad7 via lipid-protein interaction. Besides, CerS4 knockdown enhances Smo dependent cell migration by inducing Smo phosphorylation at S615.

Keywords: Metastasis, Ceramide, Smad-7, P-Smo

*Oral Presentation – 52*

# A NOVEL PALLADIUM (II) COMPLEX SELECTIVELY INDUCES CELL DEATH AND CELL CYCLE ARREST IN METASTATIC COLON CANCER CELLS

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Introduction and Aim: Colon cancer is the third most common type of cancer in the world. A large number of drugs are used in the treatment of colon cancer, but these drugs are not very effective in late stages of colon cancer. it is difficult to develop an effective treatment and new approaches are needed. Drugs should be effective on cancer cells with low doses, on the other hand they need to show low cytotoxicity on normal cells. In this study, we tested anticancer effects of 4 different new synthesized Pyridine derivative complexes that containing palladium on the SW620 colon cancer cell line and CCD-18CO normal colon cells.

Materials and Methods: First, cell lines were treated with those at ranged concentrations from 1 to 200 µm and IC50 values were calculated by performing cell vitality tests. To evaluated anti-cancer effects of those drugs, we analyzed levels of apoptosis and cell cycle upon to drug treatment. Then, we tested many marker proteins for apoptosis, cell cycle and autophagy with Westernblot.

Results: We observed that one of new synthesis drugs was the most effective at low doses on SW620 and but high doses on CCD-18Co. In addition, a novel palladium (II) complex selectively induced cell death and cell cycle arrest in SW620 cells at low concentration.

Conclusion: In conclusion, we found out that palladium (II) complex has a high potential for new colon cancer targeting therapy. However, future work will be required to observe effect of this new drug in vivo.

Keywords: Colon cancer, palladium (II) complex, apoptosis, cell cycle

Acknowledgement: This work was supported by the by Mehmet Akif Ersoy University Scientific Research Projects Coordinatorship under Grant No: 0776-YL-21. Zümre Ayça Kalabalik Supported by a fellowship from the Scientific and Technological Research Council of Turkey (TUBITAK) under Grant No: 120Z640.

*Oral Presentation – 53*

Neuroblastoma Targeted Anti-Cancer Drug Delivery

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Introduction and Aim: Everolimus is mTOR inhibitor and Tozasertib is an AURORA inhibitor. Both of these proteins plays an important role in stabilizing MYCN protein. MYCN is an important prognostic marker of neuroblastoma (NB). Ganglioside2 (GD2) is a protein that is overexpressed in tumor tissue in primary NB. Drug delivery systems that directly target tumor cells is an effective method for presenting drug combinations together. In this study effect of the GD2 targeted nanodrug including Everolimus+Tozasertib on NB was studied.

Materials and Methods: 20 mg of PEG-b-PLGA was used to form nanoparticle. $IC_{50}$ doses of therapeutics were added to the nanostructure with 1:3 rate. FTIR,size,loading capacity(LC) and zeta potential were measured. 500 µg of DTX-B (mAb) was attached to target the formed nanoparticle. LC was determined by BCA assay. Xenographt model was formed by injecting Kelly cells. After tumor was formed, $IC_{50}$ doses of nanoparticles per day administered to mice intravenously for 5 days. After treatment and sacrification, tissues were collected. Molecular analyses was performed.

Results: 7.8 mg of EVER-TOZA@PEGbPLGA/DTX-B, showed 30% viability on cells after 24 hours. A reduction in tumor size was observed in mice treated for 5 days. Compared to combination group without NP, EVER-TOZA@PEGbPLGA/DTX-B was effective. Additionally, nanostructure affected on tumor size compared to control. There was a decrease in MYCN and Aurora A gene expression.

Conclusion: Everolimus+Tozasertib combination targeted by nanostructure via GD2 antibody is shown to be a candidate therapeutic agent both in vitro and in vivo animal models in NB.

Keywords: Neuroblastoma, Nanoparticle, Drug Delivery

*Oral Presentation – 54*

Anti-growth, Antioxidant, and Hepatoprotective Properties of *Spirulina platensis* Extract

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Introduction and Aim: *Spirulina platensis*, a filamentous cyanobacterium often referred to as blue-green algae, is recognized for its biological activities, including antioxidant, immunomodulatory, anti-inflammatory properties. This study was conducted to investigate the growth inhibitory effects of the ethanolic extract of *Spirulina platensis* on PANC-1 and MIA PaCa-2 human pancreatic cancer cell lines. Additionally, the *in vivo* hepatoprotective, antioxidant properties of *S. platensis* were explored.

Materials and Methods: The ethanolic extract of *S. platensis* was lyophilized and then dissolved in DMSO. Subsequently, PANC-1, MIA PaCa-2 cell lines were treated with concentrations ranging from 0.1 to 1000 µg/ml. Cell viability was assessed using the sulforhodamine B viability assay. For *in vivo* evaluations of hepatoprotective and antioxidant properties, *Spirulina* was administered to rats via gavage at a dose of 500 mg/kg/day for four weeks. The activity levels of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were measured in the heart and liver tissues of the rats.

Conclusion: The control group that received *Spirulina* demonstrated a significant elevation in the activity of SOD, GSH-Px enzymes in heart and liver tissues. There was a significant reduction in ALT and AST enzyme levels. The extract notably hindered the growth of both examined cell lines. Future research can focus on studying the effects of *Spirulina platensis* extracts in conjunction with various chemotherapeutic agents or its impact on different cancer cell lines to more comprehensively understand its anti-growth attributes.

Keywords: *Spirulina platensis*, Pancreatic cancer, PANC-1, MIA PaCa-2, SOD, GSH-Px.

*Oral Presentation – 55*

# Investigation of the effect of toluene on nitric oxide production and protective properties of resveratrol

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Introduction and Aim: Toluene is one of the most used organic solvents in the world. Prolonged exposure to toluene, causes serious health problems. The International Agency for Research on Cancer (IARC) has classified toluene as "possibly carcinogenic to humans" (Group 2B). This classification states that toluene may have potential carcinogenic effects. Toluene exposure has been linked to the formation of reactive oxygen species and reactive nitrogen species, resulting in direct tissue damage and alteration of various antioxidant systems. Resveratrol is a naturally occurring polyphenol in many plant species known for its diverse biological effect. In this study, the effect of exposure to toluene on nitric oxide production, which has an important role as a biological regulator in cardiovascular, neurological, immunological and many other systems, and the protective properties of resveratrol were investigated.

Materials and Methods: Wistar-Albino male rats weighing 250-350g were administered toluene at a dose of 900mg/kg and three doses of resveratrol (5mg/kg, 10mg/kg, and 20mg/kg) intraperitoneally for six days. Nitric oxide levels and Nitric oxide synthase activities were investigated in liver tissue and serum.

Results: The results showed an increased nitric oxide level in the liver tissue and serum and a high nitric oxide synthase activity following toluene administration. Significant reductions in nitric oxide levels and nitric oxide synthase activity in the liver were observed after the administration of various dosages of resveratrol.

Conclusion: Our results suggested that high doses of toluene induce nitric oxide production, whereas resveratrol possesses protective properties.

Keywords: Toluene, resveratrol, nitric oxide, nitric oxide synthase

*Oral Presentation – 56*

Investigation of Anti-Cancer Potential of Omeprazole in Prostate Cancer Cells

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Introduction and Aim: Prostate cancer is the second most common type of cancer in men worldwide. Proton pump inhibitors used widely in gastritis disease are used as anti-cancer agents by drug repurposing. Omeprazole is a proton pump inhibitor and an FDA-approved anti-acidic drug used in acid-related diseases. The anti-cancer potential of omeprazole has been demonstrated in many cancer types. The aim of our study is to carry out in vitro experiments to shed light on the clinical use of omeprazole in prostate cancer.

Materials and Methods: 2D studies were performed. After 2D studies, 3D culture studies and scratch assays were performed to determine the effect of omeprazole on spheroid forming and migration capabilities of cancer cells, respectively. Finally, GLUT and V-ATPase assays were performed.

Results: The IC50 scores of PC-3, LnCap and CCD1072-sk cells were determined. Omeprazole inhibited significantly the numbers and size of PC-3 colonies. Surprisingly, a higher amount of glucose in the medium of the control group and acidity of the medium in the drug-treated group was higher than the control group. But in the CCD1072-sk cells group, the acidity of the medium in the control group was higher than the treated group.

Conclusion: Omeprazole doesn't only inhibit V-ATPase, but also suppresses the FASN enzyme, which plays an important role in lipid metabolism in cells. In the light of these findings, cancer cells may use the glycolytic pathway more actively in response to this, since omeprazole suppresses lipid metabolism.

Keywords: Prostate cancer, drug repositioning, proton pump inhibitor, omeprazole.

*Oral Presentation – 57*

Acetylsalicylic acid treatment reduces cancer promoting properties of pancreatic stellate cells

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Introduction and Aim: Pancreatic cancer is a highly aggressive type due to its unique tumor microenvironment. Active pancreatic stellate cells (PSCs) are abundant in the PaCa microenvironment and can promote cancer aggressiveness by secreting growth factors and cytokines. The daily use of acetylsalicylic acid (ASA), the active component of aspirin, has been linked to low cancer incidence in various cancers, including pancreatic cancer however, there is currently no study indicating its role on pancreatic stellate cell-mediated cancer aggressiveness. Therefore, we aimed to investigate the effect of ASA on PSCs and thereby aggressiveness of pancreatic cancer.

Materials and Methods: PSCs were evaluated for active and passive states using α-smooth muscle and Oil Red O stainings. Aspirin doses of 1.25 and 0.625 mM that were not toxic for cells selected for further experiments. PANC-1 and BxPC-3 PaCa cell lines were treated with the CM collected from non-treated (NT) PSCs and 24h ASA-treated PSCs. Changes in cell viability, migration, and invasion were evaluated using SRB assay, wound healing, matrigel invasion and colony formation assays, respectively. The difference in CM collected from PSCs after ASA pre-treatment was elucidated by ELISA and changes in released IL-6 levels were measured.

Results: The study revealed that PaCa cells exhibited increased proliferation, migration, and invasion when exposed to CM from NT PSCs, while these aggressive characteristics decreased when incubated with CM from ASA-treated PSCs.

Conclusion: ASA-treatment decreased cancer-promoting abilities of PSCs by possibly changing its secretome. Further research is needed to reveal exact mechanism of ASA on PSCs.

Keywords: pancreatic cancer, pancreatic cancer tumor microenvironment, asetylsalicylic acid.

*Oral Presentation – 58*

# Evaluation of Circulating Tumor Cell (CTC) Specific Markers and CTC Status in Metastatic Colorectal Cancer Patients by Immunomagnetic Cell-Selection Method

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Introduction and Aim: Detection of circulating tumor cells (CTC) has great potential for assessing the risk of metastatic colorectal cancer (mCRC). However, testing for CTC is not yet part of the clinical routine due to the cumbersome methodologies and concerns about sensitivity issues. Here, we evaluated the CTC status (CTC positive/CTC negative) of metastatic CRC patients and detection rates of CTC-specific markers by immunomagnetic cell-selection method to utilize the CTC status as a clinical parameter and assess the risk of metastasis development.

Materials and Methods: Peripheral blood samples were collected from 48 mCRC patients and CTC status was determined by using AdnaTest ColonCancer technology which characterizes tumor cells based on colon-specific surface markers (CEA, EGFR and EpCAM). All samples were grouped into their CTC status [CTC-positive (presence of ≥1 CRC-specific mRNA markers) or CTC-negative] and evaluated by other clinical parameters.

Results: A total of 30 (62,5%) patients were found to be CTC-positive of which 7 patients had only EGFR-positive CTC whereas CEA positivity was observed in only one patient. However, both EGFR and CEA markers were detected in 22 of 30 CTC positive. Beta-actin expression was analyzed for each sample, and all were positive. We did not detect EPCAM positivity in any samples.

Conclusion: Our preliminary results provide knowledge to liquid biopsy investigations to consider CTC status as a clinical parameter by incorporating other clinical data for precisely assessing the risk of CRC metastasis. Future studies including larger cohorts by analyzing additional biomarkers will pave the way for the development of novel translational medicine approaches.

Keywords: metastatic colorectal cancer, circulating tumor cells, liquid biopsy, tumor markers, immunomagnetic cell selection.

*Poster Presentation – 01*

# Studies on the Synthesis of Some Novel Benzamide Compounds As HDAC Inhibitor

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Introduction and Aim: Cancer is a multistage disease consisting of genetic and epigenetic factors. One of the most important epigenetic rearrangements, histone acetylation is catalyzed by histone acetyltransferase (HAT) and histone deacetylase (HDAC), which function in opposition to each other. Studies have shown that HDAC inhibitors significantly control tumor incidence and metastasis. In this study, benzamide compounds were synthesized considering their HDAC inhibitory properties.

Materials and Methods: Aminoalkylcarboxylic acid was synthesized by adding 1,1'-CDI, DBU, TEA to arylmethanol solution. The solution was acidified with HCl (pH=5) to precipitate a white solid which was collected by filtration, and purified by column chromatography to give aminoalkylcarboxylic acids. Substituted benzamide compounds were synthesized by reacting carboxylic acids with DMF, oxalyl chloride, imidazole and o-substituted phenylamine and then purified by column chromatography.

Results: The structure of the synthesized compounds was elucidated by elementary analysis, $^{1}$H NMR, $^{13}$C NMR and mass spectral data. All spectral data were in accordance with assumed structures.

Conclusion: The structures of the synthesized compounds have been elucidated; activity studies are continued.

Keywords: anticancer, benzamide, epigenetic, HDAC inhibitors,

Acknowledgement: This study is supported by TUSEB (Project No: 12220).

*Poster Presentation – 02*

High-passage LNCaP cells show castration resistance

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Introduction and Aim: Androgen deprivation is the primary treatment for prostate cancer (PCa). However, patients eventually develop castration resistance (CR), and become unresponsive to the treatment after a few years of medication. Therefore, deciphering the mechanisms of castration resistance and development of cell culture protocols for CR-PCa cells are of high importance. Here, we aimed to show the CR potential of LNCaP cells with high passage numbers ($LNCaP_{HP}$).

Materials and Methods: LNCaP cells were first cultured in RPMI1640 medium supplemented with 10% FBS and 1%pen/strep at 37°C and 5% $CO_2$. In order to obtain CR, LNCaP cells were passaged once a week until P67 in RPMI1640 medium w/o phenol red, supplemented with Charcoal Stripped Fetal Bovine Serum (CSS) and 1%pen/strep. Cell viability was determined using CVDK8 kit. PSA levels of high and low passage cells were compared using ELISA kit. Viability was analyzed in CSS-treated and untreated cells upon R1881 application. Expression levels of AR and CR-associated AKR1C3 and AR-V7 genes were determined via qRT-PCR of total RNA samples from P28, P67 and CSS-applied $LNCaP_{HP}$ cells.

Results: $LNCaP_{HP}$ cells showed decreased expression of AR and increased expression of ARV7 and AKR1C3 genes. All the gene expressions were increased in CSS-applied $LNCaP_{HP}$ cells. Although PSA levels were undetectable in $LNCaP_{LP}$ cells, the levels were consistent with CR in $LNCaP_{HP}$.

Conclusions: Our results imply that $LNCaP_{HP}$ cells show increased expressions in CR-related genes and PSA levels, therefore, can be used in in vitro assays to decipher the mechanisms of CR.

Keywords: Castration resistance, prostate cancer, LNCaP, gene expression, androgen receptor

*Poster Presentation – 03*

TNF-α-mediated effects significantly contribute to TGF-β1-induced epithelial mesenchymal transition in prostate cancer metastasis

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Introduction and Aim: TGF-β1 plays a major role on epithelial mesenchymal transition (EMT). Many factors in the tumor microenvironment contribute to EMT leading metastasis and inflammation-induced alterations are known to involve both in carcinogenesis and metastasis. NKX3.1 is a prostate epithelium-specific marker and a tumor suppressor protein, which has previously shown to undergo TNF-α-induced proteasomal degradation in inflammatory microenvironment. Recent studied revealed its regulatory role in maintenance of stemness raising new questions on the role of NKX3.1 in transition to mesenchymal phenotype. Therefore, we aimed to investigate NKX3.1-related alterations during both TNF-α- and TGF-β1-mediated cellular events in EMT including expressional changes of the EMT markers and viability and anoikis resistance of prostate cancer cells.

Materials and Methods: LNCaP cells cultured with conditioned media (CM) derived from macrophages for 8 days in order to mimic the chronic inflammatory tumor microenvironment of the prostate. Various concentrations of the mentioned cytokines were used in combination in order to distinguish the EMT-related cellular effects of TNF-α and TGF-β1. Cellular alterations such as expressional changes of EMT markers, cell viability and anoikis resistance were investigated.

Results: Overexpression of NKX3.1 resulted in significant alterations of EMT markers. Increase in fibronectin expression observed in both TNF-α- and TGF-β1-mediated inflammatory conditions was reverted by NKX3.1. Increased protein levels of Vimentin, Twist-1, Snail-1, Snail-2, and Zeb-1 in cytokine induction was detected to enhanced by NKX3.1 ectopic expression suggesting that NKX3.1 expression in inflammatory microenvironment results in induction of mesenchymal markers.

