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Cilt 4 **Sayı 2** **2024**

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IZTU JOURNAL OF MEDICAL AND HEALTH SCIENCES

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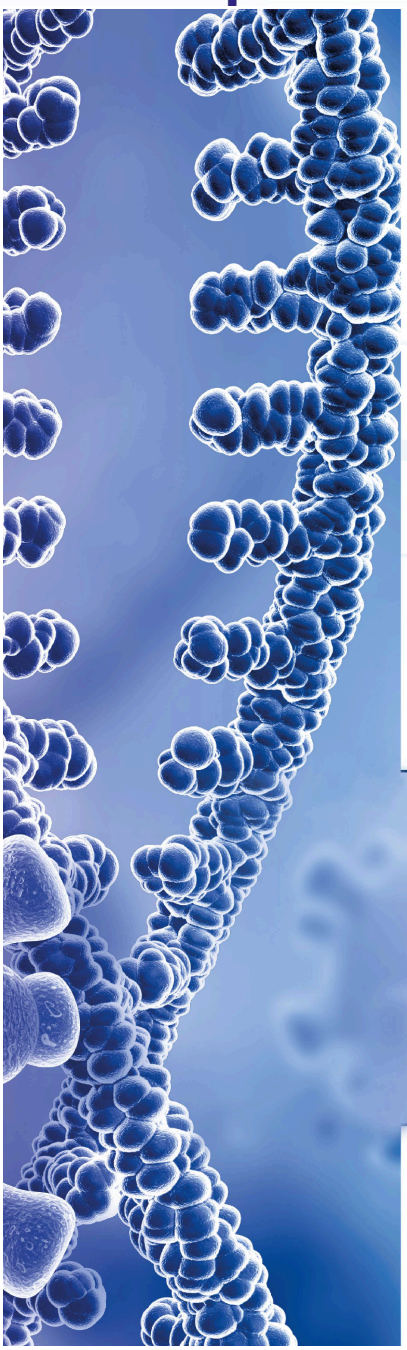

İZMİR TINAZTEPE ÜNİVERSİTESİ 2018

ULUSLARARASI SEMPOZYUM

PRECISION MEDICINE IN HEALTH AND DISEASE

KİŞİSELLEŞTİRİLMİŞ TIP ARAŞTIRMA VE UYGULAMALARI

3-4 Ekim 2024

GALEN MEDICINE DAYS - 2024
PRECISION MEDICINE IN HEALTH AND DISEASE
INTERNATIONAL SYMPOSIUM

03.10.2024	
08:30 – 09:30	**Registration**
09:30 – 10:00	**Welcome and Opening Remarks**
	Prof. Dr. Mehmet ÖZTÜRK (Chair of Galen Medicine Days-2024)
	Prof. Dr. Mustafa GÜVENÇER (Rector of Izmir Tınaztepe University)
10:00 – 10:50	**Keynote Speech** *(Session Chairs: Prof.Dr. Mehmet ÖZTÜRK, Prof.Dr. Neşe ATABEY)* Prof. Dr. Dominique BELLET - *Precision Medicine in Oncology: From Impressive Progress to Emerging Challenges*
10:50 – 11:05	**Coffee Break and Poster Session**
11:05 – 12:10	**Session 1** *(Session Chairs: Prof. Dr. Çağın ŞENTÜRK, Prof. Dr. Seyran YİĞİT)*
	11:05-11:45 Prof. Dr. Fahri SAATÇİOĞLU - Interplay of ER Stress Signaling in Cancer Cells and the Tumor Microenvironment - Implications for Immunotherapy
	11:45-12:10 Prof. Dr. Yusuf BARAN – *Molecular Mechanisms of Drug Resistance in Hematological Malignancies*
12:10 – 12:30	**Corporate Satellite Talk** *(Session Chair: Prof. Dr. Tülay AKMAN)*
	Shantanu KAUSHIKKAR - *Axiom PangenomiX Array - A Powerhouse for Predictive Genomics Research*
12:30 – 13:00	**Lunch Break**
13:00 – 14:00	**Case presentations-I: Precision Medicine for Rare Diseases** *(Session Chairs: Prof. Dr. Özlem GIRAY BOZKAYA, Prof. Dr. Esra ÖZER, Prof. Dr. Ferda ÖZKINAY)*
	Asst. Prof. Dr. Erhan PARILTAY - *Transthyretin (TTR) Cardiac Amyloidosis, Early Findings*
	Prof. Dr. Ahmet Okay ÇAĞLAYAN - *A Patient with SMA: How RNA-based Diagnosis Works*
	Assoc. Prof. Dr. Berk ÖZYILMAZ - *Two Cases and Prenatal Diagnosis Of A Rare UFM1 Associated Hypomyelinating Leukodystrophy*
	Ravza Nur YILDIRIM, PhD - *Adult Onset GalC Krabbe Disease Case*
	Assoc. Prof. Dr. Esra IŞIK - *Molecular Diagnosis Enables Effective Treatment in a Case of Late-Onset NAXE Deficiency*
14:00 – 15:20	**Session 2** *(Session Chairs: Assoc. Prof. Dr. Nilay DANIŞ, Assist. Prof. Dr. Yavuz OKTAY)*
	14:00-14:40 Assoc. Prof. Dr. Can ALKAN - *Algorithms to Characterize Genomic Structural Variation Using High Throughput Sequencing Technologies*
	14:40-15:05 Prof. Dr. Müjdat ZEYBEL - *Integrative Proteo-Transcriptomic Characterization of Advanced Fibrosis in Chronic Liver Diseases Across Aetiologies*
	15:05-15:20 Early Career Scientist Short Talk-I Dehan ÇÖMEZ, PhD - *The Effect of Ligand and Nanobody Binding Dynamics on EGFR/CXCR4 Dimerisation*
15:20 – 15:40	**Coffee Break and Poster Session**
15:40 – 16:55	**Session 3** *(Session Chairs: Prof. Dr. Necat İMİRZALIOĞLU, Prof. Dr. Banu DİLEK)*
	15:40-16:05 Prof. Dr. Uğur ÖZBEK - *Precision Medicine for Rare Diseases*
	16:05-16:30 Prof. Dr. Ahmet Okay ÇAĞLAYAN - *Multi-Omics Approach to Brain Malformations*
	16:30-16:55 Assoc. Prof. Dr. Özgür ATALAY – *Textile-Based Soft Robotics as Wearable Assistive Devices*

16:55 – 18:10	**Session 4** *(Session Chairs: Prof. Dr. Mehmet İNAN, Assoc. Prof. Dr. Elif Ebru ERMİŞ)*
	16:55-17:10 Selected Short Talk-I Asst. Prof. Dr. Mehmet BAYSAN- *COSAP: Comparative Sequencing Analysis Platform*
	17:10-17:25 Selected Short Talk-II Tunç TUNCEL, PhD - *Single-Cell RNA sequencing reveals blocking GPRC5A downregulates important cancer related genes in Mesothelioma Cell Line*
	17:25-17:40 Selected Short Talk -III Yeliz AKA, PhD - *Co-targeting DNA damage and Golgi stress induces mitochondrial apoptosis in ovarian cancer cells*
	17:40-17:55 Selected Short Talk-IV Associate Prof. Dr. Evin İŞCAN - *TAp73β induces angiogenesis and metastasis in a zebrafish xenograft model in the Hepatocellular Carcinoma*
	17:55-18:10 Selected Short Talk-V Özden ÖZ, MD PhD - *Axl Expression is Induced During the Transition from Non-Muscle-Invasive to Muscle-Invasive Stage in Bladder Cancer*
20:00	**Gala Dinner**

04.10.2024	
09:00 – 10:50	**Session 5** *(Session Chairs: Prof. Dr. Şermin GENÇ, Prof. Dr. Enver YETKİNER)*
	09:00-09:40 Dr. Stefan DIMITROV - *Mechanisms of Rare Diseases (From Altered Genome Structure to Altered Function)*
	09:40-10:20 Prof. Dr. Ahmet ÖZEN - *New Horizons in Medicine: Unveiling Novel Treatments for Protein-Losing Enteropathies*
	10:20-10:35 Early Career Scientist Short Talk-II Seval KILIÇ, PhD - *Comprehensive In Vivo Characterization of a Novel Rare Genetic Disease*
	10:35-10:50 Early Career Scientist Short Talk-III Burcu AKMAN, PhD - *Transcriptional and Molecular Drivers of Neuroendocrine Differentiation in Bladder Cancer*
10:50 – 11:10	**Coffee Break and Poster Session**
11:10 – 12:30	**Session 6** *(Session Chairs: Prof. Dr. Funda YILMAZ, Prof. Dr. Gökhan KARAKÜLAH)*
	11:10-11:50 Prof. Dr. Cengiz YAKICIER - *Comprehensive Genomic Profiling for Precision Medicine in Cancer*
	11:50-12:15 Prof. Dr. Erdener ÖZER -*Applications of Artificial Intelligence in Precision Oncology: Digital Pathology and Multiomics*
	12:15-12:30 Early Career Scientist Short Talk-IV Tugce BATUR, PhD - *AXL: A Key Player in DNA Damage Response and Epithelial-to-Mesenchymal Transition*
12:30 – 13:00	**Lunch Break**
13:00 – 14:00	**Case presentations-II: Precision Medicine in Oncology** *(Session Chairs: Prof. Dr. İbrahim PETEKKAYA, Prof. Dr. Mutlu DEMİRAY)*
	Aybike ERDOĞAN ATAY, PhD - *A case report: personalized medicine in MET and CDK6 overexpressing rare tumor*
	Ünal Metin TOKAT, PhD - *Remarkable response to dual immunotherapy and MEK inhibitor combination in a TERT and RASA1-mutant hepatocholangiocarcinoma*
	Ashkan ADIBI, MSc - *Exceptional response in a mRCC patient through precision-guided treatment involving immunotherapy rechallenge with temsirolimus and bevacizumab*

14:00 – 15:35	**Session 7** *(Session Chairs: Prof. Dr. Zümre ARICAN ALICIKUŞ, Prof. Dr. Semra HIZ KURUL)*
	14:00-14:40 Dr. Jean-Jacques DIAZ - *Ribosome Alterations in Cancer: Impact on Translational Control and Tumorigenesis*
	14:40-15:05 Prof. Dr. Nuri ÖZTÜRK - *Adjustment of Circadian Clock for Good Health*
	15:05-15:20 Early Career Scientist Short Talk-V Berkin Ersin VURAL- *Effects of F53 Hotspot Mutations on the Molecular Dynamics of MEK1 Protein the Binding of its FDA-Approved Inhibitors*
	15:20-15:35 Early Career Scientist Short Talk-VI Ezgi BAĞIRSAKÇI TEPE, PhD - *miR-181a-5p sensitizes Sorafenib resistance by attenuating the aggressive phenotype by the inverse correlation with Cav-1 in Hepatocellular Carcinoma*
15:35 – 15:50	**Coffee Break and Poster Session**
15:50 – 17:40	**Session 8** *(Session Chairs: Prof.Dr. Funda BÖLÜKBAŞI HATİP, Assoc. Prof. Dr. Duygu SAĞ)*
	15:50-16:30 Prof. Dr. Haval SHIRWAN- *Training the immune system as a universal platform for cancer prevention*
	16:30-16:55 Asst. Prof. Dr. Tolga SÜTLÜ - *Preclinical Development of Novel Natural Killer Cell-based Cancer Immunotherapies*
	16:55-17:10 Early Career Scientist Short Talk-VII Talip ZENGİN, PhD – *Investigation of Immune-targeted Cancer Biomarkers in The Cancer Genome Atlas Datasets*
	17:10-17:25 Early Career Scientist Short Talk-VIII Umut Ekin, PhD- *Stimulation of Efferocytosis by Soluble Receptor*
	17:25-17:40 Early Career Scientist Short Talk-IX Assistant Prof. Dr. Bahriye KARAKAŞ KÖSE - *Novel Monoclonal Antibodies for Stratification of Lung Cancer*
17:40 – 18:00	**Closing Remarks**

GALEN MEDICINE DAYS-2024,

PRECISION MEDICINE IN HEALTH AND DISEASE INTERNATIONAL SYMPOSIUM

September 3-4, 2024, Izmir, Türkiye

Abstract book code	**Presentation title**	**Authors**
T01	*Precision Medicine in Oncology: From Impressive Progress to Emerging Challenges*	Prof. Dr. Dominique BELLET
T02	*Interplay of Stress Signaling in Cancer Cells and the Tumor Microenvironment - Implications for Immunotherapy*	Prof. Dr. Fahri SAATÇİOĞLU
T03	*Molecular Mechanisms of Drug Resistance in Hematological Malignancies*	Prof. Dr. Yusuf BARAN
T04	*Algorithms to Characterize Genomic Structural Variation Using High Throughput Sequencing Technologies*	Assoc. Prof. Dr. Can ALKAN
T05	*Integrative Proteo-Transcriptomic Characterization of Advanced Fibrosis in Chronic Liver Diseases Across Aetiologies*	Prof. Dr. Müjdat ZEYBEL

T06	*Precision Medicine for Rare Diseases*	Prof. Dr. Uğur ÖZBEK
T07	*Multi-Omics Approach to Brain Malformations*	Prof. Dr. Ahmet Okay ÇAĞLAYAN
T08	*Textile-Based Soft Robotics as Wearable Assistive Devices*	Assoc. Prof. Dr. Özgür ATALAY
T09	*Mechanisms of Rare Diseases (From Altered Genome Structure to Altered Function)*	Dr. Stefan DIMITROV
T10	*New Horizons in Medicine: Unveiling Novel Treatments for Protein-Losing Enteropathies*	Prof. Dr. Ahmet ÖZEN
T11	*Comprehensive Genomic Profiling for Precision Medicine in Cancer*	Prof. Dr. Cengiz YAKICIER
T12	*Applications of Artificial Intelligence in Precision Oncology: Digital Pathology and Multiomics*	Prof. Dr. Erdener ÖZER

T13	*Ribosome Alterations in Cancer: Impact on Translational Control and Tumorigenesis*	Dr. Jean-Jacques DIAZ
T14	*Adjustment of Circadian Clock for Good Health*	Prof. Dr. Nuri ÖZTÜRK
T15	*Training the immune system as a universal platform for cancer prevention*	Prof. Dr. Haval SHIRWAN
T16	*Preclinical Development of Novel Natural Killer Cell-based Cancer Immunotherapies*	Assistant Prof. Dr. Tolga SÜTLÜ
C1	*Personalized Medicine in MET and CDK6 overexpressing rare tumor*	Dr. Aybike Erdoğan Atay
C2	*Remarkable response to dual immunotherapy and MEK inhibitor combination in a TERT and RASA-1-mutant hepatocholangiocarcinoma*	Dr Ünal Metin Tokat
C3	*Exceptional response in a mRCC patient through precision-guided treatment involving immunotherapy rechallenge with temsirolimus and bevacizumab*	MSc Ashkan Adibi

C4	*Transthyretin (TTR) cardiac amyloidosis, early findings*	Assistant Prof Dr Erhan Pariltay
C5	*A patient with SMA: How RNA_based diagnosis works*	Prof Dr Ahmet Okay Çağlayan
C6	*Two Cases and Prenatal Diagnosis of a rare UFM1 associated hypomyelinating leukodystrophy*	Assoc. Prof Dr Berk Özyılmaz
C7	*Adult onset GalC Krabbe disease case*	Dr. Ravza Nur Yıldırım
C8	*Molecular Diagnosis Enables Effective Treatment in a Case of Late-Onset NAXE Deficiency*	Doç. Dr. Esra Işık
ST01	*Axiom PangenomiX Array - A Powerhouse for Predictive Genomics Research*	Shantanu Kaushikkar, Lynda Kassama Demir
ST02	*The Effect of Ligand and Nanobody Binding Dynamics on EGFR/CXCR4 Dimerisation*	Dehan Comez, Stephanie Anbuhl, Raimond Heukers, Marco Siderius, Martine J. Smit, Laura E. Kilpatrick, Stephen J. Hill

ST03	*Comparative Identification of Genetic Variants*	Assistant Prof. Dr. Mehmet BAYSAN
ST04	*Single-Cell RNA sequencing reveals blocking GPRC5A downregulates important cancer related genes in Mesothelioma Cell Line*	Tunç TUNCEL, Salih Berkay BERKCAN, Tuba ÖZBAY, Ayhan DEMİR
ST05	*Co-targeting DNA damage and Golgi stress induces mitochondrial apoptosis in ovarian cancer cells*	Yeliz Aka, Ozgur Kutuk, F. Belgin Atac
ST06	*TAp73β induces angiogenesis and metastasis in a zebrafish xenograft model in the Hepatocellular Carcinoma*	Miray Sevinin, Güneş Özhan, Evin İşcan
ST07	*Axl Expression is Induced During the Transition from Non-Muscle-Invasive to Muscle-Invasive Stage in Bladder Cancer*	Ozden Oz, Umut Ekin, Burcin Tuna, Ozan Bozkurt, Seda Eryigit Kokkoz, Asli Suner Karakulah, Mehmet Ozturk
ST08	*Comprehensive In Vivo Characterization of a Novel Rare Genetic Disease*	Seval Kılıç, Kerem Esmen, Tansu Bilge Köse, Melike Sever Bahçekapılı, Emine Eren Koçak, Şeyda Demir, Ayşe Semra Hız, Gökhan Karakülah, Mehmet Öztürk, Muhammed Kasım Diril
ST09	*Transcriptional and Molecular Drivers of Neuroendocrine Differentiation in Bladder Cancer*	Burcu Akman, Ahmet Bursalı, Mustafa Gürses, Aslı Suner, Gökhan Karakülah, Uğur Mungan, Kutsal Yörükoğlu, Serap Erkek Özhan

ST10	*AXL: A Key Player in DNA Damage Response and Epithelial-to-Mesenchymal Transition*	Tugce Batur, Ece Şule Zangur, Evin İşcan, Umut Ekin, Ayse Argundogan, Hani Alotaibi, Mehmet Ozturk
ST11	*Effects of F53 Hotspot Mutations on the Molecular Dynamics of MEK1 Protein and the Binding of its FDA-Approved Inhibitors*	Berkin Ersin Vural, Cihangir Yandım
ST12	*miR-181a-5p sensitizes Sorafenib resistance by attenuating the aggressive phenotype by the inverse correlation with Cav-1 in Hepatocellular Carcinoma*	Ezgi Bağırsakçı, Peyda Korhan, Yasemin Öztemur Islakoğlu, Neşe Atabey
ST13	*Investigation of Immune-targeted Cancer Biomarkers in The Cancer Genome Atlas Datasets*	Talip Zengin, Tuğba Önal-Süzek
ST14	*Stimulation of Efferocytosis by Soluble Receptor*	Umut Ekin, Asli Babayakali, Erhan Bal, Aybike Erdogan, Mehmet Ozturk
ST15	*Novel Monoclonal Antibodies for Stratification of Lung Cancer*	Bahriye Karakas Kose, Ozden Oz, Asli Babayakali, Erdogan, Sibel Kalyoncu, Asli Kurden-Pekmezci, Sila Kahyaoglu, Erhan Bal, Fatma Tokat, Umit Ince, Mehmet Ozturk
P01	*Exploring MET-CAV1 Fusion Transcript: A Novel Biomarker Candidate for Patient Stratification for Treatment Response in Hepatocellular Carcinoma*	Yasemin Öztemur Islakoğlu, Peyda Korhan, Ezgi Bağırsakçı Tepe, Barış Keleş, Leman Binokay, Gökhan Karakülah, Neşe Atabey

P02	*HGF Promoter DATE Region Length in Liver Stellate Cells as a Prognostic Biomarker in Hepatocellular Carcinoma Progression*	Gülhas Solmaz, Peyda Korhan, Neşe Atabey
P03	*Preliminary data: Genetic polymorphism of CYP2D6 (*2,*3,*4,*5,*6,*7,*10,*41) in patients with cardiovascular disease– a cohort study**	Selim KORTUNAY, İbrahim Etem DURAL, Ömer Faruk YILMAZ, Aylin KÖSELER, Mehmet Bilgehan PEKTAŞ
P04	*3D Cell Culture Models as a Platform for Studying Tumor Progression, Testing Treatment Responses, and Discovering Biomarkers*	Peyda Korhan, Ezgi Bağırsakçı, Yasemin Öztemur Islakoğlu, Neşe Atabey
P05	*Olfactor Receptor Gene OR2A4/7 As A Potential Biomarker in Colorectal Cancer*	Tutku Güler, Ece Çakıroğlu, Barış Keleş, Peyda Korhan, Yasemin Öztemur Islakoğlu,Şerif Şentürk, Neşe Atabey
P06	*Vitamin D Administration on a Non-Alcoholic Fatty Pancreatic Disease Animal Model*	Goksan Inci Durmaz, Duygu Aydemir, Burcu Gul, Betul Zorkaya, Nargiz Bayramova, Cigdem Bayram Gurel, Nuray Ulusu, Evrim Bayrak Komurcu, Fatma Kaya Dagistanli
P07	*Effects of Exogenous Albumin on the Growth and Migration of Human Hepatoma Cells*	Helin SAĞIR, Hani ALOTAIBI, Brian I. CARR
P08	*Induction of Multiple Sclerosis Model by Oral Cuprizone Administration and Evaluation of Cognitive Functions in Wistar Albino Rats*	Sule Aydin, Cansu Kilic Tatlici, Elif Erdogan Erden, Zeynep Gul Yazici, Dilek Burukoglu Donmez, Fatma Sultan Kilic

P09	*Olfactory Receptors as Regulators of Aggressive Phenotype in Colorectal Cancer*	Barış Keleş, Tutku Güler, Yasemin Öztemur Islakoğlu, Peyda Korhan, Gökhan Karakülah, Neşe Atabey
P10	*Investigation of ALCAM's Role In Glioblastoma Senescence*	Bora Deniz Yılmaz, Tuba Sena Oğurlu, Ayça Arslan Ergül
P11	*Bioinformatics Screening of Metabolic Gene Signature Associated with c-Met Over-Expression in Colorectal Adenocarcinoma to Discover Biomarkers and Target Genes*	Bilalcan Kaya, Yasemin Öztemur Islakoğlu, Barış Keleş, Tutku Güler, Neşe Atabey
P12	*Influence of Preanalytical Factors on the Quality of Tissue Samples*	Sanem Tercan-Avci, Çağlar Çelebi, Beste Özkalay, Yeliz İnci, Ceren Ülker, Ece Uzun, Serap Birincioğlu, Neşe Atabey

Keynote Speech

GALENDAYS24-T01

Precision Medicine in Oncology: from Impressive Progress to Emerging Challenges

Prof. Dr. Dominique BELLET
Paris Descartes University, France

Abstract

This presentation will outline how fundamental achievements in cancer biology and tumor immunology, coupled with unprecedented technical improvements in gene sequencing and drug development, have converged to offer nowadays the compelling opportunity to design therapeutic approaches tailored on individual patients, namely precision medicine in oncology. Indeed, the identification of tumor molecular maps has guided the design of novel inhibitors that specifically target the altered genes and signaling pathways driving the malignant phenotypes. Molecular biomarkers should therefore be used to drive the development of effective targeted therapies and to tailor therapy to individual patients. In parallel, the discovery of immune checkpoints has spurred the development of immune checkpoints inhibitors. Composite biomarkers are used to tailor immunotherapeutic approaches to individual cancers. Despite remarkable progress, both branches of precision oncology, targeted therapy and immunotherapy, still face many challenges, including low response rates, drug resistance, high costs or a dearth of education and awareness among healthcare professionals and the public regarding this rapidly evolving field. Various strategies will be proposed to enhance the implementation of precision oncology, thereby benefiting a greater number of cancer patients.

Biography

Dr. Dominique Bellet Pharm.D., M.D., Ph.D. has been a member of the faculty at the Massachusetts General Hospital and a member of the Faculty at Harvard Medical School. He has been appointed as a physician in charge of clinical and research laboratories at Institut Gustave Roussy until 2006 before joining Institut Curie. In 1987, he was appointed Professor of Immunology at Paris Cité University. His main interest is the translation of fundamental research discoveries into clinical applications and novel therapeutics. He specializes in cancer

diagnosis or monitoring using tumor markers and in cancer treatments through immunotherapy. Since 2014, he has been a co-founder involved in the development of three educational websites dedicated to innovative cancer treatments: NetCancer, CancerConsult and AccessTrial.

Invited Talks

GALENDAYS24-T02

Interplay of ER Stress Signaling in Cancer Cells and the Tumor Microenvironment – Implications For Cancer Immunotherapy

Prof. Dr. Fahri SAATCIOGLU
Oslo University, Norway

Abstract

Endoplasmic reticulum (ER) is the largest organelle in the cell. Among others, it functions as a stress sensor responding to increased demand for protein production and folding under both physiological and pathological conditions. ER stress initiates a series of coping mechanisms that are termed the unfolded protein response (UPR), which restructures the cellular transcriptional, translational, and degradation pathways to help resolve the defects in protein folding. ER stress is common in cancer cells due to increased protein synthesis necessary for their deregulated proliferation, chromosomal abnormalities affecting membrane and secreted protein production, limited nutrient availability, and low oxygen tension; thus, UPR is often hijacked by tumor cells for their survival. However, how activation of UPR in cancer cells may affect the tumor microenvironment (TME) remain largely unexplored.

We investigated the potential role of IRE1α-XBP1s signaling, one of three canonical UPR pathways, on modulation of TME dynamics in prostate cancer (PCa). We found that IRE1α is increased in PCa patients; consistently, genetic or pharmacological inhibition of IRE1α-XBP1s signaling in multiple syngeneic or orthotopic mouse PCa models dramatically reduced tumor growth. Multiomics analysis of tumor samples upon IRE1α deletion in cancer cells showed significantly potentiated interferon (IFN) response and activation of immune system related pathways in the TME. Single-cell RNA sequencing (scRNA-seq) analysis revealed that the abundance of immunosuppressive cells, such as tumor-associated macrophages (TAMs), pericytes, cancer-associated fibroblasts, and T regulatory cells were markedly reduced in the IRE1α deficient tumors. Analysis of differentially expressed genes in various cell types in the TME demonstrated that expression signatures associated with IFN responses were significantly

enriched in TAMs, cancer cells, and dendritic cells. In addition, a novel scRNA-seq derived TAM gene signature is strongly associated with poor PCa survival. Importantly, IRE1α inhibition by the small molecule MKC8866 (ORIN1001) that is in clinical trials significantly enhanced anti-PD-1 checkpoint inhibitor therapy in syngeneic mouse PCa models. Our findings indicate that IRE1α not only promotes cancer cell growth and survival, but it also strongly inhibits anti-tumor immunity in the PCa TME.

Biography

Fahri Saatçıoğlu is a professor in molecular and cell biology at the Department of Biosciences, University of Oslo, also a senior scientist at the Institute for Cancer Genetics and Informatics, Oslo University Hospital. He focuses on basic molecular and cell biology of cancer cells, in particular prostate cancer, including translational aspects based on these findings. His laboratory studies the impact of steroid hormones and stress signaling pathways on cellular homeostasis and their role in disease states. Prof. Saatçıoğlu has been interested in the biological and physiological mechanisms of lifestyle interventions, such as yoga, breathing exercises, and meditation, also conducts research in these areas with the aim to contribute to the scientific basis of stress management for health and wellness.

GALENDAYS24-T03

Molecular Mechanisms of Drug Resistance in Hematological Malignancies

Prof. Dr. Yusuf BARAN
Izmir Institute of Technology, Türkiye

Abstract

Chemotherapy is the most widely used treatment strategy for cancer, which is the second reason for human deaths after heart-related diseases. However, cellular resistance mechanisms developed by cancer cells and tissues in the beginning or proceeding times to apply anticancer agents is a significant problem preventing successful therapy. Resistance cancer cells develop to structurally and functionally different cytotoxic agents is called multi-drug resistance.

Drug resistance mechanisms have different molecular genetics and biochemical reasons depending on the applied drug and the type of cancer. Secondary genetic alterations and disorders in cancer cells may also result in drug resistance. That is why it is vital to study and consider all signaling pathways in multidrug resistance of cancer.

Multidrug resistance occurs via many unrelated mechanisms, such as overexpression of energy-dependent efflux proteins, decrease in uptake of the agents, increase or alteration in drug targets, alterations in cell cycle checkpoints, inactivation of the agents, compartmentalization of the agents, inhibition of apoptosis, increases in DNA repair mechanisms, problems related with drug metabolism and aberrant metabolism of bioactive sphingolipids. Exact elucidation of resistance mechanisms and molecular and biochemical approaches to overcome multidrug resistance have been a significant goal in cancer research. In this talk, I will explain the mechanisms contributing to multidrug resistance in cancer chemotherapy and touch on the approaches for reversing the resistance.

Biography

Prof. Dr. Yusuf Baran is a professor in Molecular Biology and Genetics. Dr. Baran has been serving as the President of İzmir Institute of Technology and Chairman of the Board of Directors of the İzmir Technology Development Zone since October 28th, 18.

Dr. Yusuf Baran has been involved in more than 40 scientific research projects supported by national and international organizations. He has more than 500 published scientific articles and conference papers and has published one book. He also presented at over 700 national and international conferences, meetings and forums on science, technology and diplomacy.

Dr. Baran's scientific achievements have been recognized by national and international accredited institutions, and he has received more than 100 awards; including "2013 Young Scientist" award by the World Economic Forum (WEF), "Most Outstanding Young Scientific Leader of Turkey" by International Young Leaders and Entrepreneurs in 2014, "Outstanding Young Scientist Award" by the Turkish Pharmaceutical Academy in 2017, "Science Leader of the Year Award" by Turkey Innovation and Outstanding Success Awards, "Science Award" by Hacettepe University and "Innovative and Enterpreneur Acamidican Award" by Aegean Economic Development Association in 2021, and "Science Diplomacy Award" by The World Academy of Sciences in 2023.

In his research, Dr. Baran focuses on the molecular biology of cancer, science policies and science diplomacy.

GALENDAYS24-T04

Algorithms to Characterize Genomic Structural Variation Using High Throughput Sequencing Technologies

Assoc. Prof. Dr. Can ALKAN
Dept. Computer Engineering, Bilkent University, Ankara, Türkiye

Abstract

Structural variation, in the broadest sense, is defined as the genomic changes among individuals that are not single nucleotide variants. Rapid computational methods are needed to comprehensively detect and characterize specific classes of structural variation using next-gen sequencing technology. We have developed a suite of tools using a set of aligners and algorithms focused on the characterization of structural variants that have been more difficult to assay, including complex rearrangements. In this talk I will summarize our work in developing combinatorial algorithms to discover of structural variation using high throughput sequencing technologies. The algorithms we have developed will provide a much needed step towards a highly reliable and comprehensive structural variation discovery framework, which, in turn will enable genomics researchers to better understand the variations in the genomes of newly sequenced human individuals including patient genomes.

Biography

Can Alkan is currently an Associate Professor at the Department of Computer Engineering at Bilkent University since January 2012. He graduated from Bilkent University Dept. of Computer Engineering in 2000, and received his Ph.D. in Computer Science from Case Western Reserve University in 2005 after a brief visit to Simon Fraser University. During his Ph.D. he worked on the evolution of centromeric DNA, RNA-RNA interaction prediction and RNA folding problems. He then joined the Department of Genome Sciences of the University of Washington as a postdoctoral fellow. Since then his work includes computational prediction of human genomic structural variation, and characterization of segmental duplications and copy-number polymorphisms using high throughput sequencing data, and acceleration of genome analysis through hardware/software co-development.