Conclusion: NKX3.1 contributes to positive regulation of mesenchymal phenotype.

Keywords: prostate cancer, stemness, epithelial mesenchymal transition

Acknowledgement: This study was supported by Ege University (Project numbers 23063 and 23389).

*Poster Presentation – 04*

Studies on the Synthesis of Some Novel Imidazopyridine Compounds as HDAC Inhibitors

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Introduction and Aim: Epigenetic mechanisms control a variety of aspects of cancer biology, including primary tumor development and invasion by histone modification. Gene expression is regulated by the balance between histone acetyltransferase (HAT) and histone deacetylase (HDAC). HDAC inhibitors diminish angiogenesis, alter immunological response, and cause differentiation and cell death in cancer cells therefore they are considered as promising antineoplastic agents. In this study, imidazopyridine compounds were designed and synthesized considering their HDAC inhibitory properties.

Materials and Methods: Designed products were obtained by adding 1,1'-CDI, o-substituted phenylamine and trifluoroacetic acid onto imidazopyridine methanol solution in THF and purified by column chromatography.

Results: The structure of the synthesized compounds was elucidated by $^{1}H$ NMR, $^{13}C$ NMR and mass spectral data. All spectral data were in accordance with assumed structures.

Conclusion: The structures of the synthesized compounds have been elucidated; activity studies are ongoing.

Keywords: epigenetic, histon modification, HDAC inhibitors

Acknowledgement: This study is supported by TUSEB (Project No: 12220).

*Poster Presentation – 05*

3D Prostate Cancer Model to Represent Ephitelial Mesenchymal Transition

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Introduction and Aim: Use of 3D cell culture model instead of the conventional 2D has been shown to have many advantages such as better representing the in vivo cellular interactions and drug responses in a time and cost saving way enabling more reliable investigation of the molecular mechanisms of cancer and development of new therapeutics. Therefore, our study aims to develop a 3D cell culture model to represent the epithelial-mesenchymal transition (EMT) of the prostate cells induced by the inflammatory tumour microenvironment.

Materials and Methods: Prostate spheroids were formed using poly-HEMA coated U-bottom 96-well plates. Spheroid diameter was followed under microscope for 7 days in order to ensure the spheroid stability and viability experiments were performed for 2D and 3D cell culture models under inflammatory conditions. Alterations in inflammation-mediated EMT factors were studied by western blotting.

Results: EMT markers in spheroids were observed to change distinctly in comparison to 2D-cultured cells upon inflammatory conditions based on the results showing expressional changes in NKX3.1, fibronectin, E-cadherin, vimentin, Snail-1 and Twist-1. Although protein levels of NKX3.1, fibronectin, and Snail-1 differed in 2D- and 3D-cultured cells, expressional changes upon inflammation remained in the same way. However, E-cadherin, vimentin, and Twist-1 were shown to be enhanced in 3D spheroids where they suppressed in 2D cells suggesting that 3D spheroids are necessary to mimic inflammation-induced EMT of prostate cells.

Conclusion: We optimized a 3D EMT model of prostate cells, which can serve as a useful tool for prostate cancer research and anticancer drug development studies.

Keywords: Prostate spheroids, epithelial mesenchymal transition, 3D cell culture, prostate cancer

Acknowledgement: This study was supported by Scientific and Technological Research Council of Turkey (222S863) and Ege University (27410).

*Poster Presentation – 06*

# Investigation of the Protective Effect of Resveratrol on Some Enzyme Activities on Toluene Toxicity in Rats

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Introduction and Aim: Toluene is hydrocarbon and has toxic effects. Toxicity from exposure to toluene and the carcinogenicity of are a concern. About 80% of which is metabolized in the liver, triggers the production of ROS by creating oxidative stress in the body and leads to the activation of the apoptotic signaling mechanism. Against this toxic effect, in addition to the antioxidants produced by our body, the antioxidants we consume with food also provide a protective effect. Resveratrol, is a well-known antioxidant and anticancer phytochemical used in cancer research. The protective and anticancer effects of resveratrol on some biochemical parameters in brain tissues of rats were evaluated.

Materials and Methods: 36 male Wistar-albino rats were used. They were divided into 2 groups as control and experimental groups. The control groups were divided into two groups, namely physiological saline and ethanol. The experimental group, on the other hand, was grouped into 4 groups according to toluene and toluene+resveratrol administration as 5 mg/kg, 10 mg/kg and 20 mg/kg resveratrol doses. Brain tissues were prepared for analysis by homogenization, and biochemical parameters were analyzed spectrophotometrically in an autoanalyzer by enzyme kinetics.

Results: AST and ALT enzyme levels, which are increased in liver and muscle damage and metabolic disorders, as well as in tissues such as brain, kidney and heart, showed a significant increase compared to ALP and GGT enzyme levels. Dosage-dependent resveratrol administration showed that a 20 mg/kg dose was effective.

Conclusion: Aminotransferase elevation in brain tissues may be related to cancer and volatile substance use.

Keywords: Toluene, Carcinogen, Apoptosis, Resveratrol

*Poster Presentation – 07*

Anti-proliferative Effect of Dacomitinib and Retinoic Acid on Triple Negative Breast Cancer

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Introduction and Aim: Breast cancer is the most common diagnosed cancer with the leading cause of cancer-related deaths in women worldwide. Almost 15% of all breast cancer is diagnosed as triple negative breast cancer (TNBC). TNBC shows poor prognosis and high side effects of chemotherapy is seen frequently. Thus, combination therapy is generally preferred to overcome toxicity and drug resistance. Dacomitinib is an irreversible pan-HER inhibitor. Combination strategies of EGFR inhibitors show promise to overcome intrinsic resistance to these inhibitors. All-trans retinoic acid (ATRA), active metabolite of vitamin A, is a promising agent for treating breast cancer. Its anti-tumor effect is predominantly due to an inhibitory effect on growth of tumor. Therefore, in our study we investigated the anti-proliferative effects of combination therapy of Dacomitinib and ATRA on MDAMB231 cells.

Materials and Methods: The cytotoxic effects of Dacomitinib and ATRA on MDA-MB-231 cells were determined using the MTS assay. Colony formation and scratch assay was performed to investigate the clonogenic potential and migration ability of MDA-MB-231 cells, respectively.

Results: Effective combination treatment dosage was found to be 1.5 μM of Dacomitinib and 5 μM of ATRA on MDA-MB-231 cells at 48h. Combination treatment of Dacomitinib and ATRA on MDA-MB-231 cells significantly reduced the migration and colony formation compared to control group.

Conclusion: Our study showed that combination therapy of Dacomitinib and ATRA has high anti-proliferative potential in MDA-MB-231 cells. These findings suggest that this combination therapy holds promise as anticancer agent and to be used in breast cancer therapy.

Keywords: triple negative breast cancer, Dacomitinib, all-trans retinoic acid.

*Poster Presentation – 08*

# EVALUATION OF THE ANTIPROLIFERATIVE EFFECT OF SAFRANAL IN C-4 I CERVICAL CANCER CELL LINE

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Introduction and Aim: Safranal is a monoterpene aldehyde responsible for the aroma of Crocus sativus. Many studies have shown the antioxidant activity of safranal besides some pharmacological properties, including its anti-inflammatory effect. This study aimed to determine the cytotoxic effects of safranal on C-4 I, cervical cancer cell line.

Materials and Methods: To determine the cytotoxic effect of safranal on the C-4 I cell line, cells were incubated for certain times (2-72 hours) and concentrations (25-800µM). After incubation, the viability of cells and the anti-proliferation effect of safranal were determined respectively by MTT and LDH assays. In addition, Morphological changes occurring during incubation in cells were observed under inverted and light microscopes using Giemsa staining.

Results: According to the results, compared to Control group, the % viability of treated cells was decreased depending on concentration and the incubation time, and safranal significantly inhibited the growth of C-4 I cells ($p<0.05$). Some morphological changes such as nuclear condensation, and apoptotic and pyknotic cells were examined under light microscopy with Giemsa staining.

Conclusion: Based on the results obtained from this study, we can be said that safranal has an antiproliferative effect against cervical cancer-derived C-4 I cell lines.

Keywords: C-4 I, Safranal, Cervical cancer, Cytotoxicity, Antiproliferative

*Full Text - 01*

# THE EXPRESSION ANALYSIS OF SPECIFIC GENES IN OVARIAN CANCER

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## ABSTRACT

*Due to a lack of diagnostic and prognostic biomarkers, ovarian cancer (OC), the most lethal gynecologic malignancy, is frequently diagnosed at an advanced stage. Therefore, identification of OC specific biological markers is a vital step for diagnosis and treatment response. Our goal is to examine functional gene sets which are possibly markers for ovarian cancer and their expression profiles in OC patients. We also aim to determine the potential of the genes which could be possible therapeutic targets for OC patients. By using qRT-PCR, the expression profiles of seven genes (FOS, FOSL2, JUN, MMP-2, MMP-9, TIMP-2, and VEGFA) were identified. The tumor-free control group consisted of total abdominal hysterectomy (n=1) and bilateral salpingo-oophorectomy (n=9) patients who underwent gynecologic procedures. High-grade serous OC epithelial samples (n=10) were used for the experiment group. According to the qRT-PCR data, there is an increased expression of FOS (p=0.0089), MMP-9 (p=0.0029), VEGFA (p=0.0434) and decreased expression of FOSL2 (p=0.0271), JUN (p=0.0041), TIMP-2 (p=0.0062). In conclusion, the data may produce new insights regarding OC pathogenesis and treatment. The candidate genes may improve individualized diagnosis and therapy for OC in the future.*

## ÖZET

*Tanısal ve prognostik biyobelirteçlerin eksikliği nedeniyle, en ölümcül jinekolojik malignite olan over kanseri (OK), sıklıkla ileri evrede teşhis edilir. Bu nedenle, OK'ye özgü biyolojik belirteçlerin tanımlanması, teşhis ve tedavi yanıtı için hayati bir adımdır. Amacımız, OK hastalarında over kanseri için muhtemelen belirteç olan fonksiyonel gen setlerini ve ekspresyon profillerini incelemektir. Ayrıca OK hastaları için olası terapötik hedefler olabilecek genlerin potansiyelini belirlemeyi amaçlıyoruz. qRT-PCR kullanılarak yedi genin (FOS, FOSL2, JUN, MMP-2, MMP-9, TIMP-2 ve VEGFA) ekspresyon profilleri belirlendi. Tümörsüz kontrol grubu, jinekolojik prosedür uygulanan total abdominal histerektomi (n=1) ve bilateral salpingo-ooferektomi (n=9) hastalarından oluşturuldu. Deney grubu için yüksek dereceli seröz OC epitel örnekleri (n=10) kullanıldı. qRT-PCR verilerine göre FOS (p=0,0089), MMP-9 (p=0,0029), VEGFA (p=0,0434) ekspresyonunda artış ve FOSL2 (p=0.0271), JUN (p=0.0041) ve TIMP-2 (p=0.0062) ekspresyonunda azalma tespit edilmiştir. Sonuç olarak, veriler OK patogenezi ve tedavisi ile ilgili yeni yaklaşımlar geliştirilmesini sağlayacaktır. Aday genler, gelecekte OK için kişiselleştirilmiş tanı ve tedaviyi geliştirebilecektir.*

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# INTRODUCTION

Ovarian cancer (OC) is one of the most frequently diagnosed malignant carcinomas and is the leading cause of gynecological cancer-related death (Singh and Som, 2021; Zheng et al., 2019; Torre et al., 2018). Because it is asymptomatic, more than 70% of OC cases are detected at the advanced stages (Yang et al., 2020; Torre et al., 2018). Low treatment efficiency is caused by several factors, including late diagnosis, a lack of precise biomarkers, the development of drug resistance, and phenotype heterogeneity (Alshamrani AA., 2020). Identification of prognostic biomarkers and the development of personalized treatment for OC patients are significant due to the difficulties in early and effective diagnosis and the heterogeneous prognosis of OC (Liu et al., 2022; Yang et al., 2020). The gene expression profiles of tissue or liquid biopsy samples are widely used for the classification, diagnosis, or prognosis of different diseases including cancer (Gumusoglu-Acar et al., 2023; Apostolou et al., 2019; Gunel et al., 2019). In recent years, researchers have begun to investigate the risk factors underlying the etiology of OC by gene expression analysis. The dysregulation of the several genes is shown to be associated with OC (Ono et al., 2000). There has been an increasing interest in screening differentially expressed genes using various expression-based technologies (Olbromski et al., 2022; Rutter et al., 2019; Gunel et al., 2019; Narrandes and Xu, 2018).

### AIM

In this study, seven genes (*FOS, FOSL2, JUN, MMP-2, MMP-9, TIMP-2, VEGFA*) that are effective in the pathogenesis of OC were selected based on the literature. Therefore, this study aimed to screen gene expressions by qRT-PCR validation to generate novel knowledge related to OC.

# MATERIALS AND METHODS

## Sample Collection

Tissue samples of high-grade serous ovarian cancer (HGSOC) patients (*n*=10) and individuals in the control group (*n*=10) were recruited from surgical specimens at the Department of Obstetrics and Gynecology, Istanbul Medical Faculty at Istanbul University. Normal ovary tissue samples were recruited from non-tumour-related oophorectomies performed in non-cancerous patients. OC tissues were obtained from patients diagnosed with primary HGSOC, and not previously undergone chemotherapy treatment or surgery. Whole sample collection and analysis processes are ethically approved by the Istanbul University Faculty of Medicine Clinical Researches Ethics Committee (Permission No: 2014/1175) on 08.08.2014. Each tissue sample was preserved in RNA Later and stored at − 80 °C until RNA extraction.

## Total RNA Isolation

Total RNA was extracted from the tissue using the RNeasy® Plus Mini Kit (QIAGEN, GmbH) according to the manufacturer's instructions. Total RNA was diluted to a concentration of 100 ng/µl for reverse transcription. The quantity of obtained total RNA was measured by NanoDrop IMPLEN P-Class (Thermo Fisher Scientific, Inc.). The isolated total RNA was stored at −80°C until the laboratory workup.

## Reverse Transcription and qRT-PCR

Complementary DNA (cDNA) synthesis was performed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Inc.) according to the manufacturer’s instructions. The Agilent SureCycler 8800 Thermal Cycler (Agilent, Santa Clara, Inc.) performed all the reverse transcription

reactions. The qRT-PCR was performed with CFX96 C1000 Touch Real-Time PCR Detection System (Bio-Rad Laboratories, Inc.) using SsoAdvanced Universal SYBR Green PCR Master Mix (Bio-Rad Laboratories, Inc.) following the manufacturer's guidelines. *GAPDH* gene was used as an internal control for gene expression normalization for qRT-PCR. We used the comparative threshold cycle (Ct) method 2^(−ΔΔCt) was used for the analysis of the data and the results were expressed as the relative quantification (RQ) values.

### Statistical Analysis of qRT-PCR

The GraphPad Prism (v.9) was used to perform statistical analysis. P-values less than <0.05 were considered statistically significant for all tests. Quantitative variables were presented as mean values ± standard deviations (SD) and categorical variables were presented as percentages. Mann–Whitney U test was performed to analyze the difference in gene expression between OC patients and controls according to the 2^(−ΔΔCt) values of each group. *P*-value, CI, and SD were calculated using Ct values obtained from qRT-PCR results. Receiver operating characteristic (ROC) curves were also generated, and areas under the ROC curves (AUC) were calculated to obtain sensitivity and specificity. The AUC value ≥ 0.5 was set as the cut-off for inferring the diagnostic value for the distinction between control and patient outcomes.

## RESULTS

### Clinical characteristics

The demographic and clinical characteristics of patients are shown in **Table 1**. A total of 10 subjects (median age, 50) with HGSOC patients and 10 with control (median age, 56) were enrolled in the current study. None of the patients in our study had received neoadjuvant chemotherapy or had undergone assisted reproduction, and all patients had undergone primary cytoreductive surgery.

**Table 1.** Clinical characteristics of the study sample.

| | **HGSOC (*n*=10)** | **Control (*n*=10)** |
|---|---|---|
| **Mean Age** | 50 | 56 |
| **Mean of CA-125 (IU/mL)** | 1697,67 | N/A |
| **Alcohol, n(%)** | 0(%0) | N/A |
| **Smoking, n(%)** | 1 (%10) | N/A |
| **Histological Types and Stages** | 5 cases are stage 3C grade 3 serous cancer, 2 cases are stage 3B grade 3 serous cancer, 1 case is 2A endometrioid, 1 case is 2B grade 1 serous cancer, 1 case is serous cancer with unknown grade | 5 cases are leiomyomas, 3 cases are uterine polyps, 1 case is cystocele, 1 case is adnexal mass |
| **The Number of Patients with Metastasis or Other diseases Occurred After Sample Collection** | 9 patients developed metastasis | 9 cases are total abdominal hysterectomy and bilateral salpingo-oophorectomy, 1 case is total abdominal hysterectomy |
| **Survival** | 6 patients died | N/A |

1. *N/A: Not available*

## The expression analysis of specific gene set

The expression analysis of mRNA was performed on all ovary tissue samples. *FOS, MMP-9* and *VEGFA* showed an increased expression and *FOSL2, JUN* and *TIMP-2* showed a decreased expression, **Fig. 1**. Except *MMP-2,* all of the genes were significant **(Table 2)**. The highest expressed gene was *MMP-9* (*P*=0.0029).