GALENDAYS24-T05

Integrative Proteo-Transcriptomic Characterization of Advanced Fibrosis in Chronic Liver Diseases Across Aetiologies

Prof. Dr. Müjdat ZEYBEL
Koç University, Türkiye

Abstract:
Chronic hepatic injury and inflammation can lead to fibrosis, cirrhosis and may predispose to hepatocellular carcinoma. The molecular mechanisms that drive liver fibrosis and its associated carcinogenesis remain incompletely understood. In this study, we present an integrated proteo-transcriptomics approach to characterize both liver and plasma molecular pathophysiology associated with liver fibrosis in 330 individuals, including 40 healthy subjects and 290 patients with histologically characterized fibrosis resulting from chronic viral infection, alcohol consumption, and metabolic-dysfunction associated steatotic liver disease. We demonstrated the dysregulation of pathways related to extracellular matrix alterations, immune response, inflammation, and metabolism in advanced fibrotic liver independent of aetiology. Additionally, our analysis of peritumoral hepatic tissue identified transcription signatures associated with cell proliferation, survival, and inflammation in hepatocellular carcinoma carcinogenesis. We observe the plasma proteomes underwent extensive remodeling as liver fibrosis progresses and identified 132 circulating proteomics signatures for advanced fibrosis by integration with hepatic transcriptomics. Using machine learning models, we developed predictive models for advanced fibrosis and cirrhosis.

Biography

Prof Zeybel received his medical degree from Hacettepe University. Following the completion of internal medicine residency, he has been trained as gastroenterologist and started to work on epigenetic regulation of hepatic stellate cells and liver fibrosis at Newcastle University. Prof. Dr Zeybel continued his medical research career at Newcastle University by studying a Master of Research and Ph.D. on liver disease. He worked as an European Association for the Study of the Liver, Sheila Sherlock Physician Scientist Fellow on epigenetics and liver disease. He

worked as a Clinical Associate Professor of Hepatology at Nottingham University. His research topics include metabolic-dysfunction associated steatotic liver disease, epigenetic targets and treatment in chronic liver disease along with hepatocellular carcinoma.

GALENDAYS24-T06

Precision Medicine for Rare Diseases

Prof. Dr. Ugur OZBEK
Izmir Biomedicine and Genome Center-IBG , Türkiye

Precision medicine is revolutionizing the healthcare landscape by offering personalized treatments based on a patient's unique genetic, molecular, and environmental factors. This approach is particularly significant for rare diseases, which affect around 7% of the global population. These conditions often go underdiagnosed, with patients facing long diagnostic and therapeutic odysseys. Precision medicine, supported by genomic and molecular technologies, is proving essential in overcoming these challenges. Initiatives like the RareBoost project, funded by Horizon 2020 and based at the Izmir Biomedicine and Genome Center (IBG), aim to enhance research and clinical approaches to rare diseases in Turkey. RareBoost focuses on strengthening the infrastructure for rare disease research, fostering collaborations, and promoting public awareness, ultimately advancing the development of targeted therapies. Complementing these advances provides powerful research tools for modeling diseases and evaluating potential treatments. These techniques enable a more personalized therapeutic strategy, offering hope for more effective interventions. For instance, by testing drug responses in patient-derived cells, researchers can identify treatments targeting the unique molecular pathways of each patient's condition. Overall, precision medicine shifts the traditional "one-size-fits-all" model of healthcare to a more individualized and patient-centered approach, providing hope for better disease management and treatment strategies in the rare disease landscape By combining cutting-edge genomic research with collaborative platforms like RareBoost, the potential to create effective, patient-specific treatments becomes more achievable.

This project is funded by the European Union's Horizon2020 program (Grant nr: 952346).

Biography

Uğur Özbek received PhD degree on Cancer Genetics at Oncology Institute in 1995. In 1997 he became Associate Professor in Basic Oncology and in 2003 he became full Professor in Genetics. He worked as a faculty member at Istanbul University Institute of Experimental Medicine, Genetics. He became a consultant in Forensic Biology; a faculty member and director of Istanbul University Health Sciences Institute Genetics and Institute of Experimental Medicine. He worked at Acıbadem University, School of Medicine in 2016. He established Center for Rare and Undiagnosed Diseases at Acibadem University. He worked as Chair to the Horizon2020 at IBG in 2023. His research interests include delineation of the genetics and

molecular biological mechanisms underlying hereditary cancers/hematological malignancies and rare/undiagnosed diseases.

GALENDAYS24-T07

Multi-Omics Approach to Brain Malformations

Prof. Dr. Ahmet Okay CAGLAYAN
Dokuz Eylul University, Izmir, Türkiye

Abstract:

The term "omic" originates from the suffix "ome," commonly added to various fields of biological study, and refers to the examination of something in its entirety. Adopting a comprehensive molecular approach by combining analyses of various 'omic' types could enhance diagnostic outcomes, deepen our understanding of phenotypic variability and disease progression, and highlight opportunities for drug repurposing by identifying new therapeutic targets. A significant increase is seen the publication of multiomic studies of rare disease last ten years.

Malformation of Cortical Development" (MCD) was first introduced in 1996 to describe a group of disorders arising from disruptions in the normal development of the human cerebral cortex. MCDs can be caused by both monogenic and polygenic factors.

Examining expression outliers in conjunction with genomic variants and patient phenotypes offers a powerful approach for identifying promising candidate variants for clinical interpretation. Through weighted gene co-expression network analysis, we have discovered novel genes associated with structural brain malformations.

Biography:

Dr. Ahmet Okay Caglayan has dedicated almost two decades to life science research, with a primary focus on investigating structural brain malformations using next-generation sequencing technologies. Through his work, he has successfully identified rare and somatic genetic variants spanning a wide range of diseases.

Beyond his research endeavors, Dr. Caglayan has played pivotal roles within the scientific community. He has served on editorial boards, contributed as a reviewer for leading journals

and conferences, and received invitations to participate in program committees for both national and international workshops and conferences.

As an educator, Dr. Caglayan shares his expertise with undergraduate and postgraduate students, leading seminars, laboratory sessions, and lectures across various academic levels, including undergraduate, graduate, and medical school programs.

GALENDAYS24-T08

Textile-Based Soft Robotics as Wearable Assistive Devices

Assoc. Prof. Dr. Ozgur ATALAY
Istanbul Technical University, Türkiye

Abstract

Wearable soft robotic devices designed for rehabilitation and motion assistance have gained significant attention over the past decade due to their adaptable designs. Unlike rigid metal exoskeletons, soft robotics facilitate safer interactions between humans and machines. Utilizing elastomers and textiles presents an attractive, cost-effective, and flexible approach to constructing these systems. However, existing wearable solutions often suffer from bulkiness, unreliability, lack of scalability, and limited portability, hindering their practical use outside clinical settings and in daily life, where they are most needed.

The primary goal of the TEXWEAROTS project is to develop untethered, knitted soft robotic assistive devices that address these challenges related to reliability, mobility, sustainability, and integration. TEXWEAROTS aims to create a streamlined knitted robotic glove that eliminates bulky components while seamlessly integrating actuation, sensing, and self-powering capabilities. Drawing on my extensive experience in textile manufacturing, soft sensors, and soft robotics, we utilize cutting-edge digital machine knitting techniques to produce 3D actuator shells that incorporate these functionalities in a unified design. This approach will result in customizable, textile-based robotic devices that are both scalable and reliable.

TEXWEAROTS represents a convergence of textiles, electronics, robotics, and biomedical engineering. The innovative technologies and manufacturing techniques proposed will pave the way for practical applications of soft robotics in continuous assistance and rehabilitation, marking a significant advancement in the field.

Biography

Associate Professor Dr. Atalay obtained his master's and doctoral degrees in smart textiles from the University of Manchester in England. He conducted postdoctoral research in the area of soft robotic systems at Harvard University. He is awarded by Marie Curie Individual Fellowship in 2019 under the EU Horizon 2020 program. He received funding from the European Research Council (ERC) for his innovative "TEXWEAROTS" project. In this project, he is dedicated to developing a smart soft robotic glove as an assistive device, aiming to revolutionize the field of wearable technology. Currently, Dr. Atalay serves as an associate professor at Istanbul Technical University, where he continues to research in the interdisciplinary areas of smart textiles and soft robotics.

GALENDAYS24-T09

Mechanisms of Rare Diseases (From Altered Genome Structure to Altered Function)

Dr. Stefan DIMITROV
Institute of Molecular Biology, Sofia/Institute of Advanced Biosciences, Grenoble

Abstract:

Rare diseases are said to be rare when they affect one person in 2,000, i.e. more than 3 million French people and at least 30 millions Europeans. There are 7-8,000 rare diseases identified to date and the vast majority is from unknown origin. More than 90% of rare diseases are without treatment. Rare diseases are a major threat for human health and understanding of the molecular etiology of rare diseases is of primary need.

Here, I will initially shortly present some of our data on the mechanism of Rett Syndrome (RS), a very severe neuro-developmental rare disease. The genetic cause of the disease was defined as loss of function of *methyl CpG binding protein 2* (MeCP2). Nevertheless, the precise mechanism of how loss of function of this protein causes this devastating disease was not very clear. In contrast to the existing dogma claiming that MeCP2 binds to CpG conatining sequences, we observed that MeCP2 specifically recognizes and binds both *in vitro* and *in vivo* hydroxymethylated CA repeats. Moreover, we demonstrated a new function of MeCP2 as a long-range chromosome organiser, especially in chromatin domains associated with the nuclear lamina (LAD). Therefore, MeCP2, previously described as transcriptional repressor, also organizes 3D chromatin architecture, and Rett Syndrome is, indeed, an epigenetic disease.

Next, I will describe a novel work flow for deciphering the molecular etiology of rare diseases and the application of this workflow on analyzing the Rahman Syndrome (RMNS) molecular origin. RMNS is a recently described developmental disorder caused by frameshift mutations in linker histone H1.4, that produce a truncated C-terminal domain (CTD) with reduced positive charge. We found that the mutation induces nucleosome arrays to adopt a more extended, flexible conformation exhibiting phase separation behavior similar to those lacking H1.4. Molecular dynamics simulations supported by FRET analysis indicate that the mutated CTD recognizes a shorter length of linker DNA, resulting in a more open nucleosome

conformation. Correspondingly, the mutation substantially increases H1.4 mobility within cell nuclei. The combined data suggest that RS mutations alter gene expression during development by promoting a relaxed chromatin state.

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Biography:

Stefan Dimitrov graduated from the Faculty of Physics of the Sofia University Kliment Ohridsky. He got his Ph. D. in the Institute of Molecular Biology in Sofia and in the Institute of Molecular Biology in Moscow. He was a Director of Research (Full Professor equivalent) at the National Center for Scientific Research (CNRS) in France. Stefan Dimitrov worked in Izmir Biomedicine and Genomics Center (IBG) and served as IBG Scientific Director. Since 2024, Dr. Dimitrov is an European Research Area (ERA) Chair Holder in the Institute of Molecular Biology, Sofia. He is also Emeritus Director of Research at CNRS. His scientific interests are focused on the role of epigenetic factors in modelling the genome in both normal and disease conditions.

GALENDAYS24-T10

New Horizons in Medicine: Unveiling Novel Treatments for Protein-Losing Enteropathies

Prof. Dr. Ahmet OZEN

Marmara Üniversitesi Tıp Fak., Çocuk Sağlığı ve Has ABD, Allerji- İmmünoloji BD Başk., Türkiye

İstanbul Jeffrey Modell Foundation Primer Immün Yetmezlik Tanı ve Tedavi Merkezi Direktörü

Abstract

Protein-losing enteropathies (PLEs) represent a rare and challenging group of disorders characterized by the excessive loss of serum proteins into the gastrointestinal tract, leading to complications such as edema, immunodeficiency, and malnutrition. Among the most severe forms of PLE is CHAPLE (CD55 deficiency with hyperactivation of complement, angiopathic thrombosis, and protein-losing enteropathy) disease, a condition resulting from mutations in the CD55 gene. This disorder presents with significant gastrointestinal and systemic complications, including a heightened risk of life-threatening thrombosis. Recent advances in understanding the pathophysiology of CHAPLE have paved the way for targeted complement-inhibitory therapies, offering hope to patients suffering from this debilitating condition. This presentation will explore the development and clinical implications of two groundbreaking therapies: Eculizumab and Pozelimab, both of which target the complement system to reduce protein loss and alleviate other clinical symptoms. We will discuss the rapid improvements in patients' quality of life, physical function, and long-term disease management resulting from these treatments. Additionally, the session will examine the challenges, including the risk of meningococcal infections, the need for continuous treatment, and the long-term efficacy of these interventions. As we delve into the next phase of PLE research, we will explore new therapeutic strategies, including RNAi therapeutics and the role of supportive care such as dietary modifications and immunoglobulin replacement therapy. Together, these approaches mark the beginning of a new era in the treatment of protein-losing enteropathies, offering tangible hope for a cure.

Biography

Dr. Ahmet Özen, M.D., who is the Director of the Istanbul Jeffrey Modell Foundation Center, is also a Professor and Chief of the Pediatric Allergy and Immunology Division at Marmara University. Graduating from Marmara University School of Medicine, he completed his residency at Yeditepe University in the field of Allergy and Immunology. He got postdoctoral fellowship in NIH. Dr. Özen established a national network for monogenic Inflammatory Bowel Disease (mIBD) in Türkiye. His discovery of CHAPLE disease has paved the way for FDA's approval of pozelimab for patients with CHAPLE disease. He is the founder of the Marmara University Işıl Berat Barlan Center for Translational Medicine and contributes to the NIAID-MENAT Strategy for Enhanced Research Engagement Program.

GALENDAYS24- T11

Comprehensive Genomic Profiling for Precision Medicine in Cancer

M. Cengiz Yakıcıer MD. PhD.
AQUARIUS NPG Cancer Genomics Center/ Istanbul

Abstract

Precision medicine is transforming the cancer care by delivering the right therapy to the right patient, at the right dose, and at the right time. Cancer is fundamentally a genetic disease, driven by alterations in the DNA that promote uncontrolled growth and invasion. Comprehensive genomic profiling (CGP) plays a pivotal role in identifying these genetic alterations—such as mutations, copy number variations (CNV), gene fusions, and microsatellite instability (MSI)—that fuel cancer development. By leveraging this molecular information, oncologists can select targeted therapies that align with a patient's specific tumor profile, enabling more effective and personalized treatment.

One of the major challenges in treating cancers, such as lung, breast, and colorectal cancers, is their inherent inter- and intra-tumor heterogeneity. Tumors are not static; they comprise a diverse collection of genetically distinct cells. This variability within and between tumors complicates treatment, as different regions of the tumor may harbor distinct mutations, leading to variable responses to therapy. Moreover, as the cancer evolves, selective pressures from treatments can lead to the survival of resistant cell populations, which may become dominant over time and contribute to disease progression.

As cancers are treated with targeted therapies, resistant clones of cells—those that have adapted or acquired new mutations—can emerge, leading to therapeutic failure. Liquid biopsy (LB), which analyzes circulating tumor DNA (ctDNA) shed into the bloodstream, allows for real-time monitoring of these genetic changes. By detecting evolving resistance mechanisms early, ctDNA analysis enables timely adjustments to treatment, ensuring that therapy remains effective as the tumor adapts.

A real-world example illustrates the power of precision medicine in managing tumor evolution. In a melanoma case characterized by a high tumor mutational burden and a BRAF V600E

mutation, CGP initially guided the use of immunotherapy. This resulted in temporary disease stabilization, but the tumor eventually progressed. LB revealed an increased ctDNA BRAF mutation level. In response, treatment was shifted to BRAF inhibitors, leading to a significant and sustained clinical response. This case highlights the ability of precision medicine to dynamically adapt treatment strategies based on tumor evolution and heterogeneity.

The integration of CGP and LB in oncology reshapes cancer treatment by addressing these complexities. These tools allow for personalized, adaptive treatment approaches that account for genetic diversity within tumors and the ongoing evolution of resistant cell populations.
This review explores the impact of CGP and ctDNA analysis on cancer management, the challenges of tumor heterogeneity and resistance, and future directions for improving therapeutic decision-making.

Biography

Prof. M. Cengiz YAKICIER is a graduate of İstanbul University Cerrahpaşa Medical School. He completed his residency at Lyon I University Medical School, Department of Nuclear Medicine. He did PhD in molecular biology and genetics from University Lyon. He worked as a post doc at the Massachusettes General Hospital, Harvard, INSERM and subsequently in Bilkent as an assistant professor. He joined Dep. of Medical Biology at ACIBADEM University as Associate Professor, also responsible for the ACIBADEM Genetic Diagnosis Center. He was appointed as founder Dean of ACIBADEM University Faculty of Arts and Sciences. He was head of the ACIBADEM molecular pathology laboratory. He founded NPG AQUARIUS Genomics which performs genomic profiling and monitoring tests on cancer patients.

GALENDAYS24-T12

Applications of Artificial Intelligence in Precision Oncology: Digital Pathology and Multiomics

Prof. Dr. Erdener ÖZER
Izmir Tinaztepe University , Türkiye,
Sidra Medicine, Doha, Qatar

Introduction

Precision oncology represents a paradigm shift in cancer treatment, moving away from the "one size-fits-all" approach to personalized, patient-centered care. This strategy aims to tailor cancer therapies based on the individual characteristics of a patient's tumor, considering genetic, molecular, and clinical data. With the surge in data generation through advanced technologies such as digital pathology and multiomics, oncologists now face the challenge of analyzing and interpreting vast amounts of complex information. Artificial intelligence (AI), particularly machine learning (ML) and deep learning (DL), has emerged as a critical tool for handling and interpreting this data, driving significant advancements in precision oncology.

Digital pathology and multiomics are two key pillars that, when combined with AI, offer new opportunities for improving cancer diagnosis, prognosis, and treatment. Digital pathology involves the digitization of histopathological slides, which are then analyzed using AI-powered algorithms to automate the identification of cancerous cells and tissue structures. Multiomics encompasses a range of high-throughput techniques—such as genomics, transcriptomics, proteomics, and metabolomics—that generate large datasets from a single biological sample. AI's ability to process and integrate these diverse datasets enables the identification of molecular patterns, potential therapeutic targets, and personalized treatment strategies. In this paper, we explore the specific applications of AI in precision oncology through digital pathology and multiomics, highlighting the potential benefits and current limitations of these technologies.

AI in Digital Pathology: Transforming Cancer Diagnosis and Prognosis

Digital pathology has revolutionized the field of pathology by enabling the conversion of traditional glass slides into high-resolution digital images. The integration of AI, particularly deep learning models, into digital pathology workflows allows for the automated analysis of these images, significantly improving diagnostic accuracy, speed, and reproducibility.

1. Automated Image Analysis for Cancer Detection

One of the most significant applications of AI in digital pathology is the automated detection of cancerous tissues. Traditional cancer diagnosis relies on the manual interpretation of histological slides by pathologists, which is time-consuming and prone to interobserver variability. AI algorithms trained on vast datasets of annotated histopathological images can accurately classify tissue samples, identify cancerous regions, and detect specific tumor subtypes. For example, in
breast cancer, AI models have been developed to identify invasive and non-invasive cancerous cells with high accuracy. In prostate cancer, AI tools can automate the grading of Gleason scores, which are critical for determining the aggressiveness of the tumor and guiding treatment decisions.

By automating cancer detection, AI reduces the diagnostic burden on pathologists and improves the consistency of cancer diagnosis. Additionally, AI-powered image analysis can detect subtle morphological features in tissue samples that may be missed by the human eye, enabling earlier detection of cancerous changes and potentially improving patient outcomes.

2. Quantification of Tumor Microenvironment

The tumor microenvironment (TME) plays a crucial role in cancer progression and response to therapy. AI algorithms in digital pathology have the potential to analyze not only the tumor cells themselves but also the surrounding microenvironment, including immune cells, blood vessels, and stromal components. By quantifying the spatial relationships between these components, AI can provide insights into the interaction between the tumor and its microenvironment. For instance, immune cell infiltration patterns can be assessed to predict

the likelihood of response to immunotherapies, a treatment strategy that harnesses the patient's immune system to fight cancer.

AI-based image analysis can also be used to quantify other features of the TME, such as tumor vascularization or the density of stromal cells, which may have prognostic value. By integrating data from both the tumor cells and the TME, AI models can offer a more comprehensive understanding of cancer biology and help guide personalized treatment decisions.

3. AI-Driven Prognostic Models

Beyond diagnosis, AI is also being applied to predict patient outcomes based on histopathological images. By training machine learning models on large datasets of patient outcomes and corresponding pathology images, AI can develop prognostic models that predict the likelihood of cancer recurrence, metastasis, or response to specific treatments. These models can help oncologists make more informed treatment decisions and identify patients who may benefit from more aggressive therapies or closer monitoring.

In lung cancer, for example, AI-driven models have been shown to predict survival outcomes based on the analysis of histopathological features. Similar approaches have been applied to other cancers, such as colorectal and breast cancer, where AI-based prognostic models can identify patients at higher risk of recurrence after surgery or adjuvant therapy.

AI in Multiomics: Decoding the Molecular Complexity of Cancer

Multiomics refers to the simultaneous analysis of multiple "omics" layers—such as genomics, transcriptomics, proteomics, and metabolomics—to provide a comprehensive view of biological systems. In the context of cancer, multiomics data can reveal the molecular heterogeneity of
tumors, identify potential therapeutic targets, and guide personalized treatment strategies. AI's ability to analyze and integrate large-scale multiomics data is driving new discoveries in cancer biology and improving the precision of oncology care.

1. Genomics and AI: Identifying Mutations and Therapeutic Targets

Genomics, the study of the entire DNA sequence of an organism, has been central to the development of precision oncology. AI has been instrumental in analyzing genomic data to identify somatic mutations, copy number variations, and other genetic alterations that drive cancer development. AI algorithms can process vast amounts of genomic data, identifying patterns of mutations across different cancer types and uncovering potential therapeutic targets.

For example, AI models have been used to analyze genomic data from The Cancer Genome Atlas (TCGA) to identify novel driver mutations and predict patient responses to targeted therapies. These models can also predict the likelihood of resistance to certain drugs based on the presence of specific genetic alterations. AI's ability to rapidly process and analyze genomic data accelerates the identification of new biomarkers and therapeutic targets, enabling the development of personalized treatment strategies.

2. Transcriptomics: Unraveling Gene Expression Patterns

Transcriptomics focuses on the study of RNA transcripts, providing insights into gene expression patterns in cancer cells. AI can analyze transcriptomic data to identify dysregulated pathways, predict patient outcomes, and guide therapeutic decisions. By integrating transcriptomic data with other omics layers, AI can reveal how genetic alterations affect gene expression and contribute to cancer progression.

AI has been applied to analyze single-cell RNA sequencing (scRNA-seq) data, enabling the identification of rare cell populations within tumors and the characterization of intratumoral heterogeneity. This information is critical for understanding the evolutionary dynamics of tumors and identifying potential therapeutic vulnerabilities. Additionally, AI models can predict patient responses to immunotherapies by analyzing the expression of immune-related genes in tumor samples.

3. Proteomics and Metabolomics: Uncovering Functional Biomarkers

Proteomics, the study of the entire set of proteins produced by a cell or tissue, and metabolomics, the study of metabolites, provide functional insights into cancer biology that cannot be captured by genomics or transcriptomics alone. AI models are being developed to

analyze proteomic and metabolomic data, identifying biomarkers associated with cancer progression and therapeutic response.

For instance, AI has been used to analyze mass spectrometry-based proteomic data to identify protein signatures that predict patient outcomes in various cancers. Similarly, AI-driven analysis of
metabolomic data has revealed metabolic alterations that contribute to cancer growth and survival, providing new avenues for therapeutic intervention.

4. Integration of Multiomics Data

One of the most exciting applications of AI in precision oncology is its ability to integrate multiomics data to provide a holistic view of cancer biology. By combining genomic, transcriptomic, proteomic, and metabolomic data, AI models can identify complex molecular interactions that drive cancer development and progression. This integrative approach enables the discovery of new biomarkers, therapeutic targets, and resistance mechanisms that may not be apparent when analyzing individual omics layers in isolation.

AI-driven multiomics integration has been used to stratify patients into molecular subtypes, identify novel drug targets, and predict treatment responses. In breast cancer, for example, AI models have integrated genomic, transcriptomic, and proteomic data to identify distinct molecular subtypes with different prognostic and therapeutic implications. This integrative approach enables more precise treatment strategies that are tailored to the unique molecular profile of each tumors.

Challenges and Future Directions

While the applications of AI in digital pathology and multiomics offer tremendous potential, several challenges remain. One of the primary challenges is the need for large, well-annotated datasets to train AI models. The heterogeneity of cancer and the variability in data quality across institutions can also affect the performance of AI models. Additionally, the integration of multiomics data requires sophisticated algorithms that can handle the complexity and dimensionality of these datasets.

Another challenge is the need for explainable AI models that provide insights into how predictions are made. Clinicians need to understand the rationale behind AI-driven decisions to trust and effectively use these tools in clinical practice. As AI continues to evolve, efforts to develop transparent, interpretable models will be critical for its successful integration into oncology care.

Conclusion

AI is transforming the field of precision oncology by enabling the automated analysis of digital pathology images and multiomics data. In digital pathology, AI-powered tools are improving the accuracy, speed, and reproducibility of cancer diagnosis, while AI-driven analysis of multiomics data is uncovering new insights into the molecular mechanisms of cancer. By integrating these technologies, AI has the potential to revolutionize cancer care, leading to more personalized treatment strategies and improved patient outcomes. However, further researches are needed to overcome the current challenges and fully realize the potential of AI in precision oncology.

Biography

Dr. Erdener Ozer is the Division Chief of Anatomical Pathology, having joined Sidra Medicine in 2021. He is also a senior attending physician managing the digital pathology reporting service.

Prior to joining Sidra, Dr. Ozer worked at Dokuz Eylul University Hospital in Turkey as a consultant for over 20 years. He held a full-time professor position in the School of Medicine, where he taught medical students, and in the Institute of Health Sciences, where he supervised PhD candidates. He is currently an adjunct professor at Izmir Tinaztepe University School of Medicine.

Dr. Ozer also holds a PhD in Medical Biology and Genetics. He conducts research at Sidra Medicine and Research Center as a leading PI and co-PI. His special research interest focuses on the precision oncology of pediatric tumors, including neuroblastoma and pediatric brain

tumors. Dr. Ozer is particularly interested in pediatric cancer research focused on multiomics profiling, biomarker discovery, tumor microenvironment, and novel treatments like immunotherapy.

He regularly publishes in international scientific literature, with over 150 original papers and 9 textbooks to his name. He is a frequent presenter at many international pathology meetings and has been recognized with several awards, including the TUBİTAK Award.

GALENDAYS24-T13

Ribosome Alterations in Cancer: Impact on Translational Control and Tumorigenesis

Dr. Jean-Jacques DIAZ

"Ribosome, Translation and Cancer", Cancer Research Centre of Lyon (CRCL), UMR INSERM U1052 CNRS 5286, Centre Léon Bérard, Université Claude Bernard Lyon 1 (UCBL), 28 rue Laennec, 69373 Lyon cedex 08, France

Abstract:
Recent advances revealed unexpected capabilities of ribosome in controlling translation. The "specialized ribosome" concept proposes that depending on cellular and physiological contexts, ribosome variants are produced allowing differential translation of mRNAs subsets. Ribosomal RNA (rRNA) carry a ribozyme activity and are a source of ribosome heterogeneity. More than 200 chemical modifications, including 2'-O-ribose methylations have been identified so far that can be modulated individually thus opening up the possibility of numerous combinations and associated regulatory effects.

The potential role of rRNA heterogeneity in cancer will be discussed. The story starts with our discovery that modifications of rRNA methylation can occur during tumor initiation and progression (1,2). Modification of rRNA methylation pattern is associated with change in translational control of mRNAs encoding oncogenic proteins. We and others also revealed a novel facet of the tumor suppressor p53 protein, which can be now considered as a player of translational regulation through an unexpected mechanism involving the ribosome (3,4).

We developed approaches to decipher the role of rRNA methylation in controlling translation during tumorigenesis and during treatment with anti-cancer molecules such as 5-FU (5). Analyses of human samples series issued from different cancer types allowed identification of components involved in ribosome biogenesis and rRNA methylation as independent markers of poor prognosis (6-8). Interestingly, these components are involved either in the global or site-specific modulation of rRNA methylation pattern. Using bi-cistronic assays, *in vitro*

translation and translatome analysis, we show that modulation of expression of rRNA methylation components associated with cancer patients' outcome affect translational fidelity and translation initiation of subsets of mRNAs promoting tumorigenesis and escape to anti-cancer treatments (9-12).

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Biography

Jean-Jacques Diaz received his PhD in 1988 at the University of Lyon. He completed a postdoctoral fellowship at Kansas State University. In 1991 he joined the Medical University in Lyon as the group leader of the Ribosome, Translation and Cancer's team at the Cancer Research Centre of Lyon. He is specialized in the study of nucleolus, ribosomes and translational regulation in physiological and pathological conditions. He demonstrated for the first time that ribosomal RNA methylation is a key feature of cancer cells. He brought evidence of rRNA 2′-O-methylation plasticity and its role in the control of human ribosomes. He unraveled the integration of 5-fluorouracil within ribosomes favors cancer cell drug-tolerance. He is involved in clinical trials to evaluate anti-cancer molecules.

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GALENDAYS24-T14

Adjustment of Circadian Clock for Good Health

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Introduction

The idea of chronotherapy for cancer, developed in the 1970s, is based on the concept that intrinsic host rhythms cause differential tolerance to treatment. In its current form, it is a scientific discipline to improve therapeutic efficacy of treatment while limiting toxicity. However, limited studies based on chrono(radio/chemo)therapy have not produced fully consistent results. Even though there are possible explanations for this inconsistency, the mechanical studies are underway to ameliorate inconsistency problems in clinical trials by designing rational treatment regimens and to exploit those findings to target clock-interacting networks.

Material and Method

Since 10% of the transcriptome and 20-40% of the proteome are under the control of the circadian clock, the clock can be targeted in different contexts to improve therapeutic outcomes. Early studies have yielded some valuable findings, particularly with genetic targeting in animal models and later with genome editing tools in cell lines. More recently, data from genetic studies have been used to target clock proteins using small chemicals. In this talk, I summarize the related studies by mostly focusing on our works.

Results and Discussion

We showed that targeting the circadian clock can have different effects depending on the clock gene selected and the genetic background of the cells. In particular, targeting *Bmal1* gene can affect the differentiation state of cells, and its deletion has opposite effects on carcinogenesis. Similarly, interfering with another clock gene, *Cryptochrome*, using genetic tools or small chemicals delays carcinogenesis in the *p53*-null background. On the other hand, as the clock deteriorates with age, small molecules targeting the Clock:Bmal1 complex can improve clock function.

Conclusion

Modulation of the circadian clock or factors associated with the circadian clock offers opportunities in the treatment of different diseases. This raises the hypothesis that pharmacological modulation of the circadian machinery may be an effective treatment strategy or for improvement of current treatments in addition to chronotherapy.

Keywords: *chronotherapy, circadian clock, DNA damage response, drug screening*

Biography

Nuri Ozturk is graduated from Istanbul University. During his master and PhD, he studied with Prof. Mehmet Ozturk in Bilkent University about heterogeneity of hepatocellular malignant phenotype. He worked as a postdoc with Prof. Aziz Sancar at the University of North Carolina about the relationship between circadian clock and cancer and also the mechanism of the circadian clock reset by light. He worked as an Assistant Professor at the University of North Carolina. He focused on biochemical regulation of the core clock factors in mammals and Drosophila. He is working as an Assistant Professor at Gebze Technical University with the following research areas: the circadian clock and health, Interactome of the circadian clock and Light-dependent reactions of Drosophila circadian photoreceptor.