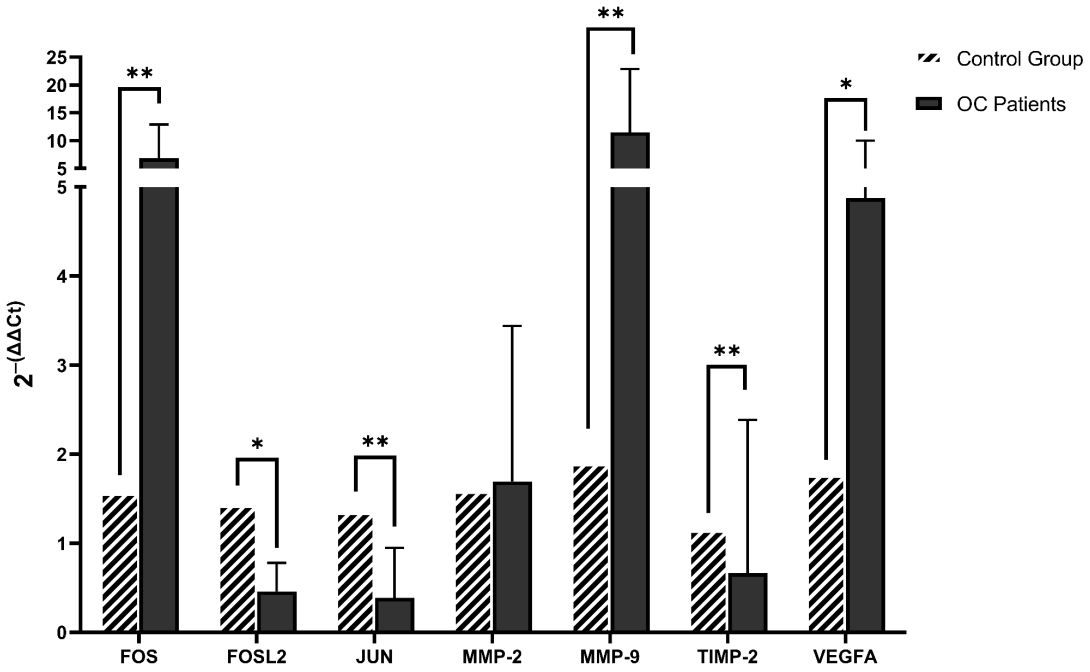


**Fig 1.** Relative expression analysis of tissue mRNAs. Significant (*P*<0.05) genes of interest. *P*-value ranges: 0.001-0.01 (very significant)**; 0.01-0.05 (significant)*; ≥ 0.05 (not significant).

**Table 2.** Average of patients and controls FC value.

| GENE | PATIENT | CONTROL | P-VALUES |
|---|---|---|---|
| *FOS* | 6.8 | 1.5 | 0.0089 |
| *FOSL2* | 0.4 | 1.4 | 0.0271 |
| *JUN* | 0.3 | 1.3 | 0.0041 |
| *MMP-2* | 1.7 | 1.5 | 0.1712* |
| *MMP-9* | 11.5 | 1.9 | 0.0029 |
| *TIMP-2* | 0.6 | 1.1 | 0.0062 |
| *VEGFA* | 4.8 | 1.7 | 0.0434 |

**: Not significant*

ROC curve analysis was performed to determine the discriminability of the genes. Highest AUC was 0.88 for *MMP-9*, and lowest AUC was 0.78 for *VEGFA*. ROC Curves can be seen in **Fig 2**.

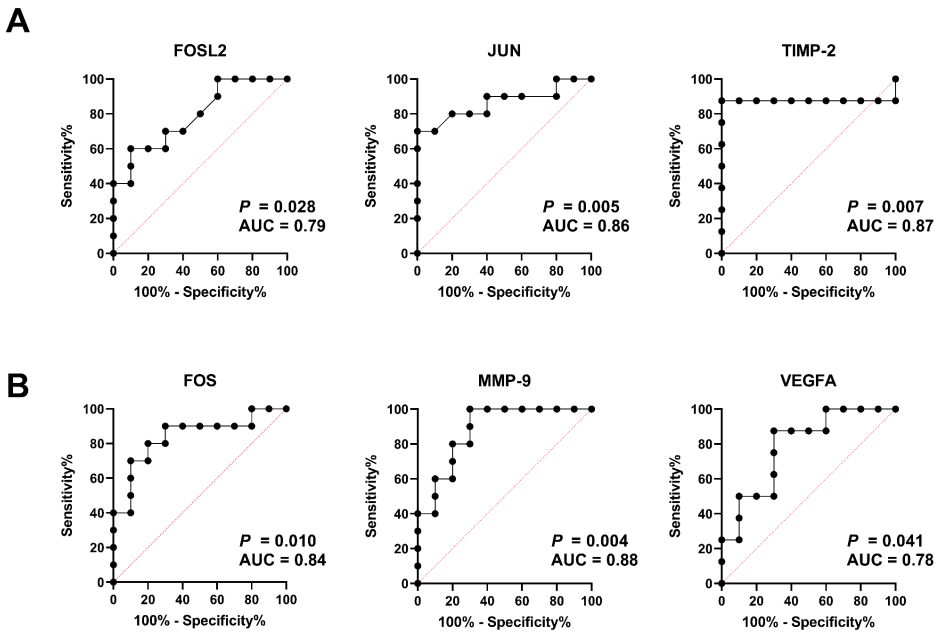


**Fig 2.** Results of receiver operating characteristic (ROC) parameters for increased and decreased putative genes OC patients were shown as A (decreased) and B (increased).

## DISCUSSION

The etiology of OC is still not completely understood for efficiently predicting disease development and designing tailored therapeutic approaches. Therefore, it is essential and urgent to investigate validated biomarkers and precise molecular pathways for the early diagnosis, treatment, and prognosis of OC. Thus, in the present study, seven genes (*FOS, FOSL2, JUN, MMP-2, MMP-9, TIMP-2, VEGFA*) expressions were determined with qRT-PCR to investigate whether common pathways influence OC pathogenesis.

***c-FOS*** is a member of the FOS protein family (c-Fos, FosB, Fra1, and Fra2) that forms the AP-1 transcription factor and its overexpression is positively associated with growth in many tumors, including ovarian cancer (Bejjani et al., 2019). Our results regarding the significantly increased expression of *c-FOS* (*P*= 0.0089) is consistent with the current literature (Olbromski et al., 2022). **c-JUN** is a member of the JUN multigene protein family (c-Jun, JunB, JunD) (Bejjani et al., 2019) and an important member of AP1 transcription factor, which has roles in many molecular processes like cell survival proliferation and differentiation. In our study, gene expression analysis showed a statistically significant decrease in *c-JUN* (*P*=0.0041). Olbromski et al. (2022) supported our results in which a significant decrease in mRNA expression level of *c-JUN*.

***MMP-2*** and ***MMP-9*** genes belong to the gelatinases mainly acting on denaturing and cleaving type IV collagen and gelatine, therefore, significantly higher in terms of facilitating cancer cell migration or invasion processes (Kicman et al., 2022; Zeng et al. 2020). *MMP-9* has been suggested as a potential serum marker for ovarian cancer diagnosis and a potential therapeutic target for ovarian cancer therapy (Zhang and Chen, 2017). In our results, the level of *MMP-9* was dramatically increased (*P*=0.0029). Although it is not statistically significant, we found that *MMP-2* is upregulated in OC patients, which is consistant with Poon et al.'s study that found significanty increased *MMP2* level in OC (Poon et al., 2011).

The ***TIMP-2*** (Tissue Inhibitor of Metalloproteinases-2) gene has been implicated in the progression and metastasis of ovarian cancer. Studies have shown that decreased expression of *TIMP-2* in ovarian cancer cells is associated with increased tumor growth, invasion, and metastasis, as well as poor patient prognosis and survival rates. *TIMP-2* is also found as differentially expressed ($P$=0.0062) in our results Moreover, it is suggested that *TIMP-2* expression may serve as a robust biomarker for prognosis in ovarian cancer (Escalona et al., 2020).

***VEGF*** signaling pathways have a key function in tumor angiogenesis and lymphangiogenesis (Saharinen et al., 2011). VEGF-A is one form of the VEGF family and has an important role in vasculogenesis and angiogenesis by regulating the activity of endothelial cells (Jang et al, 2017; Li et al, 2020). *VEGFA* can be one of the most promising angiogenic factors for clinical use as a prognostic marker of ovarian cancer (Sopo et al, 2019). Significant upregulation of *VEGFA* expression shown in our results ($P$= 0.04) supports all these findings and is associated with HGSOC.

## CONCLUSION AND RECOMMENDATIONS

In conclusion, our study suggests key candidate genes in dysregulated pathways implicated in OC. The results of this study can further enhance the molecular etiology of OC as well as can be employed in future research for biomarker and drug development-related OC.

## CONFLICT OF INTEREST

The authors have no relevant financial or non-financial interests to disclose.

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*Full Text - 02*

# ANTIOXIDANT and ANTI-GROWTH PROPERTIES of SELECTED ANATOLIAN PLANT SPECIES: EXPLORING THE POTENTIAL of *Heracleum humile*, *Doronicum reticulatum*, *Centaurea drabifolia*, and *Senecio olympicus*

**Mehmet SARIMAHMUT**[1] , **Serap CELIKLER**[2]

## ABSTRACT

*This study investigated the anti-growth and antioxidant properties of four plant species native to the Anatolian region: Heracleum humile, Doronicum reticulatum, Centaurea drabifolia, and Senecio olympicus. Plant materials were collected, authenticated, and extracted using a Soxhlet apparatus. The growth inhibitory activities of the extracts were evaluated in human breast cancer cell lines MCF-7 and MDA-MB-231, and the nonmalignant immortalized human breast cell line MCF-10A, using the sulforhodamine B (SRB) assay. Antioxidant capacities were assessed via DPPH and CUPRAC assays. The results demonstrated that C. drabifolia extract exhibited potent cytotoxic effects, while D. reticulatum displayed selective toxicity and the most pronounced antioxidant activity among the evaluated species. These findings contribute to our understanding of the therapeutic potential of these indigenous plant species in addressing various public health issues, including cancer.*

**Keywords:** cytotoxicity, antioxidant, *Heracleum humile*, *Doronicum reticulatum*, *Centaurea drabifolia*, *Senecio olympicus*

# SEÇİLEN ANADOLU BİTKİ TÜRLERİNİN ANTİOKSİDAN VE ANTİ-BÜYÜME ÖZELLİKLERİ: *Heracleum humile*, *Doronicum reticulatum*, *Centaurea drabifolia* VE *Senecio olympicus*'un POTANSİYELİNİ KEŞFETME

## ÖZET

*Bu çalışma, Anadolu bölgesine özgü dört bitki türünün anti-büyüme ve antioksidan özelliklerini incelemiştir: Heracleum humile, Doronicum reticulatum, Centaurea drabifolia ve Senecio olympicus. Bitki materyalleri toplanmış, doğrulanmış ve bir Soxhlet aygıtı kullanılarak ekstrakte edilmiştir. Ekstrelerin büyüme inhibe edici aktiviteleri, insan meme kanseri hücre hatları MCF-7 ve MDA-MB-231 ve malignan olmayan ölümsüzleştirilmiş insan meme hücre hattı MCF-10A üzerinde sülfarodamin B (SRB) testi kullanılarak değerlendirildi. Antioksidan kapasiteler DPPH ve CUPRAC testleri ile değerlendirildi. Sonuçlar, C. drabifolia ekstresinin güçlü sitotoksik etkilere sahip olduğunu gösterirken, D. reticulatum seçici toksisite sergiledi ve değerlendirilen türler arasında en belirgin antioksidan aktiviteye sahipti. Bu bulgular, söz konusu yerli bitki türlerinin, kanser de dâhil olmak üzere çeşitli halk sağlığı sorunlarına yönelik terapötik potansiyellerini anlamamıza katkı sağlamaktadır.*

**Anahtar kelimeler:** sitotoksisite, antioksidan, *Heracleum humile*, *Doronicum reticulatum*, *Centaurea drabifolia*, *Senecio olympicus*

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## 1. INTRODUCTION

Plants have increasingly gained recognition as a valuable source of novel compounds for addressing various public health issues, including cancer. However, a mere fraction of higher plants has been investigated for their biological properties (Fabricant & Farnsworth, 2001). Renowned for its remarkable floristic diversity, the Anatolian region is home to over 12,000 natural vascular plant species and boasts an exceptional endemism rate exceeding 30% (Türe & Böcük, 2010).

Numerous efforts are underway to investigate the biological activities of plant species indigenous to Anatolia. In this context, we have selected four plant species, namely *Heracleum humile* Sm. [Syn.: *Heracleum massyciticum* Stapf & Wettst. ex Stapf, *Pastinaca humilis* Calest.], *Doronicum reticulatum* Boiss. [Syn.: *Doronicum bithynicum* J.R. Edmondson, *Doronicum bithynicum* subsp. *bithynicum*, *Doronicum bracteatum* J.R. Edmondson, *Doronicum thirkei* Sch.Bip. ex Boiss.], *Centaurea drabifolia* subsp. *drabifolia* Sm. [Syn.: *Chartolepis drabifolia*, *Cheirolepis drabifolia*, *Phaeopappus drabifolius*] and *Senecio olympicus* Boiss.

*H. humile* is native to Turkey, Syria, and Lebanon (POWO, 2023). The *Heracleum* genus encompasses 90-120 species, with 24 of them found in Turkey (Logacheva et al., 2008). Traditionally, these species have been utilized for medicinal and culinary purposes (Bahadori et al., 2016). Ethnobotanical applications of *H. humile* include treatments for snakebites, fever, neurological disorders, and abdominal cramps induced by intestinal worms (Arnold et al., 2015).

*D. reticulatum* is indigenous to Turkey, Iraq, and Iran (POWO, 2023). The *Doronicum* genus has been reevaluated to include 30 taxa, 10 of which are distributed in Anatolia with a 30% endemism rate (Güven et al., 2020). Previous research investigated the anti-inflammatory and antioxidant potential of *Doronicum austriacum* root extract and several fractions of *Doronicum pardalianches* methanol extract for their efficacy against Alzheimer's disease (Marzocco et al., 2017; Manayi et al., 2021). Other *Doronicum* species have been traditionally employed in antimicrobial treatments for animal injuries (Kargıoğlu et al., 2010).

*C. drabifolia*'s distribution encompasses Turkey, Iran, Syria, and Lebanon, according to the POWO, Plants of the World Online database (POWO, 2023). *Centaurea* genus comprises 181 species within the Turkish flora, 112 of them being endemic, making it one of the most diverse genera (Candan et al., 2016). *Centaurea* has a long-standing history of traditional applications, including wound healing, common cold treatment, diabetes management, hypertension control, abdominal pain relief, ulcer treatment, and malaria therapy (Khammar & Djeddi, 2012). *C. drabifolia* subsp. *cappadocica* chloroform extract has been previously documented to possess significant antimicrobial effects (Uğur et al., 2009).

The diverse genus of *Senecio* is comprises over 1500 distinct species, with 39 of them endemic to the Anatolian region (Doral & Wink, 2002; Uğur et al., 2006). *S. olympicus* is a species exclusive to Turkey, specifically confined to Mount Uludag (Kirmizi et al., 2011). Throughout history, various *Senecio* species have been employed in an array of traditional practices, encompassing food sources, wound healing agents, and integral components in anti-emetic, anti-inflammatory, and vasodilator formulations (Kargıoğlu et al., 2010; Khammar & Djeddi, 2012; Albayrak et al., 2014). Despite their extensive historical utilization, the existing literature on the plant's biological effects remains sparse, with a solitary report on the antibacterial and antioxidant properties of *S. olympicus* (Albayrak et al., 2014).

The objective of this study is to assess the anti-growth and antioxidant properties of a select group of indigenous plant species native to Anatolia. Our results demonstrate that *C. drabifolia* possesses a potent

cytotoxic effect, while *D. reticulatum* exhibits selective toxicity and the most pronounced antioxidant activity among the evaluated species.

## 2. AIM

This research aimed to investigate the anti-growth and antioxidant properties of four plant species native to the Anatolian region: *Heracleum humile*, *Doronicum reticulatum*, *Centaurea drabifolia*, and *Senecio olympicus*. The study involved the collection, authentication, and extraction of bioactive compounds from these plants. Their anti-growth potential was evaluated on human breast cancer cell lines, while antioxidant capacities were assessed using the DPPH and CUPRAC assays. The findings aimed to offer insights into the therapeutic value of these indigenous plants, highlighting the importance of conserving Anatolia's rich floristic diversity for potential medical applications.