GALENDAYS24-T15

Training the immune system as a universal platform for cancer prevention

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Abstract

Immunotherapy has shown remarkable clinical efficacy and emerged as a standard of care for various types of cancer. Although several immunotherapeutic protocols have been approved for clinical use, significant challenges remain that include: i) limited patient response rates, ranging from 20-40% depending on the cancer type and protocol; ii) the risk of immune-related adverse events; iii) complex manufacturing and logistic issues; and iv) the high cost of treatment. The clinical efficacy of immunotherapy has provided unequivocal evidence that the immune system is a critical gatekeeper against cancer and can be trained for cancer prevention. Preventive vaccines against virally-driven cancers further reinforce this notion. However, over 80% of tumors are not caused by viruses; thus, developing preventive vaccines for these cancers remains a major challenge. This is primarily due to the unknown nature of antigens for a given tumor to formulate a vaccine. A prophylactic approach that does not require a tumor antigen will be transformative. We generated a novel recombinant form of agonist for CD137 immune checkpoint stimulator and demonstrated its robust immunoprevention efficacy as a single agent against various tumor types in preclinical models. Ongoing studies are aimed at deciphering the underlying mechanisms with the goal of discovering additional druggable pathways that could pave the way for a universal cancer prevention scheme for high-risk individuals.

Biography

Dr. Shirwan is Professor and Marvin Hall, MD Faculty Scholar in Diabetes, Department of Pediatrics and Molecular Microbiology and Immunology, Director, Division of Pediatric Research, Associate Director of Immunomodulation and Regenerative Medicine Program, Ellis Fischer Cancer Center, and Member of NextGen Precision Health Institute, University of

Missouri-Columbia, MO. Dr. Shirwan is an elected Fellow of the National Academy of Inventors and recipient of numerous awards. He earned a PhD in Molecular Biology-Biochemistry from the University of California in Santa Barbara followed by postdoctoral training at the California Institute of Technology in Pasadena. Before relocating to the University of Missouri-Columbia in 2020, he served on the faculty of various academic institutions in the United States. Dr. Shirwan with his long-term collaborator Dr. Esma S. Yolcu pioneered the concept of transient and positional display of immune ligands on biologic and non-biologic surfaces for targeted immunomodulation with application to transplantation, autoimmunity, and cancer immunotherapy /immunoprevention. He has been continuously funded by the NIH and other funding agencies. Dr. Shirwan is an inventor with 30 issued and 8 pending patents, founder/co-founder of 3 biotech startups, is widely published, has organized and lectured at numerous national/international conferences, and served on editorial boards of various scientific journals.

GALENDAYS24-T16

Preclinical Development of Novel Natural Killer Cell-based Cancer Immunotherapies

Assistant Prof. Dr. Tolga SÜTLÜ

Acıbadem Mehmet Ali Aydınlar University Dept. of Molecular Biology and Genetics

Abstract

Natural Killer (NK) cells are lymphocytes of the innate immune system that have an essential role in the immune response against tumors and virus-infected cells. The clinical potential of using NK cells for cancer immunotherapy faces many obstacles related to *ex vivo* expansion, genetic modification and antigen-specific retargeting of NK cells. Our research focuses on finding solutions to these obstacles and aims to bring *ex vivo* expanded and/or genetically modified NK cells closer to clinical application.

We have previously optimized GMP-compatible systems that have the capacity to expand polyclonal and highly cytotoxic NK cells using automated bioreactors. Furthermore, we have investigated the innate immune responses triggered in NK cells upon lentiviral genetic modification and developed novel methods for efficient *ex vivo* gene delivery. Our efforts have resulted in an optimized stimulation and genetic modification process for NK cells that greatly enhances lentiviral gene transfer. We have also evaluated different approaches to retarget NK cell cytotoxicity by genetic modification using NK cell activating receptors, chimeric antigen receptors (CARs) or T cell receptors (TCRs).

Taken together, our results demonstrate the feasibility of developing novel cancer immunotherapy protocols based on genetically modified NK cells.

Selected Publications:

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- **Sutlu T**, Gilljam M, Stellan B and Alici E. *Inhibition of intracellular anti-viral defense mechanisms augments lentiviral transduction of human natural killer cells: implications for gene therapy.* Human Gene Therapy. 2012 Oct;23(10):1090-100. doi: 10.1089/hum.2012.080.

Biography:

Dr. Tolga Sütlü received his B.Sc. in Biological Sciences and Bioengineering from Sabanci University and his Ph.D. in Medical Science from Karolinska Institutet. After his postdoctoral work in Karolinska University Hospital, he returned to Turkey to start his research at Sabanci University. He was a faculty member at the Department of Molecular Biology and Genetics of Boğaziçi University and became as an Assistant Professor position at Acibadem University. He focuses on cancer immunology, immunotherapy and also personalized medicine, with targeting of immune system cells to cancer tissue through *ex vivo* propagation and genetic modification. He is an executive board member at the Turkish Society of Immunology since 2016. He is a founding partner of the US-based immunotherapy company Vycellix.

Case Studies

GALENDAYS-C04

Transthyretin (*TTR*) Cardiac Amyloidosis, Early Findings

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Introduction

Transthyretin-related amyloidosis (ATTR amyloidosis) is a systemic condition caused by the misfolding and clumping of the transthyretin (TTR) protein. TTR plays a primary role in transporting thyroxine (T4) and retinol-binding protein. Amyloidosis occurs when amyloidogenic forms of TTR whether due to genetic mutations (hATTR-CM hereditary cardiomyopathy) or the aging process (wATTR-CM wild-type cardiomyopathy) accumulate in extracellular spaces and form amyloid fibrils, leading to dysfunction in various organs, particularly the heart, peripheral nervous system, gastrointestinal system, the kidneys and other organs. Symptoms can manifest as early as the 30s but are often noticed later in life. It has also been reported that carpal tunnel syndrome may be the first sign in these cases. It is important to diagnose ATTR-CM because Tafamidis is a specific *TTR* stabilizer approved for the treatment of cardiomyopathy associated with wild-type or hereditary transthyretin-mediated amyloidosis

Material and Method

In this study, we aim to present the cases that have been referred to our laboratory over the past year for cardiac amyloidosis. In the cases where peripheral blood samples were collected, the entire coding exons of the *TTR* gene were amplified and analyzed using next-generation sequencing (NGS).

Results and Discussion

Twenty-one cases with a diagnosis of cardiomyopathy were evaluated in terms of the *TTR* gene. Among these cases, a heterozygous c.325G>C (p.Glu109Gln) mutation was detected in only two cases (mother and daughter). This mutation has previously been identified in databases and reported as a pathogenic variant. Despite the initiation of treatment, the mother passed away in recent months.

Conclusion

Although ATTR amyloidosis is generally considered a rare disease that can be managed with medication to slow its progression, post-mortem studies have revealed that its occurrence is more common than previously anticipated. The late diagnosis of cases limits treatment options. Raising awareness about early diagnosis and treatment of ATTR is crucial for both patients and our colleagues.

Keywords: *Transthyretin, TTR, Amyloidosis, Cardiomyopathy, Tafamidis*

GALENDAYS24-05

A Patient with SMA: How RNA-based Diagnosis Works

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Abstract

Accurately identifying causal variants in rare diseases is crucial, as it not only offers patients a genetic diagnosis and concludes years of diagnostic uncertainty, but also enhances prognosis accuracy, aids in family risk assessment, and fosters research into new therapeutic approaches. Next-generation sequencing (NGS) technologies, including gene panels, whole exome sequencing (WES), and whole genome sequencing (WGS), are the most widely used approaches for diagnosing rare diseases. However, NGS technologies fail in up to 75% of cases, partly due to difficulties in detecting, prioritizing, and interpreting variants, particularly those in intronic regions. In silico tools used to predict the impact of variants on RNA expression and/or splicing outside the canonical splice sites remain imprecise, leading to these variants often being classified as variants of uncertain significance (VUSs) in clinical reports. Recently, RNA sequencing (RNA-Seq) has been successfully utilized for the genetic diagnosis of individuals with Mendelian disorders, either as a primary method or as part of a broader multi-omics approach. Studies have highlighted the effectiveness of RNA-Seq in improving diagnosis rates, with up to 36% of NGS-negative patients being diagnosed across various diseases, sample sizes, and tissue types. As a novel approach, transcriptomics via RNA-Seq aims to enhance diagnostic yield in rare diseases by enabling the analysis of the entire

transcriptome (~12,800 transcripts) in a single run. RNA-Seq technology allows for the detection of transcriptome defects, which are functional consequences of harmful variants that typically cannot be predicted from WES/WGS data alone. It facilitates the identification of abnormal gene expression levels between control and experimental samples, uncovers allele-specific expression, and provides insights into abnormal splicing events. I want to highlight the advantages of RNA-Seq in diagnosing patients with rare diseases when additional data is needed following inconclusive clinical testing. This presentation provides a brief overview of a case where a VUS in the SMN1 gene was identified through an NGS targeted panel, and RNA-Seq data supplied functional evidence that led to the reclassification of the variant as likely pathogenic. As a result, the integration of RNA-Seq as a second-line clinical test should be considered when there is strong suspicion of a causative gene, but a molecular diagnosis remains elusive.

GALENDAYS24-C06

Two cases and prenatal diagnosis of a rare UFM1 associated Hypomyelinating Leukodystrophy

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Introduction

Whole exome sequencing (WES) is a powerful diagnostic tool in previously undiagnosed patients with suspected genetic disorders. In this presentation, the diagnosis of 2 cases with WES, which were previously investigated with various tests but no specific diagnosis could be reached, and the prenatal diagnosis process for the ongoing pregnancies of the expectant mothers are presented.

Material and Method

Case I was a 2 year-old boy with seizures, laryngomalacia, microcephaly and developmental delay. Case II was a 1 year-old girl with spasticty, microcephaly and developmental delay. Both patients were investigated with WES analysis.

Results and Discussion

Even though there was no consanguinity between either the families or the parents, in both of the patients, a Homozygous c.-273_-271del variant was detected in UFM1 gene (ENST00000368300.4). UFM1 gene causes Hypomyelinating leukodystrophy-14 (HLD14) with autosomal recessive inheritance. Since this variant was detected in homozygous form, was registered as pathogenic in reliable databases, and explained the clinical findings of the patients, this variant was accepted as the
cause of the phenotype. In both families for the ongoing pregnancies invasive prenatal diagnosis were carried out with Sanger sequencing. The fetus of the family I was found to be a heterozygous carrier for the UFM1 gene c.-273_-271del variant. The pregnancy continued healthily and a healthy boy was born. The prenatal diagnosis process for the second family pregnancy has not yet been completed. When we investigated how the same pathogenic variant

was carried in these two families that were not related to each other, we found that both families came from the same ethnic origin (Romani/Gypsy). This variant detected in both of the families was previously reported in the literature to have a carrier frequency of up to 4.5% in this ethnic population.

Conclusion

WES is a powerful diagnostic tool in previously undiagnosed patients with suspected genetic disorders. Closed ethnic groups can provide surprising genetic data. Due to the high carrier frequency in the Romani/ Gypsy ethnic population, the Hypomyelinating leukodystrophy-14 (HLD14) due to UFM1 gene and especially the c.-273_-271del variant should be considered in hypotonic, microcephalic, and developmentally retarded children.

Keywords: WES, UFM1, Founder effect, Rare Diseases, mutation

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GALENDAYS24-C07

Adult Onset GALC Krabbe Disease Case

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Introduction

Krabbe disease is associated with biallelic deleterious mutations in the GALC gene and typically observed in an infantile-onset form with a lethal course. Fewer than 1% of cases are reported in adulthood. Due to the overlap of symptoms (e.g. vision loss, seizures, muscle weakness, spasticity, peripheral neuropathy, ataxia, cognitive decline, behavioral changes) with other clinical conditions, it is almost never considered as a primary diagnosis in these age groups. This presentation will focus on the diagnostic odyssey of a patient who began experiencing symptoms, such as migratory pain in the torso, arms, and legs, at the age of 35. The patient's electromyography (EMG) demonstrated progressive findings consistent with demyelinating sensorimotor polyneuropathy. He was followed with preliminary diagnoses of familial amyloid polyneuropathy (FAP), Charcot-Marie-Tooth disease (CMT), multiple sclerosis (MS), and amyotrophic lateral sclerosis (ALS), and genetic analyses related to several of these conditions were conducted. This presentation will emphasize the precision medicine approaches employed in the follow-up evaluation of a male patient diagnosed with Krabbe disease who also underwent liver transplantation due to chronic hepatitis B.

Material and Method

The patient's DNA sample isolated from peripheral venous blood was Sanger sequenced for the TTR gene to rule out FAP. The clinical findings exhibited heterogeneity consistent with CMT disease. Therefore, multiplex ligation-dependent probe amplification (MLPA) was performed on the PMP22 gene, which revealed no copy number variations. Throughout this process, the patient did not receive a definitive diagnosis and exhibited clinical progression, including difficulties in climbing stairs, speech impairment, and progressive muscle weakness. Subsequently, whole exome sequencing (WES) was performed on the Illumina platform using the Twist Human Comprehensive Exome kit, based on a preliminary diagnosis of motor neuropathy/ALS. WES analysis identified two pathogenic variants in the GALC gene: NG_011853.3(NM_000153.4):c.[857G>A];[1623G>A]. These variants were confirmed through Sanger sequencing. Parental segregation analysis indicated that the alleles were in a trans configuration with respect to each other. Ultimately, the patient was diagnosed with Krabbe disease at the age of 53, a condition that is exceptionally rare in this age group.

Results and Discussion

Late-onset Krabbe disease is a condition for which there is currently no effective treatment. As such, it is essential to discuss management strategies with a multidisciplinary team, including specialists in medical genetics, metabolism, endocrinology, neurology, and gastroenterology to ensure holistic care and optimal patient outcomes. One of the differential diagnoses for this patient was MS, leading to the administration of intravenous immunoglobulin (IVIG) therapy over a period of 15 years. However, the absence of a significant clinical response to this treatment has led to a reconsideration of the diagnosis. To prevent the progression of neuropathy, hematopoietic stem cell transplantation (HSCT) might be considered a potential therapeutic option. However, this patient has a unique clinical scenario due to having undergone liver transplantation following chronic hepatitis B infection. Consequently, the suitability and feasibility of HSCT as a treatment option in this specific context remain subjects of ongoing evaluation. Family segregation analysis was also performed. The patient’s spouse, who is his first cousin, was found to be a carrier. Additionally, it was determined that two of

his siblings are presymptomatic individuals. Comprehensive genetic counseling specifically targeted at these individuals was provided.

Conclusion

In the context of hereditary diseases, the importance of individualized approaches is significantly heightened. This is primarily because distinct therapeutic or preventive strategies may be required for the same condition, even within the same family. This case illustrates the application of diverse personalized consultations among family members who share the same genetic variant, despite have a uniform disease whether they are symptomatic or presymptomatic.

Keywords: Krabbe Disease, Late-Onset, GALC, Whole Exome Sequencing, Preventive Medicine

GALENDAYS24-C08

Molecular Diagnosis Enables Effective Treatment in a Case of Late-Onset NAXE Deficiency

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Background: Biallelic pathogenic variants in *NAXE* gene cause a severe neurometabolicdisorder known as "Progressive Encephalopathy with Early-Onset, Brain Edema, and/or Leukoencephalopathy" (PEBEL1). This disease typically begins in childhood and is characterized by rapid clinical deterioration and death, triggered by events that increase intracellular stress, such as trauma or febrile illness. For the first time, In 2020, Joanne Trinh et al. described two siblings presenting with the atypical clinical course of PEBEL1, which manifested in adulthood, and identified compound heterozygous variants in *NAXE* gene.