## 3. MATERIALS AND METHODS

### 3.1. Plant material and extraction

This study collected four plant species, namely *H. humile*, *D. reticulatum*, *C. drabifolia*, and *S. olympicus*, from Bursa and its surrounding cities. The collected plants were authenticated, and voucher specimens were deposited in the herbarium at Bursa Uludag University to ensure their accuracy and consistency. Prior to extraction, the plants were carefully inspected for any signs of contamination or foreign materials, which were removed by hand. The unwanted plant parts were separated and discarded. The remaining plant samples were dried in the shade at ambient temperature to prevent degradation of their chemical constituents.

The dried plant materials were then ground into powder, and 30 g of the powdered material was extracted via a Soxhlet apparatus using 150 ml of methanol. The extracts were then concentrated in a rotary vacuum evaporator to obtain a more concentrated solution. The resulting extracts were subsequently lyophilized using a Christ lyophilizer (Osterode am Harz, Germany) and stored at -80 °C for future use. Prior to their use in experiments, the lyophilized extracts were reconstituted in dimethyl sulfoxide to obtain a 100 mg/ml stock solution, which was further diluted with cell culture medium.

### 3.2 Cell culture

Monolayers of MCF-7, MDA-MB-231, and MCF-10A cells were cultured at 37 °C under a 5% $CO_2$ environment. MCF-7 and MDA-MB-231 human breast cancer cells were maintained in RPMI 1640 medium supplemented with 5% fetal bovine serum (FBS) and 1% penicillin-G (100 U/ml)-streptomycin (100 µg/ml). MCF-10A, a nonmalignant immortalized human breast cell line, was maintained in DMEM:Ham's F12 (1:1) medium supplemented with 5% FBS, 1% penicillin-G (100 U/ml)-streptomycin (100 µg/ml), epidermal growth factor (20 ng/ml; Gibco, PHG0315), cholera toxin B subunit (10 ng/ml; Sigma, C9903), and insulin (0.12 IU/ml; Sigma, I3536).

### 3.3. Determination of the anti-growth activity

The anti-growth properties of the extracts were evaluated using sulforhodamine B (SRB) assay as described previously (Sarimahmut & Celikler, 2023). MCF-7, MDA-MB-231, and MCF-10A cells in exponential growth were seeded at $5 \times 10^3$ cells/well. The cells were treated 48 h with serial 2-fold dilutions of the extract solutions in 96-well microplates, resulting in a final concentration range of 3.13-200 µg/ml. At the end of the treatment, ice-cold trichloroacetic acid solution was pipetted to yield a 10%

(w/v) final concentration. The cells were washed with deionized water and stained with SRB (0.4%, w/v in 1% v/v acetic acid solution). Then, the bound dye was dissolved using 10 mM Tris base. Optical density was measured at 564 nm wavelength using an ELISA plate reader (FLASH Scan S12, Analytik Jena, Germany). The reported data are the average of three independent experiments performed in triplicate. $IC_{50}$ values of the extracts were calculated using Calcusyn version 2.1 (Biosoft) software.

### 3.4. Determination of the antioxidant capacity

The antioxidant capacity of the extracts was evaluated using two methods: the DPPH radical scavenging and cupric reducing antioxidant capacity (CUPRAC) assays, as described by Molyneux (2004) and Apak et al. (2005). The DPPH assay measures the disappearance of the purple color of the DPPH radical, while the CUPRAC assay measures the reduction of copper (II) neocuproine reagent. Both assays were conducted in 96-well microplates, with a range of extract concentrations from 0.49-500 µg/ml prepared in a 1:1 v/v mixture of EtOH:$H_2O$. A 1 mg/ml stock solution was prepared from each extract. To prevent loss of antioxidant activity, all solutions were freshly prepared. The microplates were incubated on an orbital plate shaker at 600 rpm at 37 °C, and the absorbance was measured at 517 nm for the DPPH assay and 450 nm for the CUPRAC method. The results were reported as the $IC_{50}$ value for DPPH radical scavenging and Trolox equivalents (mg TE/g extract) for CUPRAC antioxidant activity.

## 4. RESULTS AND DISCUSSION

### 4.1. Anti-growth activity of the four plants

Exploring the anticancer properties of plant extracts with limited geographic distribution contributes to the conservation and untapped potential of these species. In this study, we selected *H. humile*, *D. reticulatum*, *C. drabifolia*, and *S. olympicus* species and assessed their growth inhibitory activities using the SRB assay, with the outcomes illustrated in Figure 1 and Table 1. Notably, *C. drabifolia* extract exhibited potent cytotoxic activity, whereas the other plant extracts displayed weaker cytotoxicity. *C. drabifolia* extract demonstrated dose-dependent growth inhibitory activity across various cell lines. In a related study, the ethyl acetate fraction of a dichloromethane extract from *Centaurea fenzlii*, a species belonging to the same genus, induced growth inhibitory activity against MCF-7 cells with apoptotic cell death and a comparably lower $IC_{50}$ value of 45.77 µg/ml (Yirtici et al., 2017). Hispidulin, a flavone, was identified as the major constituent of the ethyl acetate fraction. In another investigation, a nonpolar organic extract of *Centaurea drabifolia* subsp. *detonsa* from aerial parts yielded seven sesquiterpene lactones, which were tested against sensitive and resistant acute lymphoblastic leukemia cell lines, resulting in $IC_{50}$ values ranging from 0.47 to 25.36 µM in the drug-sensitive CCRF-CEM cell line (Formisano et al., 2017).

**Table 1.** Anti-growth activity of plant extracts determined by SRB assay following 48 h treatment.

| **Plant** | **$IC_{50}$ (µg/ml)** | | |
|---|---|---|---|
| | **MCF-7** | **MDA-MB-231** | **MCF-10A** |
| *C. drabifolia* | 80.26 ± 10.92 | 47.58 ± 9.34 | 52.31 ± 11.51 |
| *D. reticulatum* | 390.08 ± 60.85 | 289.26 ± 32.40 | N/A* |
| *H. humile* | N/A | N/A | N/A |
| *S. olympicus* | 716.39 ± 37.34 | 104.63 ± 18.42 | 554.16 ± 53.39 |

*: Not calculated due to low cytotoxicity.

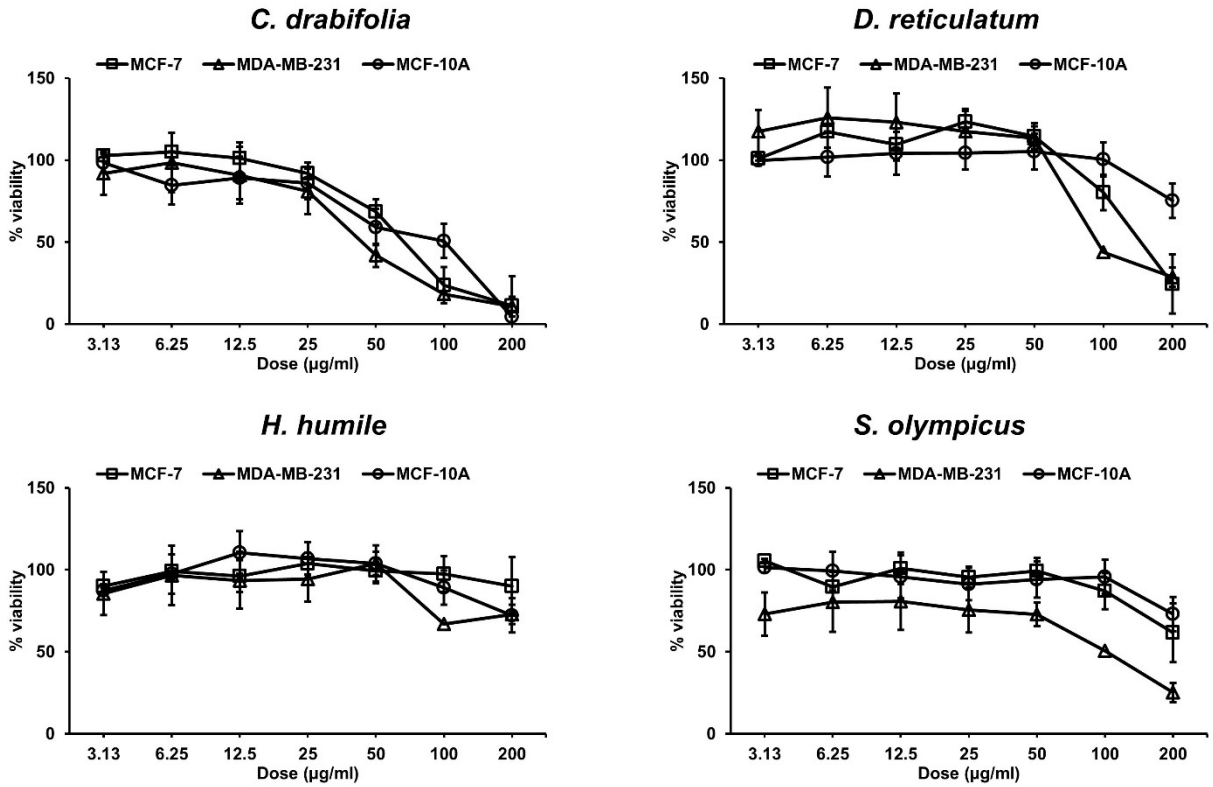


**Figure 1** Dose response curve of plant extracts determined by SRB assay following 48 h treatment.

Interestingly, *D. reticulatum* displayed selective toxicity towards cancer cells with 3-fold selectivity at the highly growth-inhibitory concentration of 200 µg/ml. No data regarding the selective cytotoxicity or anticancer effects of *D. reticulatum* currently exists. Species from the *Doronicum* genus are known to contain pyrrolizidine alkaloids, flavonoids, essential oils as hydrocarbon sesquiterpenes, and primarily thymol derivatives with ester groups, which may contribute to both cytotoxic effects and selective cytotoxicity (Badalamenti et al., 2021).

*S. olympicus* showed weak cytotoxicity ($IC_{50}$ > 100 µg/ml) across all cell lines, although relatively higher cytotoxic activity was observed against MDA-MB-231 cells. This study represents the first investigation into the cytotoxic activity of *S. olympicus*. Research on other *Senecio* species has uncovered that *Senecio stabianus* exerted cytotoxic activity against MCF-7 cells, with $IC_{50}$ values of 91.1, 87.2, and >100 µg/ml from methanol, *n*-hexane, and ethyl acetate extracts, respectively (Tundis et al., 2009). Additional species within the *Senecio* genus are known to contain bioactive compounds, such as the cytotoxic quinone molecule jacaranone, its derivatives, and pyrrolizidine alkaloids, which have the potential to induce hepatotoxicity (Loizzo et al., 2007; Wang et al., 2010; Acito et al., 2022).

In contrast, *H. humile* extract did not exhibit significant cytotoxic activity against any of the breast cell lines ($IC_{50}$ > 200 µg/ml). A recent study analyzed three *H. humile* extracts obtained via an ultrasound-assisted extraction method and tested them against MCF-7 and MDA-MB-231 cell lines using the MTT cell viability assay (Ocal et al., 2022). Consistent with our findings, the methanol extract of the plant did not display substantial growth inhibitory activity at a concentration of 125 µg/ml. However, the ethyl acetate extract exhibited more potent cytotoxicity, with $IC_{50}$ values of 97.94 and 103.9 µg/ml against MCF-7 and MDA-MB-231 cells, respectively. Numerous *Heracleum* species are utilized in culinary

practices worldwide, and the majority of previous studies report weak to moderate cytotoxicity from various *Heracleum* species (Bahadori et al., 2016). The essential oil of common hogweed (*Heracleum sphondylium* L. subsp. *ternatum*) displayed weak cytotoxic activity against MDA-MB-231 and the glioblastoma multiforme cell line T98G ($IC_{50}$ > 200 µg/ml), while it demonstrated moderate activity against A375 (melanoma) and HCT116 (colon carcinoma) cells, with $IC_{50}$ values of 48.69 and 95.83 µg/ml, respectively (Maggi et al., 2014). Furthermore, Amani et al. (2019) discovered that treatment with the ethanolic extract of *Heracleum persicum* resulted in a 60% reduction in human lymphocyte viability at 250 µg/ml and induced DNA damage at the same concentration.

### 4.2. Antioxidant potential of the four plants

The antioxidant capacities of the plant extracts were quantified using DPPH and CUPRAC assays (Table 2). The most potent antioxidant activity was observed in *D. reticulatum*, followed sequentially by *S. olympicus*, *C. drabifolia* and *H. humile* extracts. The outcomes of both DPPH and CUPRAC assays exhibited consistency in evaluating antioxidant activity.

**Table 2.** Antioxidant capacity of the plant extracts determined by DPPH and CUPRAC methods.

| Plant extracts | DPPH radical scavenging activity ($IC_{50}$) (µg/ml) | CUPRAC (mg TE/g extract) |
|---|---|---|
| *C. drabifolia* | 210.60 ± 29.51 | 83.04 ± 5.10 |
| *D. reticulatum* | 97.39 ± 1.48 | 148.71 ± 7.37 |
| *H. humile* | 257.84 ± 30.66 | 78.02 ± 1.32 |
| *S. olympicus* | 171.35 ± 0.85 | 137.74 ± 14.79 |
| Ascorbic acid | 5.01 ± 0.06 | 1817.83 ± 39.86 |
| Gallic acid | 1.29 ± 0.04 | 3924.71 ± 185.29 |

CUPRAC: Cupric reducing antioxidant capacity; DPPH: 2,2-Diphenyl-1-picrylhydrazyl; TE: Trolox equivalent.

Concurring reports highlight the antioxidant capacity of various *Doronicum* species. CUPRAC reducing power of an ethanol/water (7:3, v/v) extract of *Doronicum orientale* showed 151.15 mg TE/g extract; while the $IC_{50}$ value for DPPH scavenging activity of *Doronicum hookeri* methanol extract was reported as 217 µg/ml (Gupta et al., 2011; Zengin et al., 2022). 3- and 5-caffeoylquinic acid were detected as the predominant compounds in the ethanol/water extract of *Doronicum orientale* (Zengin et al., 2022).

The antioxidant capacity of *C. drabifolia* subsp. *drabifolia* methanolic extract was previously measured as 102.35 mg TE/g extract using the CUPRAC assay, which aligns with our findings (Zengin et al., 2018). Phenolic compounds, such as protocatechuic, caffeic, monocaffeoylquinic acids, monoferuloylquinic acid, and flavonoids, have been identified as likely contributors to the antioxidant activity (Zengin et al., 2022).

The *H. humile* methanol extract demonstrated an antioxidant capacity of 47.15 mg TE/g extract according to the CUPRAC assay (Ocal et al., 2022). Additionally, the DPPH radical scavenging activity of *S. olympicus* methanol extract was reported to have an $IC_{50}$ value of 46.81 µg/ml (Albayrak et al., 2014). The observed discrepancies in the antioxidant capacities of *H. humile* and *S. olympicus*, when compared to our study's findings, may be attributed to variations in extraction methods as well as seasonal and ontogenetic factors.

## 5. CONCLUSION

Our study examined the anti-proliferative and antioxidant properties of four plant species native to northwestern Anatolia. Our findings reveal that *C. drabifolia* extract displayed potent cytotoxic activity against breast cancer cells, while *D. reticulatum* extract demonstrated selective cytotoxicity towards cancer cells at relatively elevated concentrations. Moreover, *D. reticulatum* possessed the highest antioxidant capacity among the investigated plants. This research contributes to the understanding of the biological importance of Anatolia's floristic diversity and emphasizes the necessity of preserving these species within the region.

## ACKNOWLEDGEMENTS

This work was funded by Bursa Municipality within the scope of bilateral cooperation with Bursa Uludag University. The authors would like to thank Prof. Engin Ulukaya (MD, PhD) for his valuable support and Prof. Gurcan Guleryuz (PhD) for his support in collection and identification of the plants.

## CONFLICT OF INTEREST

The authors declare no competing interests relevant to the content of this article.

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*Full Text - 03*

# ANTI-GROWTH, ANTIOXIDANT, AND HEPATOPROTECTIVE PROPERTIES OF *SPIRULINA PLATENSIS* EXTRACT

**Sedef ZİYANOK-DEMİRTAŞ[1]**

## ABSTRACT

*Spirulina platensis, a filamentous cyanobacterium often referred to as blue-green algae, is recognized for its biological activities, including antioxidant, immunomodulatory, anti-inflammatory properties. This study was conducted to investigate the growth inhibitory effects of the ethanolic extract of Spirulina platensis on PANC-1 and MIA PaCa-2 human pancreatic cancer cell lines. Additionally, the in vivo hepatoprotective, antioxidant properties of S. platensis were explored. The ethanolic extract of S. platensis was lyophilized and then dissolved in DMSO. Subsequently, PANC-1, MIA PaCa-2 cell lines were treated with concentrations ranging from 0.1 to 1000 µg/ml. Cell viability was assessed using the sulforhodamine B viability assay. For in vivo evaluations of hepatoprotective and antioxidant properties, Spirulina was administered to rats via gavage at a dose of 50 mg/kg/day for four weeks. The activity levels of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were measured in the heart and liver tissues of the rats. The control group that received Spirulina demonstrated a significant elevation in the activity of SOD, GSH-Px enzymes in heart and liver tissues. There was a significant reduction in ALT and AST enzyme levels. The extract notably hindered the growth of both examined cell lines. Future research can focus on studying the effects of Spirulina platensis extracts in conjunction with various chemotherapeutic agents or its impact on different cancer cell lines to more comprehensively understand its anti-growth attributes.*

**Keywords:** *Spirulina platensis*, Pancreatic cancer, PANC-1, MIA PaCa-2, SOD, GSH-Px.

# *SPİRULİNA PLATENSİS* EKSTRESİNİN ANTİ-BÜYÜME, ANTİOKSİDAN VE KARACİĞER KORUYUCU ÖZELLİKLERİ

## ÖZET

*Spirulina platensis, sıklıkla mavi-yeşil alg olarak adlandırılan filamentöz bir siyanobakteri olup, antioksidan, bağışıklık düzenleyici, anti-inflamatuar özellikler dahil biyolojik etkinlikleriyle tanınır. Bu çalışma, Spirulina platensis'in etanol ekstresinin PANC-1 ve MIA PaCa-2 insan pankreas kanseri hücre hatları üzerindeki büyümeyi engelleme etkilerini araştırmak amacıyla gerçekleştirildi. Ayrıca, S. platensis' in in vivo karaciğer koruyucu ve antioksidan özellikleri incelendi. S. platensis'in etanol ekstresi liyofilize edildi ve ardından DMSO'da çözüldü. Daha sonra PANC-1 ve MIA PaCa-2 hücre hatları, 0.1 ila 1000 µg/ml arasında değişen konsantrasyonlarla muamele edildi. Hücre canlılığı, sülforhodamin B canlılık testi kullanılarak değerlendirildi. Karaciğer koruyucu ve antioksidan özelliklerin in vivo değerlendirmeleri için Spirulina, sıçanlara günde 50 mg/kg dozunda dört hafta boyunca gavaj yoluyla uygulandı. Sıçanların kalp ve karaciğer dokularındaki süperoksit dismutaz (SOD), glutatyon peroksidaz (GSH-Px), alanin aminotransferaz (ALT) ve aspartat aminotransferaz (AST) aktivite seviyeleri ölçüldü. Spirulina alan kontrol grubunda kalp ve karaciğer dokularındaki SOD ve GSH-Px enzim aktivitesinde belirgin bir artış görüldü. ALT ve AST enzim seviyelerinde önemli bir azalma oldu. Ekstrakt, her iki incelenen hücre hattının büyümesini önemli ölçüde engelledi. Gelecekteki araştırmaların, Spirulina platensis ekstrelerinin çeşitli kemoterapötik ajanlarla birlikte kullanımının veya farklı kanser hücre hatları üzerindeki etkilerinin daha kapsamlı bir şekilde anlaşılması için odaklanabileceğini düşündürmektedir.*

**Anahtar Kelimeler:** *Spirulina platensis*, pankreas kanseri, PANC-1, MIA PaCa-2, SOD, GSH-Px

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## INTRODUCTION

Cancer represents a significant category of diseases and remains a leading cause of death globally. Natural products play a crucial role as valuable reservoirs of potential anticancer compounds. While several plant-derived anticancer drugs have achieved clinical success, the emergence of drug resistance and the associated side effects have underscored the need for continued and extensive exploration of novel anticancer agents from natural sources for cancer treatment (Demain et al., 2011, Mondal et al., 2012, World Health Organization, 2010).

Antioxidant enzyme levels are one of the key indicators used to assess the antioxidant effects of certain dietary components within the body. From a biochemical perspective, the activation of antioxidant enzymes in the body and the equilibrium between oxidants and antioxidants are closely associated with the intake of antioxidative compounds through the diet. Among the most recognized antioxidant enzymes used to assess oxidative stress in the body are glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) (Yegin et al. 2013).

The liver is the body's largest and most essential metabolic organ, consisting of various functional and anatomical structures. When it comes to liver enzymes, the ones that come to mind are ALT (alanine aminotransferase) and AST (aspartate aminotransferase).

Aminotransferases are involved in the interconversion of amino and keto acids in carbohydrate and nitrogen metabolism. ALT is a cytosolic enzyme and is liver-specific.

AST is an enzyme found in the liver, heart, pancreas, and muscles, among other parts of the body. Although this enzyme is present throughout the body, it is most commonly associated with liver health. An elevation in serum aminotransferase levels can occur not only as a result of hepatocellular necrosis, where the intracellular enzyme is released into the bloodstream but also due to increased membrane permeability in cases of non-necrotic cellular injury (Ersoy, 2012).

In recent years, studies have shown that an increase in free oxygen radicals and lipid peroxidation plays a role in the pathogenesis of many diseases. The relationship between oxidative stress and various diseases, including diabetes, cancer, cardiovascular and neurological disorders, asthma, and aging, has been demonstrated. Oxidative stress can lead to changes in the levels of antioxidant enzymes. Interest in plant-based products with antioxidant properties is growing day by day, both in metabolic diseases and in healthy individuals.

Microalgae are important organisms due to their ability to produce various chemical and biological compounds. Vitamins, pigments, proteins, minerals, lipids, and polysaccharides are the main products obtained from them. Compared to other living sources, algae are particularly rich in compounds such as polyunsaturated fatty acids (PUFA), gamma-linolenic acid (GLA), allophycocyanin, zeaxanthin, and mixoxanthophyll among their pigments. Microalgae are being studied for their rich content of proteins, fatty acids, minerals, vitamins pigments, and many other valuable cellular metabolites. In this context, the production and harvesting of the blue-green algae species *Spirulina platensis* have become more popular, mainly because of its ease of production and harvest (Alavi et al., 2017, Andrade et al., 2018, Chen et al., 2019, Chopopani et al., 2016).

*Spirulina platensis* is a planktonic organism characterized by high carbonate and bicarbonate levels and a high pH in aquatic environments. It is known for its high protein content, gamma-linolenic acid, $B_{12}$, E, and C vitamins, as well as elements such as zinc, iron, calcium, manganese, and selenium. Its low-fat content also contributes to its commercial significance. Research studies have shown that Spirulina exhibits antiviral, anticancer, antibiotic, antioxidant, immune-boosting, cardiovascular protective,

hypocholesterolemic, and anti-allergic effects. *S. platensis* has been suggested as a dietary supplement that may lower blood glucose levels and alleviate oxidative stress due to its antioxidative properties (Tokusoglu et al., 2003).

This study was conducted to investigate the growth inhibitory effects of the ethanolic extract of *Spirulina platensis* on PANC-1 and MIA PaCa-2 human pancreatic cancer cell lines. Additionally, the in vivo hepatoprotective, antioxidant properties of S. platensis were explored.

**AIM**

The aim of this study was to investigate the growth inhibitory effects of the ethanolic extract of Spirulina platensis on PANC-1 and MIA PaCa-2 human pancreatic cancer cell lines. Additionally, the in vivo hepatoprotective and antioxidant properties of S. platensis were explored.

**METHODS**

**Preparation of extracts**

*Spirulina* was provided in powdered from Mugla, Turkey. The ethanolic extract of *S. platensis* was lyophilized and then dissolved in dimethyl sulfoxide (DMSO).

**Cell culture**

PANC-1 and MIA PaCa-2 human pancreas carcinoma cells were kindly provided by (Bursa Uludag University, Turkey). PANC-1 and MIA PaCa-2 cells were cultured in RPMI 1640 medium supplemented with penicillin G (100 U/mL), streptomycin (100 mg/mL), L-glutamine, and 10% fetal bovine serum at 37°C in a humidified atmosphere containing 5% CO2.

**Cytotoxicity assay**

PANC-1 and MIA PaCa-2 cell lines were treated with concentrations ranging from 0.1 to 1000 µg/ml. Cell viability was assessed using the sulforhodamine B viability assay. Then, PANC-1 and MIA PaCa-2 cells were seeded at a density of 5x103 cells per well of 96-well plates. Subsequently, cells were incubated with various concentrations of the extracts for 48 h. The assay was terminated by the addition of ice-cold 50% (w/v) trichloroacetic acid. SRB 0.4% (w/v) in 1% (v/v) acetic acid staining was then performed. The bound dye was extracted using 10 mM unbuffered Tris and optical density was measured at 564 nm with an ELISA plate reader. Viability of treated cells was calculated in reference to the untreated control cells by using the following formula:

Cell viability (%) = [100×(Sample Abs)/(Control Abs)].

**The Test Animals**

Eighteen adult male Wistar rats were used (Bursa Uludag University, Bursa, Turkey). The weights of the rats were approximately 350-400 g. The rats were allowed free access to standard laboratory chow and three rats were housed in per cage. Animals were randomized and divided into two groups as the healthy rats (control group) “C”, the healthy rats fed with Spirulina “C+SPE”. *Spirulina platensis* extract was administered to rats via gavage at a dose of 50 mg/kg/day for four weeks.

**Sampling and Measuring of Blood Parameters**

Blood sampling was made under anaesthesia from the chest area. Blood samples were centrifuged (1500 rpm, Nuve NF 200, Turkey) to separate the serum and plasma for 10 min. Separated samples were stored

-20 °C. The organ tissues including heart and liver were taken just after blood collection, washed with saline solution and stored at -20 °C to use for the analyses.

The parameters including glutathione peroxidases (GSH-Px) and superoxide dismutase (SOD) were determined by ELISA kit. Blood samples were transferred into EDTA-containing tubes at first, followed by centrifugation, and then the plasma phase was separated and analysed. ALT (alanine aminotransferase) and AST (aspartate aminotransferase) were measured in the autoanalyzer (TCHO-P 238608 kit for TG; TG-P 223104 kit for TC - Fuji Dri-Chem, Japan).

**Statistical Analysis**

The data obtained from biochemical tests will have their mean values and standard deviations determined, and they will be statistically evaluated using one-way analysis of variance (ANOVA) and multivariate analysis of variance (MANOVA). Differences between groups will be determined at a significance level of $p<0.05$ using the Tukey Honestly Significant Difference (HSD) test. The statistical analyses will be conducted using the SPSS 23.0 software package.

**FINDINGS AND DISCUSSIONS**

It was found that the extracts inhibited Figure 1. The cytotoxic effects with different concentrations of *S. platensis* extract on PANC-1 and MIA PaCa-2 cell lines.

The cytotoxic effects with different concentrations of *S. platensis* extract on PANC-1and MIA PaCa-2 cell lines growth of cells in a dose-dependent manner and prominently reduced the cell viability at the 1000 ug/mL. The cytotoxic effects after the treatment with the extract against human pancreas adenocarcinoma cell lines were shown in Figure 1. Due to its antioxidant properties, *Spirulina* can neutralize free radicals, thereby reducing cellular damage. This could potentially help in reducing the free radical damage that contributes to the development of cancer. Furthermore, our findings in the study have indicated that *Spirulina* may have the potential to inhibit the growth of cancer cells and promote their apoptosis. The present investigation represents a preliminary screen for the cytotoxic effect of *S.platensis* in human pancreas cancer cell lines. The synergistic and/or antagonistic effects with chemotherapeutic agents can be supported by different studies.

GPx and SOD levels were significantly increased compared with the control rats. The levels of GSH-Px and SOD in the tissues (liver and heart) are expressed in Table 1. Test animals were fed with *Spirulina* diet; GSH-Px and SOD were found to increase 28 and 80% in the liver tissue and GSH-Px and SOD were found to increase 19 and 33% in the in the heart tissue in the rats. In addition AST and ALT levels were significantly lower in the C+SPE group compared with the control group (Table 1). AST and ALT enzyme levels were found to decrease 15% in the C+SPE group. When PUFA's are metabolized in the body, are converted to the sub-components having antioxidant capacity in the body. Thus, they play role to activate some antioxidant enzymes such as glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD). It is obvious that the antioxidant components available in *Spirulina,* caused to a significant antioxidant effect in the rat bodies ($p \leq 0.05$). It was probably occurred due to cumulative effect of the phenolics and polyunsaturated fatty acids available in *Spirulina*. As mentioned earlier, *Spirulina* is a natural food supplement known for its significant antioxidant capacity. Food components with antioxidant potential typically encompass bioactive compounds characterized by numerous double bonds, including antioxidant vitamins and minerals, flavonoids, phenolic compounds, as well as ω-3 and ω-6 polyunsaturated fatty acids. Our findings also substantiate these pieces of information. In the given control group receiving Spirulina, a decrease in AST and ALT levels was observed. Considering that the levels of AST and ALT enzymes tend to increase as a result of conditions such as chronic illness, medication usage, intense exercise, or even in the early stages of a chronic liver disease that may not

produce significant symptoms, the observed reduction in these enzymes in healthy groups receiving *S. platensis* extract is significant. *Spirulina* may contain components that can support the detoxification processes of the liver. However, further clinical research is needed to better understand the full effects of *Spirulina* on liver health.

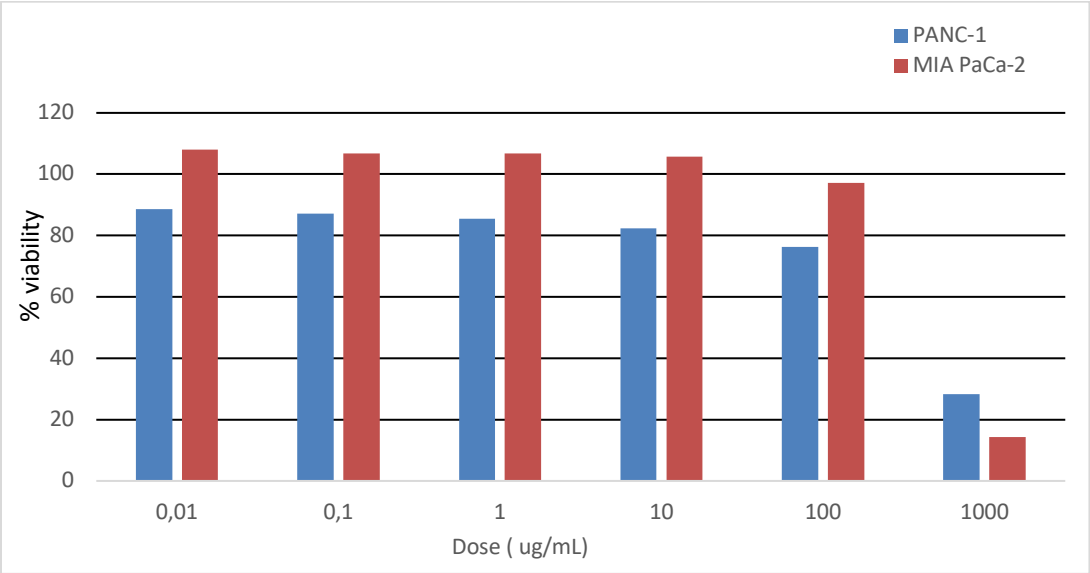


**Figure 1. The cytotoxic effects with different concentrations of the S. platensis extract on PANC-1 and MIA PaCa-2 cell lines.**

**Table 1. GSH-Px and SOD levels in heart and liver tissue and AST and ALT levels in blood.**

| | Liver GSH-Px (ng/mL) | Heart GSH-Px (ng/mL) | Liver SOD (ng/mL) | Heart SOD (ng/mL) | AST (IU/L) | ALT (IU/L) |
|---|---|---|---|---|---|---|
| Control | 14.3 ± 2 | 15.4 ± 1 | 1.0 ± 0.1 | 1.5 ± 0.2 | 131.3 ± 4.8 | 70.7 ± 6.8 |
| Control+SPE | 18.5 ± 1[a*] | 18.2 ± 1 [a*] | 1.8 ± 0.1 [a*] | 2.0 ± 0.2 [a*] | 111.8 ± 2[a*] | 60.2 ± 5.9 [a*] |

a; compared to the control group. $p<0.05$*

## CONCLUSION

In conclusion, our study has yielded findings indicating that *Spirulina* suppresses the growth of pancreatic cancer cell lines while increasing antioxidant enzyme levels in liver and heart tissues. In light of this information, it emphasizes the belief that this plant-based form, rich in antioxidants, may play a significant role in assisting treatment not only in healthy individuals but also in metabolic and chronic diseases.

## ACKNOWLEDGEMENT

I extend my thanks to the Scientific Research Projects Unit of Bursa Uludağ University.

## CONFLICT OF INTEREST

There is no conflict of interest.