Case Report: A 16-year-old female patient presented to Ege University Pediatric Neurology Department with complaints of progressive imbalance and inability to walk, blurred vision, and backward contractions in the neck and back muscles, which had started 5-6 months earlier. Two to three days prior to these symptoms, she described flu-like symptoms and severe headaches. When the imbalance symptoms first appeared, the patient was evaluated at an external center, where all tests were normal, and she was referred to child psychiatry. It was noted that the symptoms began after an emotional trauma, and antidepressant medication was initiated, but without benefit. With the patient's imbalance and walking problems worsening over the last week, she was admitted to our department. There were no notable features in her medical and family history, except for parental consanguinity. While the patient's physical examination was normal, her neurological examination revealed dysarthria, bradykinesia, truncal dystonia, and pyramidal signs. The patient was followed up with preliminary diagnoses of juvenile parkinsonism and hereditary spastic paraplegia. Detailed metabolic screenings and tests for Wilson's disease, including copper and ceruloplasminlevels, were normal. Ophthalmologic consultation revealed no Kayser-Fleischer ring. Both cranial MRI and awake sleep EEG were normal. Baclofen and L-dopa were started as symptomatic treatments for spasticity and parkinsonism, and a significant clinical response was observed. Due to parental

consanguinity and the described neurodegenerative process, whole exome sequencing (WES) was planned, and the patient was discharged.

Approximately 1.5 months after the initial presentation, the patient was admitted to the hospital's intensive care unit with severe headaches, dysphagia, and speech impairment that began the day before. She presented with altered consciousness, loss of ambulation, and swallowing dysfunction. Glasgow Coma Scale was E4M3V2. Neurological examination revealed vertical nystagmus, pyramidal signs, and dystonia. Awake-sleep EEG performed due to encephalopathy showed normal background rhythm without epileptic abnormalities. Cranial MRI showed T2A hyperintensity (Figure 2A) and diffusion restriction (Figure 2B), more pronounced in the periphery of the bilateral ventricle-striate nuclei and caudate nucleus. Due to the clinical and radiological findings, the patient was diagnosed with suspected biotin-thiamine-responsive basal ganglia disease, and high-dose thiamine, biotin, and a mitochondrial cocktail were initiated. A lumbar puncture was performed to rule out possible autoimmune encephalitis, and the autoimmune encephalitis panel was sent. Pulse methylprednisolone therapy (1g/day for 5 days) was started. The patient's encephalopathy resolved within 24 hours of starting treatment. The autoimmune encephalitis panel, including dopamine-2 receptor antibody, was negative, so maintenance oral steroid therapy was discontinued. Thiamine and biotin treatments were continued.

Whole exome sequencing revealed a homozygous c.757G>A (p.Gly253Ser) variant in NAXE gene. This rare variant had been previously reported by Joanne Trinh et al. as responsible for the NAXE-associated clinical picture. In-silico analyses predicted that the variant disrupts protein function. Family segregation studies showed that both parents were heterozygous, however her two healthy brothers carried the same variant in a homozygous state. Based on all these data, the NAXE c.757G>A (p.Gly253Ser) variant was classified as likely pathogenic in accordance with ACMG criteria.

With the identification of *NAXE* variant through molecular analysis, treatment was continued with niacin and coenzyme Q10. The patient was discharged with full consciousness and independent mobility. She has been followed up for 22 months without sequelae or ataxia while continuing niacin and coenzyme Q10 treatment.

Discussion: In this family, the proband's clinical findings are consistent with the NAXE-associated adult-onset atypical phenotype, and the clinical response to niacin and coenzyme Q10 treatment supports the genotype-phenotype correlation. However, despite the fact that the

variant is homozygous in the proband's three adult siblings, no neurological symptoms are present. This suggests that the c.757G>A (p.Gly253Ser) variant only mildly affects NAXE gene function, and that the other three siblings may not have encountered the level of stress necessary for symptom onset.

Conclusion: Importantly, symptoms in patients with NAXE variants may improve with vitamin B3 and coenzyme Q administration. This case has been reported to emphasize the invaluable impact of molecular diagnosis in selecting effective treatment.

Short Talks

GALENDAYS14-ST01

Axiom PangenomiX Array - A Powerhouse for Predictive Genomics Research

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Introduction

Predictive Genomics is a powerful application which precision medicine that is helping researcherspredict disease risk and understand drug response to improve health outcomes and manage healthcarecosts. Thermo Fisher Scientific is at the forefront of this wave of Predictive Genomics, where in then ot-so-distant future, genomic testing brings actionable insights and greater understanding of diseasea cross different global ancestries. Today, Axiom Arrays are being deployed in biobanking, population stratification, calculation of polygenic risk scores, pharmacogenomics, and more recently in research for blood typing antigens. We will present on the NEW Axiom™ PangenomiX™ Array, an array which offers the capability to perform research in several areas including non-communicable diseases,pharmacogenomics, blood typing, and human leukocyte antigen (HLA) typing

Material and Method

The Axiom PangenomiX Array utilizes the Axiom 2.0 Assay and workflow (Figure 3). The Axiom 2.0 workflow is a standard 3- to 4-day workflow, inclusive of whole-genome amplification through array hybridization, staining, and scanning on the Applied Biosystems™ GeneTitan™ MC Fast Scan Instrument. The Axiom PangenomiX Plus Array utilizes the Axiom 2.0 Plus Assay and workflow with an extra step introduced for gene-specific amplification for PGx markers that are in highly homologous regions of the genome

Results and Discussion

The Axiom PangenomiX Array's genotyping performance has been evaluated on 192 samples from the International HapMap Project using stringent quality control metrics that cover average sample call rate, sample concordance, and reproducibility. We validated the array's efficacy in genotyping complex regions for blood typing and pharmacogenomics using 89 diverse HapMap samples. Results show that out of 129 variants analyzed in CYP2D6, the array genotyped all samples correctly, whereas short-read NGS caused errors in 5 variants and failed to call 43

Conclusion

Designed with powerful imputation across diverse populations and copy number variant detection, this array and its simple data analysis, allow you to advance your research goals in Predictive Genomics. The Axiom PangenomiX Array brings ethnic diversity to researchers' fingertips, accounting for global population coverage without compromising on directly genotyped variants, copy number analysis, and other important content such as HLA and blood types. This array will help enable researchers to identify potential population-specific associations for better understanding of complex diseases, leading to diverse genomic datasets and inclusive outcomes for the genomics community and predictive genomics applications. In conclusion, our results show that the PangenomiX array outperforms short-read sequencing and technologies such as LPS that require imputation, suggesting microarrays are well suited for predictive genomics research applications.

For research use only.

Keywords: Axiom, PangenomiX, Pharmacogenetics, biobanking, HLA,, blood typing, carrier screening, microarrays, Predictive Genomics, Precision Medicine

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GALENDAYS14- ST03

COSAP: Comparative Sequencing Analysis Platform

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Introduction

Recent improvements in sequencing technologies enabled detailed profiling of genomic features. These technologies mostly rely on short reads which are merged and compared to a reference genome for variant identification. These operations need to be done using computers due to the size and complexity of the data. The need for analysis software produced many programs for mapping, variant calling, and annotation. A high level of disagreement was observed among popular mapping and variant calling algorithms in multiple studies, making reliance on any single algorithm unsound. User-friendly, open-source software tools that offer comparative analysis are a necessity, given the growth of sequencing technologies.

Material and Method

COSAP1 was developed using Python (core and backend) and React (frontend) and integrates popular open-source bioinformatics tools for genomic analysis. The library was validated using the SEQC2 gold standard somatic variants dataset, with pipeline comparisons performed using VCF Observer2. All elements of the platform are containerized using Docker to allow seamless integration.

Results and Discussion

Here, we propose Comparative Sequencing Analysis Platform (COSAP). This open-source platform provides popular sequencing algorithms for SNV, indel, and structural variant calling, alongside copy

number variation, microsatellite instability, and fusion analysis as well as their annotations. COSAP is packed with a fully functional user-friendly web interface and a backend server that allows fully independent deployment. Pipelines that combine algorithms can be customized and new algorithms can be added with minimal coding through COSAP's modular structure.

Conclusion

COSAP simplifies and speeds up the process of DNA sequencing analysis, providing commonly used algorithms for SNVs, indels, structural variant calling, microsatellite instability, and fusion analysis, as well as their annotations. COSAP is packed with a fully functional user-friendly web interface and a backend server allowing fully independent deployment for individual and institutional scales. Standardized implementations of popular algorithms in a modular platform provide comparisons, making it much easier to assess the impact of alternative pipelines, which is crucial in establishing the reproducibility of sequencing analyses.

Keywords: NGS, Variant Detection, Reproducibility

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GALENDAYS24-ST04

Single-Cell RNA Sequencing Reveals Blocking GPRC5A Downregulates Important Cancer Related Genes in Mesothelioma Cell Line

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Introduction

Malignant Mesothelioma (MM) is rare, aggressive type of cancer and effective treatment options are limited. Asbestos is main risk factor for Mesothelioma and gene vs environment interactions are critical for developing personalized treatment options. Although it has been suggested that some gene mutations may affect treatment outcomes in MM, our knowledge on the subject is still limited. There is a need for detailed molecular profiling of an individual tumor at the cell level which will pave the way for finding effective personalized treatment targets for MM. To address this issue, we aimed to identify expression levels in mesothelioma tumor cells by single cell RNA sequencing to detect an effective gene target which can be applied all tumor clones in a tumor sample.

Material and Method

We used single-cell RNA sequencing in MM cell line (NCI-H28) with 10X Genomics Single Cell 3'Gene Expression protocol. Starting Cell Dilutions are prepared (700-1,200 cells/µl) for GEM generation with cell viability above % 90. 10x Genomics Chromium Controller utilized for GEM generation, capturing and labeling cells. Reverse transcription was performed for each cell. Then cDNA was fragmented, amplified, and the library was constructed with dual indexes. Library sizes were measured by Tapestation 4200 (Agilent Technologies) and the QubitTM dsDNA HS Assay Kit (Thermo Fisher) for quantification. Average library product size was detected around 500 bp. Illumina NovaSeq 6000 system (Illumina) was used for sequencing. Sample demultiplexing, barcode processing, single cell gene-level unique

molecular identifier (UMI) count achieved via cell ranger 8.0. Sequencing reads were aligned to GRCh38. Resulting cloupe files loaded to loupe browser for further analyses. Expression levels of genes are analyzed with their log2 transformed UMI counts for every cluster. Genes with same expression patterns in each cell cluster were selected as a suitable target. To understand the functional effects of the selected gene target, CRISPR experiment performed.

Results and Discussion

Elevated G protein-coupled receptor class C group 5 member A (GPRC5A) expression was detected in all cell clusters of NCI-H28 cell line. Due to high expression levels in NCI-H28 clusters we knocked out GPRC5A to assess its effects on transcript levels of genes especially in Hippo Signalling including YAP1 and c-myc. We also detected downregulation of important cancer genes including KRAS, STAT3, PIK3CA and NFKB1. These downregulated genes are known as the positive regulators of cell proliferation and highly expressed various cancers.

Conclusion

Based on our data GPRC5A may serve as a novel target for treating MM. Since GPRC5A is a membrane receptor protein, repressing its activity may reduce cancer cell proliferation by decreasing transcript levels of important oncogenes like YAP1, c-myc, KRAS, STAT3, PIK3CA and NFKB1.

Keywords: Malignant Mesothelioma, Single Cell RNA sequencing, GPRC5A, CRISPR

GALENDAYS24-ST05

Co-targeting DNA damage and Golgi stress induces mitochondrial apoptosis in ovarian cancer cells.

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Introduction

Ovarian cancer has the highest mortality rate among all gynecologic cancers due to its diagnosis at the metastatic stage and developing chemotherapy resistance. Despite surgical removal of the tumor and platinum-based chemotherapy, many ovarian cancer patients succumb to the disease due to cancer recurrence. Therefore, new treatment strategies are essential to improve the prognosis of ovarian cancer patients.

Material and Method

Apoptotic cell death response was performed by Annexin V/PI staining and flow cytometry, caspase activation tests and monitoring cytoplasmic translocation of cytochrome c. Expression levels of proapoptotic and antiapoptotic BCL-2 family members were determined by immunoblot and RT-qPCR. The results obtained in 2-dimensional cell culture were confirmed in Algimatrix 3-dimensional cell culture system. Golgi stress was determined by GM130 immunofluorescence staining and DNA damage was determined by H2AX immunofluorescence staining.

Results and Discussion

In this study, it was revealed how the combined application of AZD6738/Brefeldin A (DNA damage/Golgi stress inducing molecule), which was determined as the most effective cell death inducing combination when used simultaneously in ovarian cancer cells as a result of high-capacity small molecule screening, regulates programmed cell death pathways in ovarian cancer cells. Experimental studies have shown that simultaneous application of AZD6738/Brefeldin A causes apoptotic cell death in SKOV3 ovarian cancer cells, together with caspase-3 and caspase-9 activation. AZD6738/Brefeldin A exposure also induces cytochrome c translocation to the cytosol, which is a characteristic feature of the mitochondrial cell death pathway. The most important molecular process here is that the combinatorial use of AZD6738/Brefeldin A in SKOV3 and IGROV1 cells leads to a decrease in the expression levels of anti-apoptotic BCL-2 family members BCL-2, BCL-XL and MCL1, while it leads to an increase in the expression levels of pro-apoptotic BCL-2 proteins, especially BIM and PUMA.

Conclusion

Altogether, these findings suggest that AZD6738/Brefeldin A is a potential therapeutic approach for ovarian cancer.

Keywords: ovarian cancer, DNA damage, Golgi stress, programmed cell death, BCl-2 proteins.

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GALENDAYS24-ST06

TAp73β Induces Angiogenesis and Metastasis in a Zebrafish Xenograft Model in the Hepatocellular Carcinoma

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Introduction and Objective

HCC is a hypervascular cancer with angiogenesis that promotes growth, progression and invasion. Advanced therapies particularly targeting tyrosine kinases, vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) have been used as a strategy. The molecular mechanisms underlying angiogenesis in HCC need to be elucidated in order to develop new therapeutic targets for the treatment of HCC. The aim of this study was to investigate the role of TAp73β in angiogenesis in a HCC zebrafish xenograftmodel.

Materials and Methods

To test the effect of TAp73β expression on angiogenesis in HCC cell lines, Flia1:GFP zebrafish larvae were used. The western blot, immunofluorescence and luciferase studies were performed

to analyze β-catenin activation in HCC. The β-catenin rescue assay was performed by ectopic expression of Axin-1.

Results and Discussion

Our results showed that TAp73β induced angiogenesis in HCC celllines. Moreover, overexpression of TAp73β 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 signaling pathway in HCC cells and overexpression of TAp73β increased the nuclear localization of active p-β-catenin (Ser675) in the zebrafish xenograft model. Overexpression of Axin-1 caused degradation of β-catenin and inhibited TAp73β-induced metastasis and angiogenesis. In this study, we demonstrated that TAp73β induces angiogenesis and metastasis in the zebrafish xenograft model by activating the Wnt/ β -catenin signaling pathway.

Conclusion

Consequently, our results indicate that TAp73β increases angiogenesis and metastasis through β-catenin activation in a zebrafish xenograft model.

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

zebrafish xenograft

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GALENDAYS24-ST07

Axl Expression is Induced During the Transition from Non-Muscle-Invasive to Muscle-Invasive Stage in Bladder Cancer

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Introduction

The most critical step in bladder cancer (BC) pathogenesis is the transition from a non-muscle invasive (NMI) to a muscle-invasive (MI) state. According to the BC staging system, the progression from pTa to the pT1 stage is proper to the NMI stage, whereas pT2 represents the entry phase into the MI state. This is why the differentiation of the pT2 tumor from the PT1 tumor is critically important, for which there is no established biomarker.

Material and Method

We analyzed six cell lines representing non-muscle-invasive (NMIBC) and muscle-invasive (MIBC) and the clinical cohort of 102 pT1 and 100 pT2 stage patients diagnosed between 2010-2017 with a minimum five-year follow-up. Three-dimensional organoid-like structures derived from the cell lines, as well as tissue microarray sets from the initial diagnostic biopsies of these patients, were investigated. Statistical analyses employed included Kaplan-Meier survival estimates and Cox regression analysis.

Results and Discussion

We have identified the Axl tyrosine kinase receptor as a selective biomarker for pT1/pT2 differential diagnosis. Its high expression rate was 10-fold higher in pT2 tumors ($p<0.001$). Using our in vitro model based on the 3D growth pattern in Matrigel, we also demonstrate that Axl expression perfectly correlates with the 3D spreading and invasion capacity of BC cell lines.

Conclusion

Axl is a mechanistically relevant biomarker for differential diagnosis of muscle invasion in BC. Our findings that showed significant upregulation of Axl in T2 tumors provide strong evidence for considering Axl-targeting as a first-line therapy in these tumors representing the specific subclass of MIBC tumors, especially in the initial diagnosis phase.

Keywords: Bladder Carcinoma, Axl, Immunohistochemistry, Spheroid, Matrix Invasion, Prognosis.

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GALENDAYS24-ST08

Comprehensive In Vivo Characterization of a Novel Rare Genetic Disease

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Introduction

Autophagy, a fundamental eukaryotic cellular degradation pathway, plays an important role in trophoblast migration/invasion in human placental development, and misregulation of autophagy is linked with preeclampsia. Mutations in core autophagy genes ATG5 and ATG7 have been previously reported to cause rare genetic disorders with Mendelian inheritance. Here we describe a novel rare genetic disease and present our findings in cellular and genetically modified mouse models.

Materials and Methods

The genomic DNA from patient blood samples was isolated and subjected to WES and bioinformatical analysis for the determination of candidate gene variants. A novel disease causing variant was confirmed and selected for functional characterization. Cellular ectopic expression studies for WT and mutated genes were performed to investigate their expression and localization. Furthermore, a genetically modified mouse model was generated by CRISPR knock-in technology, histological, transcriptional, and behavioral characterization of the mutation was performed.

Results and Discussion

The Blue Gene Project aimed to determine novel gene variants in pediatric patients and their families. A homozygous deletion mutation in the ATG9B gene was detected in two pediatric patients (siblings) with neurodevelopmental disorders. Their consanguineously married parents were heterozygous for the deletion. The deletion caused a frameshift and a premature STOP codon, truncating the ATG9B protein. This is a novel autosomal recessive genetic disorder, also the first genetic disease linked with ATG9B. ATG9B is a homolog of ATG9A and the literature is rich on ATG9A and its roles in autophagy. ATG9B, whose expression is restricted to the placenta, is not well studied. When expressed in human cell lines, the mutant ATG9B was not stable and displayed aberrant localization. Immunohistochemistry in human term placentas showed high expression of endogenous ATG9B protein in syncytiotrophoblasts. The histomorphometry analysis of the knock-in mouse model revealed no major abnormality in the placentas and the RT-PCR analysis of genes related to syncytiotrophoblast formation was similar in placentas homozygous or heterozygous for the knock-in. The analysis of memory and anxiety-related behaviors were similar in WT and homozygous knock-in mice while a reduction trend was detected in fear memory in homozygous knock-in mice.

Conclusion

In summary, we identified a novel rare genetic disease and characterized the causative mutation by cell and mouse models. While the mutated protein was not stable when expressed in cells and displayed abnormality in localization, mouse models of the disease did not show a major

phenotype in tissue-level characterization of placentas. Moreover, behavioral characterization of adult knock-in mice showed a reduction trend in behavioral assays.

Keywords: rare genetic disease, autophagy, genetically modified mouse models, CRISPR, ATG9B

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GALENDAYS24-ST09

Transcriptional and Molecular Drivers of Neuroendocrine Differentiation in Bladder Cancer

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Introduction

Muscle-invasive bladder cancer (MIBC) exhibits a range of molecular subtypes, including a rare neuroendocrine-like (NE-like) phenotype, associated with poor prognosis and resistance to therapy. Recent studies have implicated β-catenin, a key player in the Wnt signaling pathway, in the development of neuroendocrine features in various cancers. Our previous study demonstrated that β-catenin and related TCF/LEF transcription factor motifs are enriched in NE-like active chromatin regions. Additionally, mutation analysis revealed a statistically significant increase in the frequency of oncogenic β-catenin exon 3 mutations in NE-like bladder tumors. In the current study, we investigated the role of β-catenin localization and its contribution to neuroendocrine differentiation in MIBC.

Material and Method

We conducted immunohistochemical analyses on 169 MIBC patient samples to assess β-catenin localization and its correlation with clinicopathological features. Gene expression profiling, gene set enrichment analysis (GSEA), and gene co-expression network analysis were performed to identify key pathways and transcription factors associated with β-catenin positivity. The consensus molecular classification tool was applied to classify molecular subtypes and compare the neuroendocrine scores between β-catenin positive and negative groups.

Results and Discussion

Our analysis revealed nucleocytoplasmic β-catenin localization in 18.9% of MIBC cases. β-catenin positive tumors exhibited an increase in neuroendocrine markers and showed higher NE-like molecular subtype scores. Gene co-expression analysis highlighted the enrichment of pathways associated with neurogenesis, chromatin remodeling, and cell cycle regulation in β-catenin positive tumors. Transcription factor binding motif analysis revealed significant involvement of YY1 and E2F family members, both known to regulate NE differentiation and cell cycle dynamics. Additionally, polycomb repressive complex (PRC) components were upregulated in β-catenin positive tumors, aligning with findings in other NE-like cancers, and TCGA bladder cancer cohort.

Conclusion

This study underscores the role of β-catenin in driving neuroendocrine differentiation in bladder cancer. The enrichment of neuroendocrine-related pathways, chromatin remodeling processes, and cell cycle regulation in β-catenin positive tumors suggests that these tumors may share molecular characteristics with other NE-like cancers. Future studies should focus on elucidating the mechanistic links between β-catenin, PRC complexes, and transcription factors to identify potential therapeutic targets for managing NE-like bladder cancer.

Keywords: Bladder cancer, neuroendocrine differentiation, molecular subtype, transcriptomics, Polycomb repressive complex

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GALENDAYS24-ST10

AXL: A Key Player in DNA Damage Response and Epithelial-to-MesenchymalTransition

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Introduction

Axl is a key receptor kinase whose expression is upregulated in tumors that acquire resistance to chemotherapy, tyrosine kinase inhibitors and even radiotherapy often in conjunction with the process of epithelial-to-mesenchymal transition (EMT). While Axl's role in EMT is well-established, the mechanisms driving its increased expression remain largely unknown. In this study, we establish a connection between AXL activation and the DNA damage response. Normally, p53 is a key regulator of the DNA damage response, but in many cancers, its function is lost due to mutations. In such cases, is thought to assume p73, which remains functional in cancer cells mediating the DNA damage response. Our previous research demonstrated that Axl inhibition sensitized hepatocellular carcinoma (HCC) cells to DNA damage (Batur et al., 2021). Here, we provide experimental evidence showing that p73 can transactivate the AXL promoter**.**

Material and Method

We manipulated p73 expression in HCC cells through overexpression with TET- inducible systems and siRNA-mediated silencing. Changes in AXL expression were assessed via western blotting across different HCC cell lines. To evaluate AXL promoter activity, we used a luciferase reporter assay in TET-inducible p73-overexpressing HEP3B cells. Additionally, chromatin immunoprecipitation (ChIP) assays were performed to investigate p73 binding and histone modifications at the AXL promoter.

Results and Discussion

Overexpression of p73 led to an increase in Axl protein levels and siRNA- mediated silencing of p73 caused decrease in Axl protein across various HCC cell lines. Analysis of the AXL promoter revealed p73 binding motifs was found and conserved across species. Luciferase reporter assays showed significant transactivation of the Axl promoter by p73, which were confirmed to be enriched through ChIP assays. Additionally, high enrichment of H3K4Me3 and low enrichment of H3K27Me3 in AXL promoter showed active gene transcription of AXL . These result showed p73 plays a critical role in activation of AXL, yet the specific DNA damage pathway responsible for driving this activation of Axl remains unclear. Further research is needed to determine which DNA damage response pathways are involved in this regulatory mechanism.

Conclusion

Our novel findings clearly established Axl as a p73 transactivation target in HCC cells. Taken together our recent observations strongly suggests that Axl involvement both in resistance to DNA damaging therapies and in EMT is related to a potential role in DNA damage response mediated by p73 in cancer cells.

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GALENDAYS24-ST11

Effects of F53 Hotspot Mutations on the Molecular Dynamics of MEK1 Protein and the Binding of its FDA-Approved Inhibitors

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Introduction

Targeted cancer therapy aims to combat cancer cells by focusing on specific proteins that are crucial for disease progression by minimizing off-target effects. As cancer cells frequently depend on proto-oncogenic genes, it makes these nodes as prime targets within the scope of personalized therapy. Importantly, mutations in the targeted protein can disrupt drug binding and/or activity. MEK1, a key protein activated by various signalling pathways, is linked to cancer through mutations in the MAP2K1 gene. Its role in cancer has led to the development of several small molecule inhibitors, four of which (trametinib, cobimetinib, binimetinib, selumetinib) have received FDA approval. However, the impact of F53 hotspot mutations on drug binding/activity and MEK1's structural dynamics remains unclear. We investigated this using molecular dynamics (MD) simulations of wild type and mutated (F53L/V/C/I/Y) MEK1 in the presence and absence of FDA-approved inhibitors.

Material and Method

Homology modeling was performed to obtain complete using Modeller software. After the wild-type modeled MEK1 structure was verified using PROCHECK and Pro-SA web servers, the F53L/V/C/I/Y

mutations were introduced via Pymol mutagenesis tool. Trametinib, cobimetinib, binimetinib and selumetinib were docked to all MEK1 structures. Totally, we performed 30 Molecular dynamics simulationsfor each complex (WT/F53L/V/C/I/Y MEK1+ATP+Mg2+±Inhibitor) via Gromacs. RMSD, RMSF, SASA, DSSP, DCCM, PCA, FEL, MM-PBSA and H-bond analyses were applied.

Results and Discussion

We realised changes on the motion of MEK1 where a longer duration in the lowest energy state conformation was observed during the simulations in the presence of F53 mutations. This was complemented by cross-correlated motions of amino acids of MEK1. More importantly, the binding affinities of inhibitors were affected in particular cases. There was a marked reduction in the calculated binding affinity of trametinib in the presence of F53C mutation. On the other hand, cobimetinib and selumetinib tolerated F53 mutations generally well in terms of drug binding. Binimetinib interestingly exhibited an increased binding affinity when F53C/I mutations were present**.**

Conclusion

Our results provide a comprehensive perspective on the structural and drug-binding effects of observed mutations on the hotspot residue F53 within MEK1; the groundwork for the personalization of MEK inhibitors.

Keywords: MEK1; F53; inhibitors; molecular dynamics

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GALENDAYS24-ST12

miR-181a-5p Sensitizes Sorafenib Resistance by Attenuating the Aggressive Phenotype by the Inverse Correlation with Cav-1 in Hepatocellular Carcinoma

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INTRODUCTION

Hepatocellular carcinoma has one of the highest death rates among all cancers, and the reasons behind this are metastasis, recurrence, and therapy resistance. Sorafenib is the first multi-kinase inhibitor used for HCC treatment and is still the most preferred and accepted drug. However, the long duration of therapy generates resistance to sorafenib. The mechanism behind this is yet to be elucidated, but non-coding RNA-based mechanisms are counted as one of the reasons for therapy resistance. Recent studies have highlighted the influence of miRNAs in the development, progression, metabolic reprogramming, and therapy responses in HCC. Our previous studies showed that decreased expression of miR-181a-5p causes a more aggressive phenotype in HCC. Cav-1 overexpression causes a more aggressive phenotype, as well. In this study, we aimed to enlighten the Sorafenib resistance mechanism, which made us think about the Cav-1 and miR-181a-5p axis in Sorafenib resistance.

MATERIAL AND METHOD

We first determined Cav-1 expression levels and the expression changes of miR-181a-5p in sorafenib-resistant cells that we generated from HuH-7, SNU-449, and Mahlavu HCC cell lines. By overexpression and silencing of miR-181a-5p via transfecting mimic or inhibitor of miR-181a-5p, we investigated the role of miR-181a-5p level in Sorafenib resistance and its biological responses to HCC cells. We investigated publicly available transcriptome data of HCC patients who received SOR/placebo (GEO accession ID:GSE109211) and analyzed MIR181A and CAV1 expressions in Sorafenib responder and non-responder patients.

RESULTS AND DISCUSSION

In Sorafenib-resistant cells, miR-181a-5p expression was decreased. When we increased miR-181a-5p expression by mimic transfection in Sorafenib-resistant cells, they became more sensitized to Sorafenib, and their motility and colony formation capability were decreased besides Cav-1 expression. When we inhibited miR-181a-5p expression, all the results turned the opposite including Cav-1 expression. In the patient dataset, MIR181A expression was significantly lower in non-responders who are resistant to Sorafenib whereas CAV1 expression was the opposite. We analyzed Cav-1 and miR-181a-5p expressions that were negatively correlated.

CONCLUSION

Since Cav-1 and miR-181a-5p contribute to aggressive phenotype individually, they may contribute to Sorafenib resistance together by their relation and enlightening this relation may help develop new approaches to managing Sorafenib resistance mechanisms.

Keywords: HCC, Sorafenib Resistance, Cav-1, miR-181a-5p

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GALENDAYS24-ST13

Investigation of Immune-targeted Cancer Biomarkers in The Cancer Genome Atlas Datasets

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Introduction

The Cancer Genome Atlas (TCGA) project provides a comprehensive dataset of 33 cancer types, including molecular profiles such as single nucleotide variations (SNVs), copy number variations (CNVs), DNA methylation, RNA expression, miRNA expression, and clinical data such as drug response and survival outcomes. This dataset is essential for advancing cancer research, particularly in biomarker discovery and drug response analysis. In this study, we used this rich dataset to explore immune-targeted cancer biomarkers using integrative clustering.

Materials and Methods

We applied the iCluster method, an advanced integrative clustering approach, using TCGA data to integrate multiple omics datasets including SNVs, CNVs, gene expression, miRNA expression and DNA methylation to identify distinct tumor clusters based on their molecular profiles. We focused on correlating these clusters with immune-targeted proteins to understand their role in immune response mechanisms and therapeutic applications. We analyzed the expression patterns of these proteins, their associations with immune subtypes and their correlations with tumor-infiltrating immune cell fractions. In addition, we developed a web

tool that allows users to query and visualize cancer-specific data, including iClusters, literature cohorts and biomarkers.

Results and Discussion

Our analysis revealed significant patterns of immune-targeted protein expression across tumor clusters. We observed correlations between iClusters and these proteins, different immune subtypes, and differences in tumor-infiltrating immune cell fractions. These findings reveal multiple interactions between tumors and the immune system that influence the efficacy of immunotherapy. This approach provided insights into the different immune landscapes of patient clusters. We also investigated how these clusters relate to immunotherapy response and patient survival, with the aim of identifying predictive and prognostic markers for personalized treatment strategies.

Conclusion

Our study highlights the value of using integrative clustering to uncover complex patterns in cancer data and gain new insights into immune interactions and therapeutic outcomes, revealing potential biomarkers and prognostic indicators. In addition, our web tool, TCGAnalyzeR, enhances the ability to combine candidate biomarkers and assess their impact on survival statistics across different patient clusters or sub-cohorts.