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*Full Text - 04*

# EVALUATION OF THE ANTIPROLIFERATIVE EFFECT OF SAFRANAL IN C-4 I CERVICAL CANCER CELL LINE

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**ABSTRACT**

*Safranal is a monoterpene aldehyde responsible for the aroma of Crocus sativus. Many studies have shown the antioxidant activity of safranal besides some pharmacological properties, including its anti-inflammatory effect. This study aimed to determine the cytotoxic effects of safranal on C-4 I, cervical cancer cell line. To determine the cytotoxic effect of safranal on the C-4 I cell line, cells were incubated for certain times (2-72 hours) and concentrations (25-800µM). After incubation, the viability of cells and the anti-proliferation effect of safranal were determined respectively by MTT and LDH assays. In addition, Morphological changes occurring during incubation in cells were observed under inverted and light microscopes using Giemsa staining. According to the results, compared to Control group, the % viability of treated cells was decreased depending on concentration and the incubation time, and safranal significantly inhibited the growth of C-4 I cells ($p<0.05$). Some morphological changes such as nuclear condensation, and apoptotic and pyknotic cells were examined under light microscopy with Giemsa staining. In conclusion, based on these results safranal has an antiproliferative effect against cervical cancer C-4 I cell lines.*

**Key Words**: C-4 I, Safranal, Cervical cancer, Cytotoxicity, Antiproliferative

**ÖZET**

*Safranal, Crocus sativus'un aromasından sorumlu monoterpen bir aldehittir. Safranalın antiinflamatuar etkisi dahil olmak üzere farmakolojik özelliklerinin yanı sıra antioksidan aktivitesi birçok çalışmada gösterilmiştir. Çalışmamızda, safranalın serviks karsınoma kökenli C-4 I hücre hattı üzerindeki sitotoksik etkilerini tespit etmek amaçlanmıştır. Safranalın C-4 I hücre hattı üzerindeki sitotoksik etkisini belirlemek için hücreler belirli sürelerde (2-72 saat) ve konsantrasyonlarda (25-800µM) inkübe edildi. İnkübasyon süresi bitiminde hücrelerin viabilitesi ve safranal antiproliferatif etkisi sırasıyla MTT ve LDH testleri ile belirlendi. Bununla birlikte, inkübasyon süresince meydana gelen morfolojik değişiklikler Faz kontrast ve ışık mikroskopları aracılığıyla Giemsa boyama kullanılarak gözlemlendi. Sonuçlara göre, safranal ile tedavi edilen hücreler kontrol grubu ile karşılaştırıldığında hücrelerin canlılık yüzdesi doza ve inkübasyon süresine bağlı olarak azaldı ve safranal C-4 I hücrelerinin çoğalmasını anlamlı bir şekilde inhibe etti ($p<0.05$). Nükleer yoğunlaşma, apoptotik ve piknotik hücreler gibi bazı morfolojik değişiklikler ışık mikroskobu altında Giemsa boyama ile incelendi. Elde edilen sonuçlara dayanarak safranalın serviks kanseri kökenli C-4 I hücre hatlarına karşı antiproliferatif bir etkiye sahip olduğu söylenebilmektedir.*

**Anahtar Kelimeler**: C-4 I, Safranal, Serviks karsinoma, Sitotoksisite, Antiproliferatif

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## INTRODUCTION

Cancer is a group of diseases characterized by uncontrolled cell division and invasiveness. Cancer is one of the major public health problems worldwide and the second leading cause of death (Gavas et al., 2021). Each year, cancer incidence and death rates are increasing by approximately 1% and this increase is expected in 2030 to 26.4 million new cancer cases and 1.7 million cancer deaths (Siegel et al., 2020). Cervical cancer is the second most common malignancy and the second leading cause of cancer death in women worldwide. Cervical cancer is a virus-borne disease caused by the integration of high-risk human papillomavirus (HPV) into the host genome (Martín, 2007). The treatment methods used in patients with cervical cancer are regional treatments such as surgery, radiotherapy, and chemotherapy (Liontos et al., 2019). Surgery, chemotherapy, radiotherapy, and hormone therapy methods which are used commonly in clinics cause side effects during and after treatment.

Safranal, abundant in *Crocus sativus* essential oil, is a monoterpene aldehyde responsible for the aroma and odor of the plant. Safranal has antioxidant activity (Rezaee and Hosseinzadeh, 2013; Cerdá-Bernad et al., 2022) as well as pharmacological effects such as anti-inflammatory, antidepressant, anxiolytic, anti-asthmatic, antihypertensive, anticonvulsant, antitussive and antigenotoxic (Mollazadeh et al., 2015; Nanda and Madan, 2021; Esmaeilzadeh et al., 2023). Many studies have shown that safranal has a therapeutic effect against diabetes (Maeda et al., 2014), cardiovascular diseases (Toledo-ibelles and Mas-oliva, 2018), malignant diseases, and Alzheimer's. (Samarghandiana et al., 2016). In addition, some studies have shown its antitumor activity in some cell lines (Samarghandian and Shabestari; Jabini et al., 2017; Al-Hrout et al., 2018; Zarrineh et al., 2022; Burak and Güven, 2023).

The main goal of most cancer treatment research is finding an effective therapeutic agent thus minimizing damage to normal cells and very therapeutically very effective while destroying cancerous cells. In this context, our study aimed to determine the cytotoxic effect of safranal on the human cervical cancer C-4 I cell line. We investigated the effects of safranal treatment on general aspects of C-4 I such as cell viability, proliferation, and morphology.

## MATERIALS AND METHODS

### Cell culture

C-4 I (ATCC, CRL1594) cell line was cultured in Waymouth MB 752/1 (Gibco) medium supplemented %10 Fetal Bovine Serum (Gibco) with containing %1 of 100 µg/ml of penicillin and 100 µg/ml of streptomycin at 37°C in 95% humid atmosphere containing 5% $CO_2$ (Ozcan et al., 2016).

### Evaluation of Cell morphologies

C-4 I cells (120 x $10^4$ cells/800 µl) were seeded in a 24-well plate. After treatment with different concentrations of safranal for 24, 48, and 72 h, cells were fixed with Carnoy's fixative (1:3; acetic acid: ethanol) for 10 minutes at the end of the incubation periods of the control and experimental groups. It was then washed twice for five minutes with 70% alcohol and left to dry. Afterwards, fixation, cells were stained with Giemsa at +4°C for 6mn. Morphological changes in cells were observed under the phase-contrast and light microscopies (10X25, 10X40) (Özcan et al., 2016).

### MTT Assay

C4-I cells were seeded in 96-well cell plates at 3 x $10^4$ cells/200 µl. After 24 hours, medium was removed and cells were incubated with different concentrations of safranal (Sigma, CAS: 116-26-7, Indian) (25 µM, 50 µM, 100 µM, 200 µM, 400 µM, and 800 µM) for 24, 48, and 72 hours. After incubation time, 40µl of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltratrazolium bromide (MTT, Duchefa Biochemie)

were added to the cells. In order to dissolve the formazan crystals formed after a 4 h incubation period with MTT, 160 µl of dimethylsulfoxide (DMSO; BioFroxx, CAS: 67-68-5) was added to each well and incubated overnight in the dark at 37°C in 95% humid atmosphere containing 5% CO2. After incubation, absorbance values were measured at 570 nm with reference to the 690 nm in the µQuant ELISA plate reader (µQuant, Bio-tek Instruments, INC.) (Ozcan et al., 2016; Ozsoylemez and Ozcan, 2018).

**LDH assay**

Lactate dehydrogenase (LDH) is a cytoplasmic enzyme found in almost every cell in the body. When cells are exposed to a toxic substance, the integrity of their plasma membrane is disrupted and the LDH enzyme escapes from the cells and passes into the medium. LDH is a very important biomarker to show the presence of secondary apoptosis and necrosis (Tokur and Aksoy, 2017; Erkekoğlu and Baydar, 2021). In our study, LDH kit (Cytotoxicity Detection Kit, Roche, Ref:11644793001; Lot:51751400) was used to measure the amount of LDH released into the medium as a result of the disruption of plasma membrane integrity in secondary apoptotic/necrotic cells to examine the cytotoxic effects of safranal in C-4 I cell lines. The cells used in the experiments were seeded to 96 well plates at 3 x $10^4$ cells/200 µl per well. Cells were treated with different concentrations (25-800 µM) of safranal for 24, 48, and 72 h. After incubation, 100µl of the medium was withdrawn from the medium whose cells were incubated and transferred to a new 96-well plate. 100µl of the freshly prepared reaction mixture in the kit was added to the wells and kept in the dark for 30 minutes at room temperature. At the end of this period, absorbance values at 490-492 nm were read on the µQuant ELISA Plate reader (µQuant, Bio-tek Instruments, INC.)

**Statistical Analysis**

All results are expressed as mean with the mean and standard deviation of the average of all 3 independent analyses. Significant data were determined statistically with ANNOVA test: (ANNOVA: Single Factor) according to the value of $p<0.05$.

**RESULTS**

**Morphological Assessment**

Concentration and time-dependent morphological changes were observed under an inverted microscope in C4-I cells treated with safranal (25µM, 50µM, 100µM, 200µM, 400µM, and 800µM) were applied (Figure 1). Morphological changes such as cell shrinkage, nuclear condensation, pyknosis, and apoptotic bodies were observed with Giemsa staining under light microscope (Figure 2).

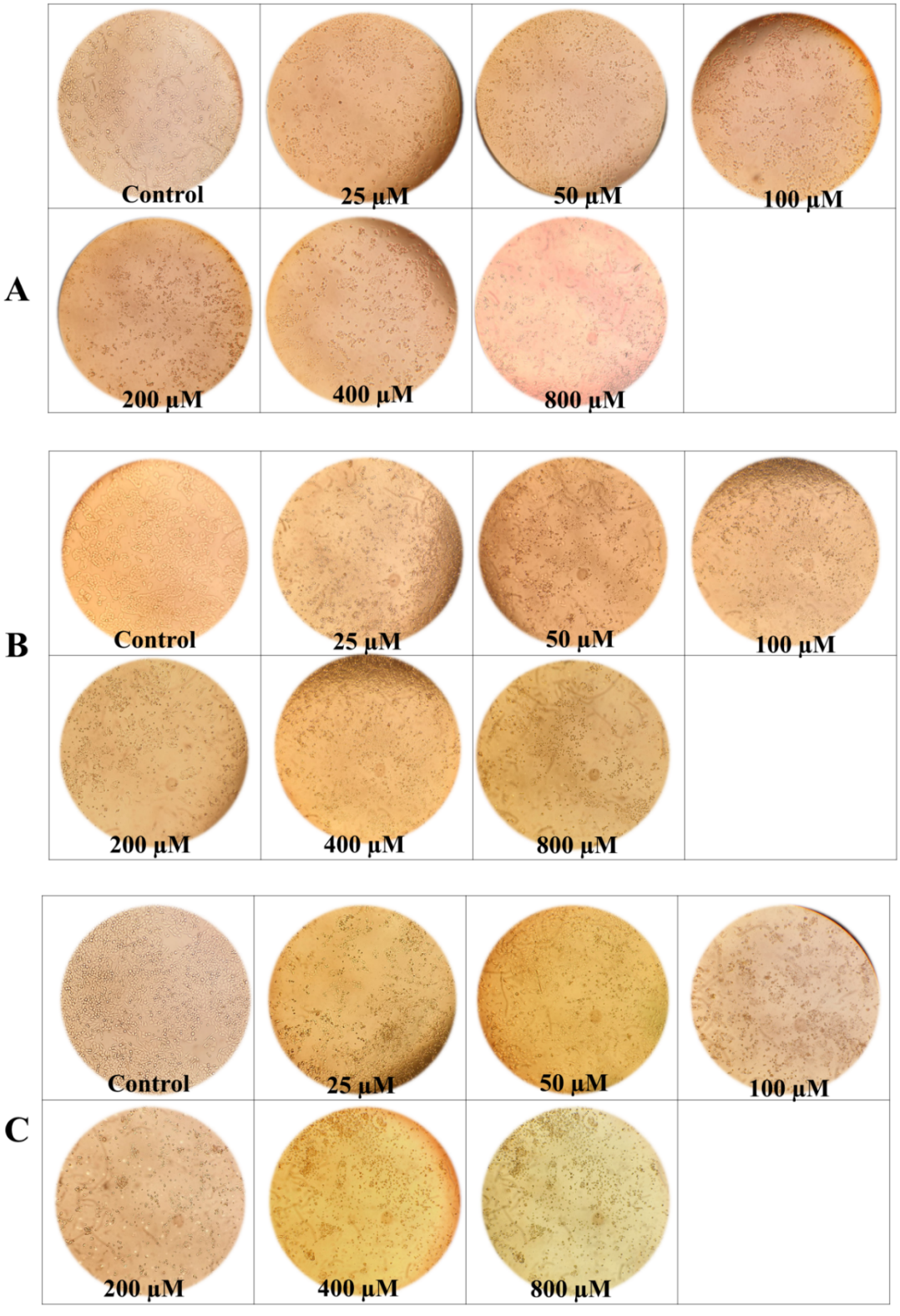


Figure 1. Inverted microscopy images of control group and C-4 I cells treated with safranal for 24 (A), 48 (B), and 72 (C) hours (10x25).

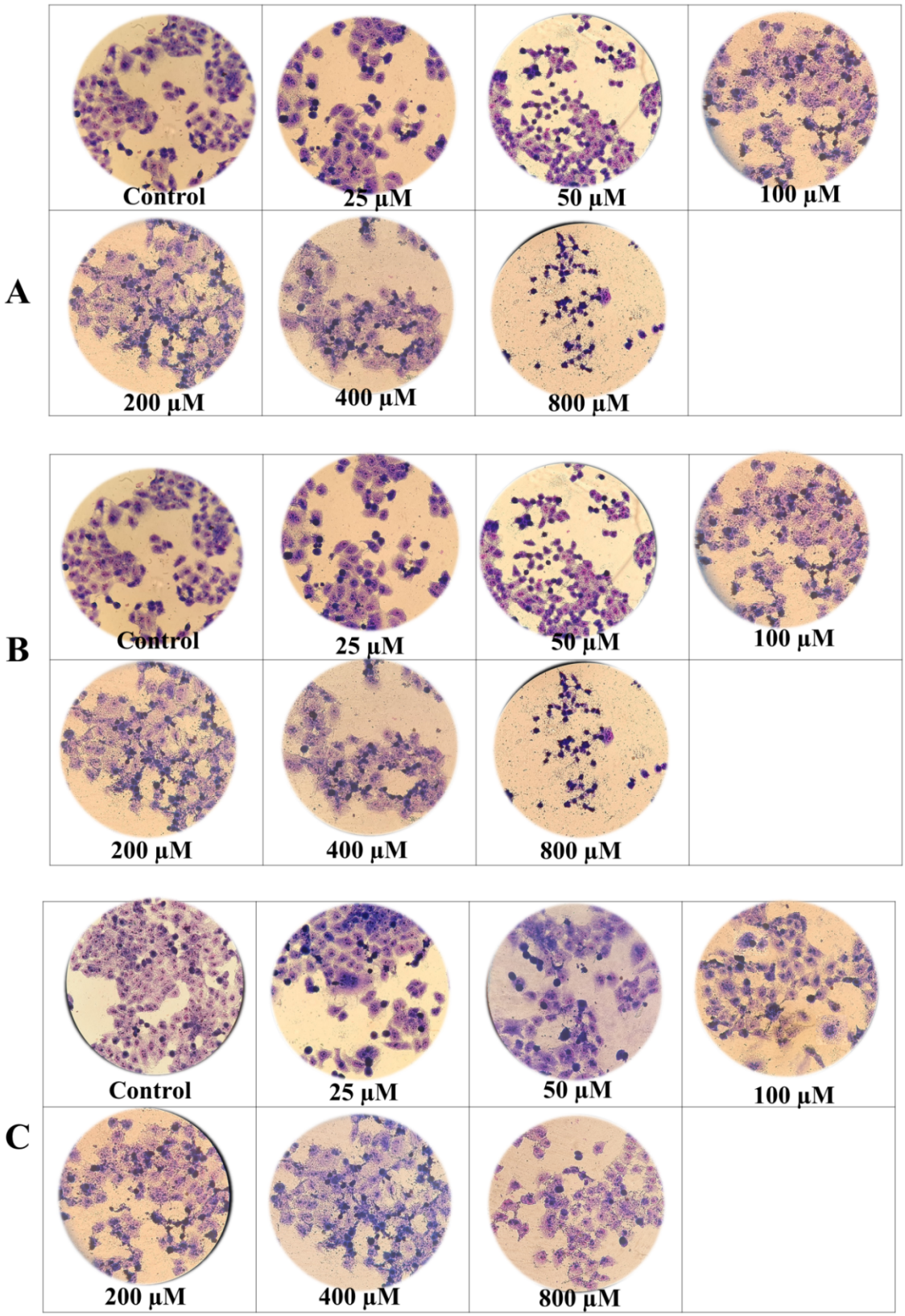


Figure 1. Giemsa staining images of control group and C- 4 I cells treated with safranal for 24 (A), 48 (B), and 72 (C) hours (Light microscope; 10X40).

**MTT Assay**

MTT assay was applied to assess the cytotoxic effect of safranal on cervical cancer, C4-I cells were treated for 24, 48, and 72 hours with a range of concentrations (25-800 µM). The % viability values were calculated based on absorbance values determined at the end of the incubation period. The viability of cells treated with safranal compared to the control group was decreased depending on concentration and time (*p<0.05) (figure 3, Table 1).

Table 1. Absorbance values of C- 4 I cells treated with safranal at 24, 48, and 72 h (D1=25µM, D2= 50µM, D3=100µM, D4=200µM, D5=400µM, D6=800µM) of the MTT assay (* p<0.05, compared to Control group).

| | **Absorbance values (570 – 690 nm). Mean ±SD** | | |
|---|---|---|---|
| **Treatment groups** | 24 h | 48 h | 72h |
| **Control** | $635.1x10^{-3} \pm 0.02$ | $795.5x10^{-3} \pm 0.01$ | $768.07x10^{-3} \pm 0.01$ |
| **D1** | $510.2\ x10^{-3} \pm 0.03$* | $548\ x10^{-3} \pm 0.02$* | $390.28\ x10^{-3} \pm 0.02$* |
| **D2** | $441.7x10^{-3} \pm 0.01$* | $389x10^{-3} \pm 0.01$* | $338.07x10^{-3} \pm 0.01$* |
| **D3** | $444.7\ x10^{-3} \pm 0.01$* | $310.1x10^{-3} \pm 0.02$* | $291.88x10^{-3} \pm 0.02$* |
| **D4** | $373.9\ x10^{-3} \pm 0.03$* | $290.3x10^{-3} \pm 0.02$* | $260.47\ x10^{-3} \pm 0.02$* |
| **D5** | $329.7\ x10^{-3} \pm 0.03$* | $215\ x10^{-3} \pm 0.02$* | $200.27\ x10^{-3} \pm 0.02$* |
| **D6** | $267.3\ x10^{-3} \pm 0.01$* | $199\ x10^{-3} \pm 0.01$* | $156.4x10^{-3} \pm 0.01$* |

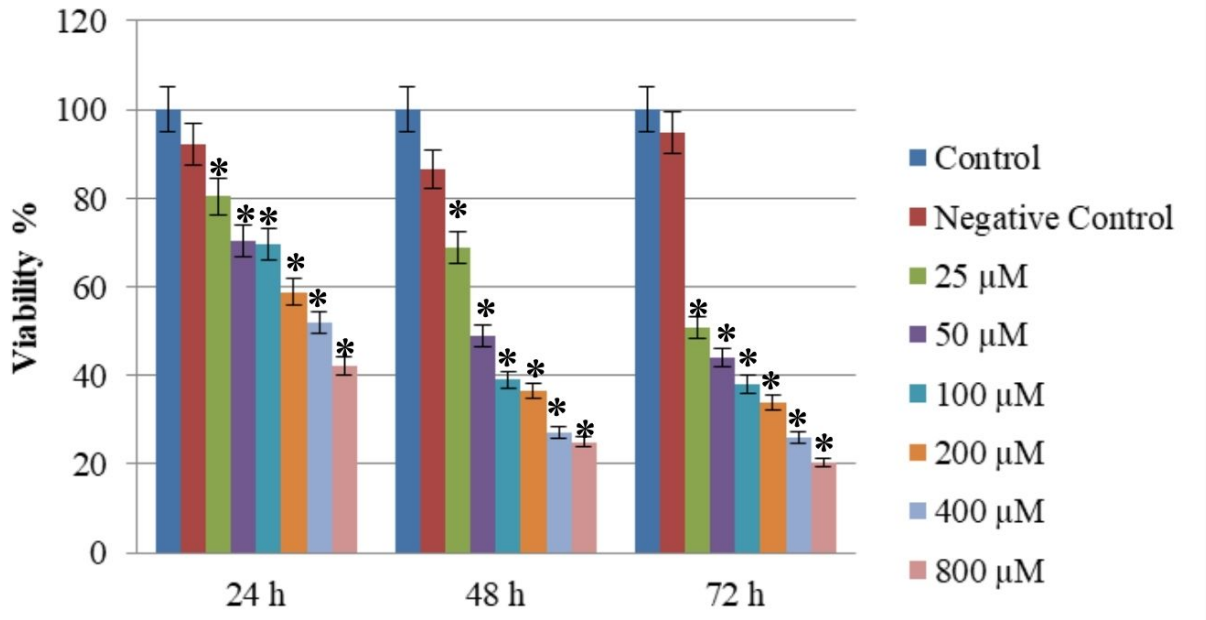


Figure 3. Viability values (%) of C-4 I cells treated with safranal for 24, 48 and 72 hours (negative control = DMSO; *p<0.05).

**LDH Assay**

Lactate dehydrogenase is a cytoplasmic enzyme present in most cells. When a cell is exposed, the LDH enzyme is released from the cytoplasm. LDH is released when cells are dead at seconder apoptosis and necrosis. LDH assay was used to approve the results obtained in the MTT assay. In order to assess the cytotoxic effect of safranal applied to cervical cancer C4-I cell lines for 24 to 72 hours, the percentage of inhibition of proliferation and growth of cells treated with safranal is calculated based on the

determined absorbance values (Figure 4). It was observed that safranal inhibited the proliferation and growth of cervical cancer C-4 I cell line by seconder apoptosis and necrosis.

Table 2. Absorbance values of C- 4 I cells treated with safranal at 24, 48, and 72 h (D1=25µM, D2= 50µM, D3=100µM, D4=200µM, D5=400µM, D6=800µM) of the LDH assay (* $p<0.05$, compared to Control group).

| | **Absorbance values (490-492 nm). Mean ±SD** | | |
|---|---|---|---|
| **Treatment groups** | 24 h | 48 h | 72h |
| **Control** | $111.27x10^{-3} \pm 0.02$ | $632.9 \times 10^{-3} \pm 0.02$ | $1107.9x\ 10^{-3} \pm 0.02$ |
| **D1** | $173.97 \times 10^{-3} \pm 0.04$* | $1010.6 \times 10^{-3} \pm 0.01$* | $2340.4 \times 10^{-3} \pm 0.03$* |
| **D2** | $222.37 \times 10^{-3} \pm 0.04$* | $1182.3 \times 10^{-3} \pm 0.04$* | $2845.7x\ 10^{-3} \pm 0.03$* |
| **D3** | $256.43 \times 10^{-3} \pm 0.02$* | $1271.1 \times 10^{-3} \pm 0.01$* | $3021.83 \times 10^{-3} \pm 0.02$* |
| **D4** | $290.03 \times 10^{-3} \pm 0.04$* | $1377 \times 10^{-3} \pm 0.02$* | $3342.9 \times 10^{-3} \pm 0.36$* |
| **D5** | $325.73 \times 10^{-3} \pm 0.03$* | $1413.1 \times 10^{-3} \pm 0.02$* | $3844.6x\ 10^{-3} \pm 0.01$* |
| **D6** | $364.6 \times 10^{-3} \pm 0.02$* | $1445 \times 10^{-3} \pm 0.02$* | $4245.86x10^{-3} \pm 0.01$* |

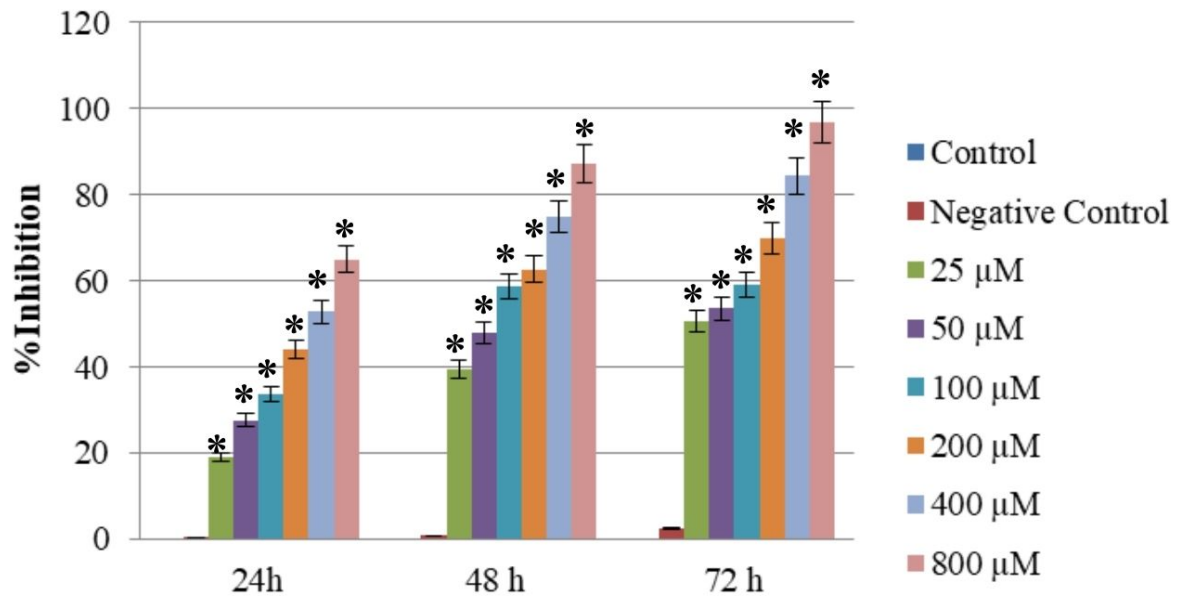


Figure 4. Inhibition values (%) of C-4 I cells treated with safranal for 24, 48 and 72 hours (Negative control = DMSO; *$p<0.05$)

## DISCUSSION

Medicinal plants are the main source of treatment in traditional medicine. Their natural antiseptic properties make them important in medical research on the study of the potential properties of plant extracts for the treatment of many diseases, including cancer. In many studies, plant species focusing on compounds contained in plants and exhibiting anti-cancer properties have been identified in developing countries (Chermahini et al., 2010). Plants used in traditional medicine are considered one of the main sources for the discovery and development of cancer chemopreventive drugs due to their potential to inhibit oxidative stress (Greenwell and Rahman, 2015). Crocus sativus L. is a rootless annual herb of the well-known saffron family Iridaceae. It is obtained from the stigmas of the saffron flower, one of the most expensive aromatic herbs in the world (Mollazadeh et al., 2015). safranal, one of the important compounds of this plant, is a monoterpene aldehyde responsible for the aroma and odor of the plant

which has potential antioxidant properties as well as photoprotective properties. Studies have shown that, pharmacologically, safranal has the ability to neutralize free radicals. However, the protective effect of safranal against oxidants caused by ischemia-reperfusion injury has also been determined (Hosseinzadeh and Sadeghnia, 2005; Cerdá-Bernad et al., 2022). In addition, the antitussive and anticonvulsant activities of safranal have been mentioned in numerous studies (Malaekeh-Nikouei et al., 2013; Kyriakoudi et al., 2015; Moratalla-Lopezet al., 2019).

In this study, the cytotoxic effects of safranal on the Cervical C-4 I cancer line were investigated. At 24, 48, and 72 hours, we observed a decrease in cell density, nucleic condensation, cell shrinkage, cell fragmentation, apoptosis-specific morphological markers inverted microscopy, and Giemsa staining in treated with safranal compared to control. Results obtained in MTT Assay showed a decrease in the viability of cells treated with safranal. This effect is approved by results obtained with LDH which showed that safranal C-4 I cells have the ability to initiate apoptosis and necrosis by inhibiting proliferation. Our data confirmed that safranal has a cytotoxic activity effect dependent on dose and time against C-4 I cells.

Several studies have shown its antitumor activity in some cell lines. Many of them have reported the selective toxicity of saffron extract and its derivatives against cancer cells and its non-existent toxicity against normal cells (Milajerdi et al., 2016). In the Jabini et al. (2017) study, the cytotoxic effect of safranal has been shown on oral squamous cancer cell lines NIH 3T3 (Healthy) and KB (Cancer). This cytotoxicity effect against oral squamous cancer cells acts by inhibiting the growth of KB cell lines. It was determined that this effect was partially selective against KB cells because it was biologically very low on health (NIH 3T3) cells. The investigation of Samarghandian and Shabestari (2013) on the effect of Safranal on human prostate cancer cells concluded that safranal has a cytotoxic effect and the ability to initiate apoptosis against prostate PC-3 cancer line cells by inhibiting the proliferation and growth of PC-3 cancer cell. Cheriyamundath et al. (2018) study showed that safranal has the potential to inhibit HeLa cell viability. In Zarrineh et al. (2022) study, the anti-proliferative activity of safranal on triple negative MDA-MB-231 breast cancer cell lines was investigated. Safranal induces a considerable decrease in the viability of MDA-MB-231, MCF10A, and MDA-MB468 cell lines in different concentrations. These findings correlate with previous studies which demonstrated that safranal has an antiproliferative effect against cancer cells.

## CONCLUSION AND RECOMMENDATIONS

According to the results obtained in our study, it can be said that safranal has an anti- proliferative effect against cervical cancer C-4 I cell lines. This antiproliferative effect can be induced by cell death types such as apoptosis and necrosis. However, more research will be needed to fully delineate and clarify the molecular mechanism of safranal anticancer effects.

## ACKNOWLEDGEMENT

This study was funded by Scientific Research Projects Coordination Unit of Istanbul University Project number: FDK-2022-38981.

## CONFLICT OF INTEREST

The authors declare that they have no competitive interests.

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*Full Text - 05*

# MIR-145's REGULATORY ROLE IN BREAST CANCER PROGRESSION

**Boran Can DİNÇER[1], Şeref Buğra TUNÇER[2]**

## ABSTRACT

*Delayed diagnosis of breast cancer leads to reduced treatment success rates and lower survival rates. Every year, millions of women are diagnosed with breast cancer, and many lose their lives due to this disease. However, with the discovery of biomarkers that enable early diagnosis, it is aimed to improve the success of treatment processes and increase survival periods. In recent years, studies have been conducted on the use of microRNAs as biomarkers for early breast cancer diagnosis. Research indicates that microRNAs could serve as potential biomarkers for breast cancer diagnosis and prognosis prediction. Alongside ongoing research on the potential use of microRNAs as biomarkers for early breast cancer diagnosis, our study also analyzed the potential utility of miR-145 as a biomarker in breast cancer. In our study, RNA samples were isolated from the blood-serum samples of 200 breast cancer patients and 100 individuals in a healthy control group, and the specific expression levels of miR-145 were determined using the RT-qPCR technique. As a result of these analyses, it was found that miR-145 had lower expression levels in breast cancer patients compared to the healthy control group. After further investigation of miR-145's biomarker potential in a larger cohort of breast cancer patients, it is aimed to use it as a target biomarker for early breast cancer diagnosis.*

**Key Words**: Breast cancer, microRNA, miR-145

## ÖZET

*Meme kanseri tanısı geç konulduğunda tedavi ve sağkalım oranları azalmaktadır. Her yıl milyonlarca kadın meme kanseri teşhisi almakta ve bu hastalık nedeniyle birçok insan hayatını kaybetmektedir. Ancak, erken teşhisi sağlayacak biyobelirteçlerin keşfi ile tedavi süreçlerinin daha başarılı olacağı ve sağkalım sürelerinin arttırılması hedeflenmektedir. Son yıllarda, mikroRNA'ların biyobelirteç olarak meme kanseri erken teşhisinde kullanımı ile ilgili çalışmalar yapılmaktadır. Araştırmalar, mikroRNA'ların meme kanseri teşhisi ve prognoz tahmini için kullanılabilecek biyobelirteçler olabileceğini göstermektedir. mikroRNA'ların meme kanseri erken tanısında biyobelirteç olarak potansiyel kullanımı üzerine devam eden araştırmalarla birlikte, çalışmamızda da miR-145'in meme kanserinde biyobelirteç olarak kullanılabilme potansiyeli analiz edildi. Çalışmamızda, 200 meme kanseri hastasının ve sağlıklı kontrol grubunda yer alan 100 kişinin kan-serum numunelerinden RNA örnekleri izole edilerek, mir-145 özgün ekspresyon seviyeleri RT-qPCR tekniği ile tespit edildi. Bu analizler sonucunda, miR-145'in meme kanseri hastalarında sağlıklı kontrol grubuna göre daha düşük düzeylerde ekspresyon seviyeleri bulundu. miR-145'in biyobelirteç olabilme niteliğinin daha yüksek sayılı meme kanseri hasta kohortunda araştırıldıktan sonra meme kanseri erken teşhisinde hedef biyobelirteç olarak kullanılabileceği hedeflenmektedir.*

**Anahtar Kelimeler:** Meme kanseri, mikroRNA, miR-145

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## INTRODUCTION

MicroRNAs (miRNAs) are small non-coding RNA molecules that have garnered significant attention in molecular biology and biomedical research in recent years. These small, non-coding RNA molecules play a critical role in regulating gene expression. miRNAs form a complex network in controlling cellular functions, and as a result, they are actively involved in the pathogenesis of various diseases (Macfarlane, L. A., & Murphy, P. R. 2010).

It has been observed that miRNAs are involved in different mechanisms and pathways in various diseases. They have been reported to play an active role in processes such as the progression of diseases, tumor formation, metastasis, including breast cancer. MicroRNAs have been shown to regulate the proliferation of breast cancer cells by targeting genes involved in cell cycle control and growth factor signaling pathways. Additionally, they have been found to regulate apoptosis by targeting genes involved in cell death pathways, and furthermore, they have been determined to induce the invasion of breast cancer cells by regulating genes related to epithelial-mesenchymal transition (EMT) and cell migration (Goh, J. N., & Kumar, A. P., 2015; Hu, J. et al., 2012).

miR-145 is defined as a tumor suppressor miRNA and there is an increase in cancer cells in case of loss of expression. This downregulation is associated with an increase in cancer cells, emphasizing the pivotal role of miR-145 as a tumor suppressor (Otmani, K., & Lewalle, P., 2021). Therefore, it has been reported that miR-145 can be evaluated as a potential biomarker that can be used in cancer diagnosis. The molecule's potential as a biomarker for early detection, prognosis and staging of breast cancer has been investigated. According to the literature, miRNA-145 (miR-145) plays an important role in tumorigenesis and progression. This miRNA interacts with signaling pathways involved in cell invasion and migration (Xu, W. X. et al., 2019). miR-145 is hypothesized to be a tumor suppressor and has been observed to be downregulated in direct association with the p53 protein in several types of cancer, such as breast cancer (Sachdeva, M. et al., 2009). In another study, it was observed that miR-145 directly targets ROCK1 and its downregulation increases the mechanism involving ROCK1 causing cancer and invasion (Zheng, M., & Zuo, W. 2016). In another study, it was shown that downregulation of miR-145 causes increased expression of TGF-β1 in breast cancer, resulting in cell proliferation and migration (Ding, Y. et al. 2017).

In conclusion, miR-145 works as a tumor suppressor and its increased expression prevents invasion and cell proliferation in cancerous cells and tissues. Decreased miR-145 levels in cells, along with increased expression of associated pathways, favor tumor development and invasion. These data suggest that miR-145 may be an important target for cancer therapy.

**AIM**

Breast cancer ranks among the most common types of cancer in women worldwide, highlighting the critical importance of early detection. Advances in biotechnology and cancer research have revealed the significant role of microRNAs (miRNAs) in the diagnosis and prognosis of breast cancer. Particularly, miR-145 is recognized as a tumor-suppressive miRNA, and its deficiency is associated with increased cancer cell proliferation. Therefore, ongoing research into the potential utility of miR-145 as a biomarker for breast cancer diagnosis holds promise for innovative approaches to early detection, prognosis, and staging of the disease. Our study evaluates the expression profile of miR-145 in serum samples obtained from 200 breast cancer patients matched for age, gender, and ethnicity with 100 healthy control individuals. This research aims to investigate the potential of miR-145 as a valuable biomarker in breast cancer diagnosis and treatment, offering new insights into its role in disease management.

## MATERIAL AND METHODS

### Materials

• NEB Monarch Quick-DNA/RNA Miniprep kit

• NEB ProtoScript® II Reverse Transcriptase

• NEB Q5® High-Fidelity DNA Polymerase

• Ampliqon TEMPase Hot Start DNA Polymerase

• NEB Quick-Load® Taq 2X Master Mix

• Fastruler 6x Loading Dye

• FastRuler DNA Marker

• ThermoScientific Gel Stain

• BIOMATIK Agarose Powder

• Meridian Sensifast No Rox SYBR qPCR kit

• Forward Primers

• Reverse Primers

• Stem Loop Primers

## Methods

A Power and Sample Size program was utilized to determine the sample group in the study. Following the power analysis, where a Type I Error of 0.05 and Test Power (Confidence Interval) of 80% were considered, it was calculated that a minimum of 182 participants were necessary for the patient analysis. The decision was made to enroll 200 patients in the study, who had been diagnosed with breast cancer, sought treatment at Istanbul University Institute of Oncology, agreed to take part voluntarily, and provided informed consent by signing the required documents. Similarly, a control group of 100 healthy individuals with no history of cancer in their families and no diagnosis of breast-related disorders, matched for age and gender with the patient group, were included in the study. miRNA-145 expression studies were conducted using blood serum samples from both the patient group and the control group. As a method, firstly, total RNA was obtained from serum samples. Then, cDNA synthesis was performed with stem loop primers using Reverse Transcriptase from the total RNAs obtained. Reactions were incubated at 42°C for 30 minutes and then at 95°C for 5 minutes using Thermal Cycler. Real-time qPCR was performed on the resulting cDNAs to analyze the difference in expression levels of miRNAs. The assumption of normality of the distribution between the groups in the study was determined using the Kolmogorov-Smirnov Test. Therefore, the Mann-Whitney U Test, which is a non-parametric test, was used in the analysis of the data. The Mann-Whitney U Test was performed using 2-(ΔΔCt) values between patients and control group.

## RESULTS

The expression results obtained showed that the expression levels of target miRNAs were statistically significant compared to the control group of the patients. When we look at the results, there are statistically significant differences between the groups in which miR-145 expression differences were examined. Means for the healthy control group, stage 1, stage 2, stage 3 and stage 4 patient groups were compared and statistically significant differences were found for each ($p<0.001$ for each). The mean miR-145 expression of the control group was statistically significantly higher than the mean of stage 1, stage 2, stage 3, and stage 4 patient groups ($p<0.001$ for each) fold change in stage1, stage 2, stage 3 and stage 4 patient groups It is seen that the fold change 2-ΔΔCT ratios decreased compared to the control group.

## DISCUSSION

According to the results, miR-145 decreases inversely with the progression of breast cancer stages. Statistically significant findings ($p<0.001$ for each) underscore the significance of changes in miR-145

expression in breast cancer. Based on these results, it is thought that miR-145 expression analyzes can bring a different perspective to the known clinical staging method of breast cancer and can also be used in cancer diagnosis. In addition, it is thought that the inclusion of different miRNA molecules in the study can be used as a more sensitive diagnostic tool in cases where a single miRNA expression data is insufficient as a result of varying expression levels in breast cancer patients at different stages, and their evaluation together can provide a comprehensive analysis.

## CONCLUSION AND RECOMMENDATION

The results of this study demonstrate that miR-145 plays a tumor-suppressive role in breast cancer and that the expression of miR-145 significantly decreases at different stages of the disease. These findings support the potential use of miR-145 as a potential biomarker for the diagnosis and staging of breast cancer. From a clinical perspective, further research and clinical studies are required to validate the role of miR-145 in breast cancer diagnosis and prognosis. If these findings are confirmed, miR-145 expression analysis could become a part of routine clinical procedures to enhance the accuracy of breast cancer diagnosis and staging. In terms of diagnostic tests, it is advisable to consider a panel of different miRNAs as miRNA expression profiles can vary among breast cancer patients. Combining multiple miRNAs may contribute to a more comprehensive and accurate evaluation of the disease. Given the tumor-suppressive role of miR-145, it is important to investigate therapeutic strategies aimed at restoring miR-145 expression in breast cancer cells. miR-145-based therapies may offer potential contributions to the development of new treatment options for breast cancer.

Finally, long-term studies should be conducted to monitor changes in miR-145 expression during the course of treatment and disease progression. These studies can provide valuable insights into the dynamic role of miR-145 in breast cancer. Therefore, further research and clinical applications are needed to better understand and utilize the potential of miR-145 in the diagnosis and treatment of breast cancer.

## CONFLICT OF INTEREST

Authors declare that there is no conflict of interest regarding this work.

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*Full Text - 06*

# Biological Effects of CRISPR/Cas9-mediated Knockout of RAB27A in SCLC

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## ABSTRACT

*Small cell lung cancer (SCLC) is characterized by rapid growth and early metastasis. Identifying new molecular targets are important in the pathogenesis of SCLC in order to develop new treatment strategies. RAB27A is the critical protein for intracellular exosome trafficking and is a driver of tumour progression. However, demonstrating the potential impact of suppressing RAB27A in SCLC as therapeutic approach is an important deficiency. RAB27A gene knockout SCLC cell lines were generated using a CRISPR/cas9 system. qRT-PCR, Western blotting and Sanger sequencing were performed to confirm RAB27A knockout in SCLC cells. TEM and EXOCET assays were used to detect the alteration of exosomes. Proliferation and colony formation were detected by MTT and microscopy. Subsequently, we intrapulmonally injected N417 and H524 SCLC cells(control and RAB27A knockout for each cell) into SCID mice. The effects of RAB27A knockout on mouse tumor model were analysed using 18F-FDG PET/CT scans.Knocking out RAB27A significantly decreased the expression of CD9, CD63, Tsg101, exosome secretion and exosomal protein in SCLC($p<0.0001$). We found that RAB27A knockout dramatically reduced proliferation and colony formation in SCLC cells($p< 0.001$, $p<0.0001$). Furthermore, RAB27A knockout decreased proliferation and especially metastasis in mouse model ($p<0,0001$). These studies clearly demonstrated that RAB27A plays an important role in the pathogenesis of SCLC, and targeting the RAB27A gene in SCLC cell lines significantly reduces the activity of the exosomal pathway. RAB27A, therefore, can be a promising cancer therapeutic strategy.*

**Key Words:** RAB27A, exosome, SCLC, CRISPR/Cas9, Carcinogenesis

## ÖZET

*Küçük hücreli akciğer kanseri (KHAK) hızlı büyüme ve erken metastaz ile karakterizedir. Yeni tedavi stratejileri geliştirmek için KHAK patogenezinde yeni moleküler hedeflerin belirlenmesi önemlidir. RAB27A, hücre içi eksozom trafiği için kritik bir proteindir ve tümör progresyonunda rol oynar. Bununla birlikte, KHAK'de RAB27A'nın baskılanmasının terapötik yaklaşım olarak potansiyel etkisinin gösterilmesi önemli bir eksikliktir. RAB27A geni nakavt SCLC hücre hatları CRISPR/cas9 sistemi kullanılarak üretildi. qRT-PCR, Western blotlama ve Sanger sekanslama SCLC hücrelerinde RAB27A nakavtını doğrulamak için gerçekleştirildi. Eksozomların nicel ve nitel değişimini tespit etmek için TEM ve EXOCET testleri kullanıldı. Proliferasyon ve koloni oluşumu MTT ve mikroskopi ile tespit edildi. Daha sonra, N417 ve H524 SCLC hücrelerini (her hücre için kontrol ve RAB27A nakavtı) SCID farelerine intrapulmonal olarak enjekte edildi. RAB27A nakavtının fare tümör modeli üzerindeki etkileri 18F-FDG PET/BT taramaları kullanılarak analiz edildi. RAB27A'nın nakavt edilmesi KHAK'de CD9, CD63, Tsg101, eksozom salgısı ve eksozomal protein ekspresyonunu önemli ölçüde azaltmıştır ($p<0.0001$). RAB27A nakavtının KHAK hücrelerinde proliferasyonu ve koloni oluşumunu önemli ölçüde azalttığını bulduk ($p< 0.001$, $p<0.0001$). Ayrıca, RAB27A nakavtının fare modelinde proliferasyonu ve özellikle metastazı azalttığı görülmüştür ($p<0,0001$). Bu çalışma, RAB27A'nın KHAK patogenezinde önemli bir rol oynadığını ve KHAK hücre hatlarında RAB27A geninin hedeflenmesinin eksozomal yolağın aktivitesini önemli ölçüde azalttığını açıkça göstermiştir. Bu nedenle RAB27A, umut verici bir kanser terapötik stratejisi olabilir.*

**Anahtar Kelimeler:** RAB27A, eksozom, KHAK, CRISPR/Cas9, Karsinogenezis

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## INTRODUCTION AND AIM

Small cell lung cancer (SCLC) is one of the histopathological subtypes of lung cancer. SCLC is of neuroendocrine origin and accounts for 15% of lung cancers. High proliferative index, early metastasis and poor prognosis. The average survival time for patients with SCLC is ~ 10 months, with a 2-year survival rate of only 6%. Many oncogenic genes and signalling pathways are involved in the development of SCLC and intra-tumour heterogeneity is high (Rudin, et.al., 2021). The Rab27 sub-family is a member of the Rab family of proteins, which consists of two isoforms, RAB27A and RAB27B. As essential proteins for vesicle exocytosis and exosome release, which are critical for the regulation of the tumour microenvironment, Rab27 proteins have also been reported to be involved in regulating carcinogenesis (Ostrowski et.al., 2010; Guo et.al., 2019; Van Solinge et.al., 2020; Tang et.al, 2016) However, there is a lack of information in the literature on the impact of targeting RAB27A in the development of a treatment strategy for SCLC. Accordingly, to investigate the biological changes that may occur in SCLC when the RAB27A gene is silenced, in vitro and in vivo studies are being conducted.

## MATERIALS AND METHODS

### Cell Culture, Generating of SCLC cell lines knocked out of RAB27A and Validation

The cell lines H889, H1963 and H524 were obtained from the American Type Culture Collection (ATCC). H889, H524, H1963 and N417 small cell lung cancer cell lines were cultured in RPMI-1640 media with 10% fetal bovine serum (FBS) and 1% Penisilin/Streptomycin (Gibco, USA). All cells were grown in 5% $CO_2$ humidified air at 37ºC. SCLC cell lines were co-transfected with RAB27A-targeting gRNA+Cas9+GFP and HDR+RFP vectors. Sanger sequencing, qRT-PCR and Western blotting were used to validate the application of CRISPR/Cas9 to the *RAB27A* gene.

### EXOCET Assay

The amount of exosomes derived from SCLC cell lines was quantified using the EXOCET assay (System Biosciences, USA) according to the manufacturer's protocol.

**Proliferation assay**

The effect of knocking out RAB27A on proliferation of SCLC cells was investigated.

**In Vivo SCLC model and 18F-FDG PET/CT Analysis**

The in vivo experimental phase of our study was realised by intrapulmonary injection of the N417 control and N417 RAB27A KO group cell lines into SCID mice. PET-CT analyses at the end of the 6th week to analyse possible changes in tumour growth and metastatic activity of the N417 cell lines in vivo after RAB27A manipulation.

**RESULTS**

**There was a statistically significant reduction in RAB27A gene expression in SCLC cell sequences after the CRISPR/Cas9 procedure.**

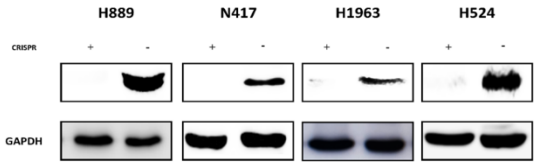


**Figure 1.**Expression of RAB27A protein in SCLC cell lines after CRISPR/Cas9 application.

In small-cell lung cancer cell lines, the Rab27A gene was found to be dramatically decreased in expression at both protein and RNA levels. Mutation in the 6th exon DNA region of the RAB27A gene was detected by Sanger sequencing after knockout by CRISPR/Cas9 application.

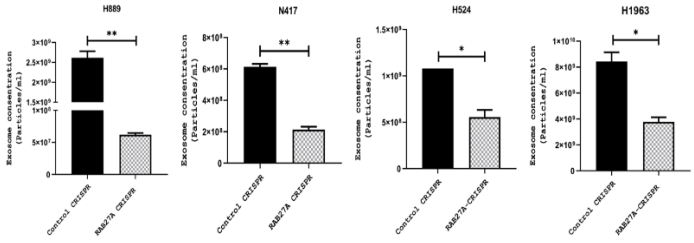


**Figure 2.** Change in the amount of exosomes.

There was also a statistically significant decrease in the total amount of exosomal protein secreted by the cells following suppression of the exosomal pathway by targeting RAB27A in CHAC cell sequences ($p<0.01$; $p<0.001$). We determined that the size of exosomes secreted by the RAB27A KO SCLC cell lines was reduced in comparison to the control groups.

**Knockout of RAB27A in SCLC cell lines results in reduction of cell proliferation.**

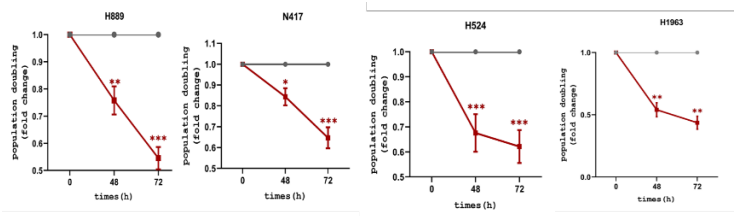


Figure 6. Proliferative activity of SCLC cell lines.

To investigate the function of RAB27A on SCLC cell proliferation, Cell Counting Kit-8 (CCK-8) was used to measure the growth of the established cell lines. As shown in Figure 6, the optical density (OD) value in RAB27A knockout N417, H889, H524 and H1963 cells was significantly lower than that of the control cells 48 hours after cell seeding, indicating that RAB27A knockout inhibits the proliferative activity of SCLC cell lines.

**In vivo SCLC Mouse model studies**

At the end of 6 weeks, 18F-FDG PET-CT analyses were performed on 3 mice from each group and the macro- and micro-metastatic status of the mice was analysed. In SCID mice injected with control vector-bearing N417 cells, higher than normal 18F-FDG uptake was observed in distant lymph nodes and bones. Furthermore, It was found that the 18F-FDG uptake of metastatic tumours was lower in mice that were injected with RAB27A knockout cells. Therefore, RAB27A gene silencing reduced metastatic activity in the SCLC in vivo model.

## DISCUSSION AND CONCLUSION

Small cell lung cancer(SCLC) is a subtype of lung cancer with aggressive neuroendocrine characteristics. The success rate of translating potential treatment strategies into clinical practice is low due to its genetic structure. Based on our findings, the RAB27A gene has the potential to become a new targetable molecule for the treatment of SCLC. Suppression of the exosome pathway by inhibition of RAB27A may be an effective treatment strategy for SCLC.

## ACKNOWLEDGEMENT

This study was supported by the TUBITAK under the project number 220S104.

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