Keywords: TCGA, Integrative clustering, SNV, CNV, Expression, DNA Methylation, Immune therapy

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GALENDAYS24-ST14

Stimulation of Efferocytosis by Soluble Receptor

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Introduction

Efferocytosis, a cellular process crucial for maintaining tissue homeostasis, involves the engulfment of apoptotic cells by phagocytes. This process is regulated by "eat me" and "don't eat me" surface signals on the apoptotic cells, recognized by receptors on phagocytes. The CD47-SIRPα interaction is a critical checkpoint in the immune system that prevents the destruction of healthy cells by macrophages. CD47 acts as a "don't eat me" signal, inhibiting phagocytosis. Disruptions in this interaction can lead to autoimmune diseases and impaired immune function. However, cancer cells often hijack this checkpoint by overexpressing CD47, allowing them to evade immune surveillance. Targeting this interaction with therapeutic agents has shown promise in preclinical studies and clinical trials, demonstrating its potential as a novel strategy for cancer immunotherapy. Here we engineered a chimeric soluble SirpA receptor protein that binds to CD47, thereby promoting macrophage-mediated efferocytosis.

Material and Method

A chimeric SirpA protein was constructed, comprising the extracellular domain of the SirpA receptor fused to the Fc region of mouse IgG2a with a hinge region. The synthesized construct was cloned into the pDAD2.0 vector, patented by our laboratory. Both SirpA-Fc and a Control-Fc protein were expressed in HEK293T cells and validated by ELISA and Western blotting. Subsequently, the recombinant proteins were purified using Protein A affinity chromatography. Prior to co-culture experiments and efferocytosis assays, CD47 expression and SirpA-Fc binding on 4T1 cancer cells was assessed by flow cytometry.

Results and Discussion

The chimeric SirpA-Fc protein was engineered, expressed, and purified with high yield. It displayed enhanced affinity for CD47 compared to the natural receptor-ligand interaction. The recombinant SirpA-Fc protein bound effectively to CD47 on live 4T1 cancer cells, leading to a significant increase in macrophage-mediated engulfment of these cells. These results suggest that SirpA-Fc holds promise as a therapeutic agent for cancer treatment.

Conclusion

In conclusion, the SirpA-Fc chimeric protein represents a promising therapeutic candidate for the treatment of cancer. Its high binding affinity for CD47, coupled with its ability to enhance macrophage-mediated engulfment of cancer cells, suggests that it could be a valuable addition to the arsenal of cancer immunotherapies. Further studies are needed to evaluate the efficacy and safety of SirpA-Fc *in vivo* and to explore its potential for combination therapies with other cancer treatments.

This study was supported by TUBITAK grant-No 122S045

Keywords: *Efferocytosis, SirpA, CD47, Recombinant Chimeric Protein, Cancer*

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GALENDAYS24-ST15

Novel Monoclonal Antibodies for Stratification of Lung Cancer

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Introduction:

Lung cancer is the leading cause of cancer-related mortality worldwide. Each year around 41.000 patient is diagnosed with lung cancer in Turkiye. Adenocarcinoma and squamous cell carcinoma are the most frequent lung cancer types. To distinguish of these two subtypes are crucial for personalized therapy options. Due to their high selectivity and specificity, monoclonal antibodies(mAb) against tumor associated epitopes are promising tools for identification of novel biomarkers. The mAb industry has grown exponentially and in 2026, global mAb market value is estimated to be around 292 billion dollars. Since Turkiye imports the majority of its mAbs used for diagnostics and therapeutic applications, TUBİTAK has initiated project calls for domestic production of such mAbs. The goal of this project was to design, produce and characterize monoclonal antibodies against a panel of antigens including Napsin A and P40 that would be suitable for immunoassays to distinguish adenocarcinoma from squamous cell carcinoma.

Material and Method:

5-6 weeks old BALB/c mice were immunized at 3-5 times at two-week intervals with the corresponding antigen. Serum antibody levels of mice were subjected to serial dilution and determined by indirect ELISA method. Fusion of SP2/F0 myeloma and spleen cells was performed in the presence of polyethylene glycol, then cells were seeded in 96-well plates in selective hypoxanthine-aminopterin-thymidine medium. Screening was performed by indirect ELISA using supernatants of hybridoma colonies that had grown in an average of 2 weeks. Colonies that gave positive signals for the antigen of interest were subjected to single cell cloning and hybridoma clones were obtained. Monoclonal antibodies were purified by protein A affinity chromatography and their binding to the antigen of interest was further confirmed by western blotting or flow cytometry. Selected antibodies were tested in immunohistochemistry (IHC) using the Roche Ventana device.

Results and Discussion:

We could obtain satisfactory results with numerous different hybridoma clones for Napsin A and P40 monoclonal antibodies. We select the best antibodies according to the immunohistochemistry staining. Napsin A monoclonal antibody gave specific cytoplasmic staining pattern in agreement with commercial Napsin A antibody currently used to select adenocarcinoma cases in routine diagnostics. On the other hand, p40 monoclonal antibody IHC staining showed specific nuclear staining that is similar to commercial antibody’s staining pattern in squamous cell carcinoma cases.

Conclusion:

The histopathological subtyping is important in determining the targeted treatment option for non-small cell lung cancer. Newly developed Napsin A and P40 spesific monoclonal antibodies verified onautomated immunohistochemical staining platform showed exquisite agreement with often used commercial antibodies in routine diagnostics. Our studies and results show that Turkey has the capacity to produce monoclonal antibodies allowing application in clinical pathology for cancer patients.

Keywords: Lung cancer, personalized therapy, diagnostics, immunohistochemistry, monoclonal antibody, hybridoma, Napsin A, P40

Posters

GALENDAYS24-P01

Exploring MET-CAV1 Fusion Transcript: A Novel Biomarker Candidate for Patient Stratification for Treatment Response in Hepatocellular Carcinoma

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Introduction

Liver cancer is one of the most prevalent malignancies globally, with hepatocellular carcinoma

(HCC) being the predominant form of liver cancer. Recent advancements in "-omics" technologies and related analytical methodologies have significantly enhanced the identification of potential biomarkers associated with various diseases. Despite these advancements, the mechanisms underlying the progression of HCC remain inadequately understood. Early diagnostic and treatment options for HCC are currently limited, highlighting the critical need for reliable biomarkers in clinical practice. Fusions play an important role in cancer progression, and fusion transcripts (FTs) have been effectively used as diagnostic biomarkers and aiding in treatment decision-making in hematologic malignancies. Research

focusing on fusions is limited in HCC. This study aims to investigate HCC-associated FTs through the application of bioinformatics approaches and perform wet-lab validation analysis.

Material and Method

In this study, we re-analyzed independent HCC RNA-Seq datasets (meta-dataset) obtained from the NCBI GEO (Gene Expression Omnibus) and TCGA (TCGA-dataset) by using ChimerDB 4.0. The meta-dataset analyzed encompassed 328 tissues, and the TCGA-dataset encompassed nearly 12.000 samples from 33 different cancer types. Our analysis algorithm involved the use of STAR-Fusion and Chimer DB analysis for the identification of FTs. The MET-CAV1 fusion transcripts were analyzed using ORF finder, which searches for open reading frames (ORFs) in the sequence you enter, and the program returns the range of each ORF, along with its protein translation. Additionally, we designed specific primers for different versions of the MET-CAV1 fusion transcript for the wet-lab validations and expression of partner genes individually examined by qRT-PCR in MV, Huh7, and SNU449 HCC cell lines, along with their Sorafenib-resistant versions. Lastly, we investigated MET and CAV1 transcript expression in transcriptome data of patients who received Sorafenib (GSE109211). We identify MET and CAV1 levels between Sorafenib-resistant and sensitive patients to further investigate cell line results.

Results and Discussion

124 FTs were found in at least five or more tumor samples in the meta-dataset, 15 of them are specific to cancer tissues, and 777 HCC-related FTs in LIHC (Liver Cancer – Hepatocellular Carcinoma) TCGA-dataset samples. When we further analyzed the MET-CAV1 fusion transcripts, Four different FTs with distinct breakpoints were identified, and two of these FTs were found to be capable of producing a fusion protein. These fusions were identified as novel and unique candidates that have not been previously associated with HCC. Our analysis reveals that this fusion was also identified in STAD (stomach), GBM (glioblastoma), BRCA (breast), and UCEC (endometrial) cancers. Additionally, when analyzing the expression of the partner genes (MET, CAV1) involved in this fusion, it was observed that they exhibited higher expression levels in fusion-negative tumors. Patient transcriptome analysis also revealed that MET and CAV1 expressions are higher in

Sorafenib-resistant patients compared to sensitives. Finally, validation analyses conducted with HCC cells demonstrated that the expression levels of these genes and MET-CAV1 fusion transcript were particularly higher in the SNU-449 cell line and significantly increased in the Sorafenib-resistant SNU-449 cell line.

Conclusion

This study highlights the potential for identifying HCC-specific fusion candidates or transcript profiles to discover new druggable FTs for HCC treatment. Further studies are needed to understand the mechanistic roles of the MET-CAV1 transcript in patient stratification for treatment response in HCC. This study is supported by the TUBITAK (The Scientific and Technological Research Council of Turkey) project, 120C216.

Keywords: HCC, hepatocellular carcinoma, fusion transcript, chimeric RNA

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GALENDAYS24-P02

HGF Promoter DATE Region Length in Liver Stellate Cells as a Prognostic Biomarker in Hepatocellular Carcinoma Progression

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BACKGROUND:

Hepatocyte Growth Factor (HGF) produced by stromal and mesenchymal cells binds to c-Met receptors of neighboring epithelial cells and activates HGF/c-Met signaling pathway whose abnormal activation leads to tumor formation, uncontrolled cell growth, invasion and metastasis in several cancers, including Hepatocellular Carcinoma (HCC). Moreover, elevated HGF levels in tumor microenvironment are known to be associated with poor survival, metastasis, and relapses. Abnormal HGF promoter activity was detected in breast, colon and bladder cancer cells as a result of mutations in a specific region of HGF promoter known as deoxyadenosine tract element (DATE), which normally consists of approximately 30 deoxyadenosine mononucleotide repeat (30A). However, there is no study in the literature yet presenting the role of HGF-DATE polymorphisms in stromal cells on tumor cells to gain an aggressive phenotype. In this study, we tested the importance of HGF-DATE region length of hepatic stellate cells on their HGF expression and as well as the biological behaviors of HCC cells.

MATERIALS and METHODS:

The effect of HGF-DATE region length on transcriptional activity of HGF promoter was examined using luciferase reporter vectors containing different lengths of DATE region. Then, expression vectors containing deletion mutants were produced. LX-2 hepatic stellate cells (HSC) were transfected with these vectors having different DATE lengths to obtain HGF-DATE LX-2 clones. The effect of DATE length on transcriptional activity of HGF promoter

was examined by luciferase analysis, and the gene expression of HGF and other HSC activation markers were examined by qPCR. Finally, HUH-7 cells, an HCC cell line, were treated with conditioned media (CM) obtained from HGF-DATE LX-2 clones. The effects of the factors present in CM on EMT phenotype, adhesion, proliferation, 2D colony formation and migration capacity of HUH-7 cells were determined.

RESULTS:

HGF-DATE region in WT LX-2 cells was determined as 28A. HGF-DATE deletion mutants containing 28A, 20A and 14A were successfully produced and LX2 cells transiently transfected with these vectors. LX-2 cells transfected with 20A and 14A HGF-DATE vectors showed significantly reduced HGF and activation markers gene expression compared to those transfected with the 28A vector. It was determined that HUH-7 cells exposed to CM obtained from 14A HGF-DATE LX-2 clones showed reduced gene expression of epithelial (E-cadherin&EpCAM) and mesenchymal markers (N-cadherin&Vimentin), increased proliferation and migration properties and decreased surface adhesion and colony formation capacities.

CONCLUSION:

Importance of HGF-DATE length in HSCs and the role of HGF-DATE length in these cells in HSC activation and aggressiveness of HCC cells were determined for the first time in the literature. This data has the potential to lead to the investigation of the potential of HGF-DATE polymorphisms in stellate cells as a new prognostic marker in HCC prognosis.

This study was supported by TÜBİTAK BİDEB 2218 - National Postdoctoral Research Scholarship Program (Project #121C380).

GALENDAYS24-P03

Preliminary data: Genetic polymorphism of CYP2D6 (*2,*3,*4,*5,*6,*7,*10,*41) in patients with cardiovascular disease– a cohort study*

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Introduction:

Cardiovascular diseases are multifactorial diseases that are influenced by both environmental and genetic factors. CYP2D6 found in the liver and the heart is involved in the metabolism of many drugs and some endogenous substances responsible for maintaining homeostasis. This raises an interesting hypothesis that it may have a role in the development of or protection against cardiovascular diseases (1). To study the distribution of genotypes of CYP2D6 among patients with cardiovascular diseases in Afyonkarahisar (Turkey).

Material and Method

Ethics committee approval was obtained before the study (AFSÜ Non- Interventional Scientific Research Ethics Committee Decision No: E.98935). The informed consent form was read and signed by the patients. CYP2D6 enzyme gene polymorphisms (*2,*3, *4, *5,*6,*7,*10 and *41 alleles) were detected by DNA Sequence Analysis method in blood samples (2 ml) taken from 200 patients who applied to AFSU Hospital Cardiology polyclinic due to their cardiological disease.

Results and Discussion

Six patients were genotyped homozygous CYP2D6*7/*7 predicting a poor metabolizer (PM) prevalence 3%. 83.5% of the all cases were determined as normal metabolizers (NM), 13.5% as intermediate metabolizers (IM). There were no significant differences in genotype

frequencies of CYP2D6 in cardiovascular disease subgroups. In the later stages of the study, the contribution of CYP2D6 genetic polymorphism to metoprolol efficacy will be determined in the subgroup of patients using metoprolol. It will be investigated whether there is a difference in susceptibility to adverse effects of metoprolol in different metabolizer subgroups.

Conclusion

Our study suggests that there is no involvement of CYP2D6 genotypes in cardiovascular system diseases. Since the rate of cases with changes in enzyme activity was 16.5% in our study, it would be useful to determine CYP2D6 gene changes in cases before treatment of CYP2D6 substrate drugs. *This study was supported by Afyonkarahisar Health Sciences University BAP Commission with project number 23.GENEL.026.

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GALENDAYS24-P04

3D Cell Culture Models as a Platform for Studying Tumor Progression, Testing Treatment Responses, and Discovering Biomarkers

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Introduction

The 3 dimensional (3D)-models have become significant research tools, as they lie between 2D systems and in vivo organismal models. They empower mimicking and studying several biological processes promoting innovation in disease modeling, drug development, and precision medicine. Especially, Multicellular Tumor Spheroids (MCTSs) formed by co-culturing cancer cells with heterogeneous cell types (such as immune cells, endothelial cells, or fibroblasts) have become an appealing model for in vitro tumor biology studies. This study focused on designing different 3D MCT models to investigate tumorigenesis, discover biomarkers, and test new treatment options.

Material and Method

Hepatocellular Carcinoma Cells (HCC), and Colorectal Cancer (CRC) cell lines and hepatic stellate cells (HSC), were cultivated as described (1,2). For investigation of tumorigenesis HCC

caveolin clones were embedded into collagen as described before (2) For treatment testing studies either a control group or treated group was embedded in 3D collagen gel that contained SU11274 and/or HGF. For disease modeling, treatment testing, and biomarker discovery studies, HSCs cells were embedded in collagen gel. The following day, cells were treated with either a metabolic medium or control medium. On day 5, upper chambers were integrated into the system and loaded HCC clones. On day 8, HCC cells that passed the membrane were collected and transferred to a new culture dish. On day 8, RNA was obtained from HSCs cells. RNA and protein isolations were performed when HCC cells reached 70% confluency. For disease modeling and biomarker studies, HSCs cells were embedded in collagen in the upper chambers, and CRC cells were embedded in collagen in the bottom chamber. They were incubated for 8 days. The proteomics and transcriptomic analysis were performed to determine. protein expression/activation profiles and RNA profiles. Western Blot (WB) and RT-PCR analysis were used for validation assays (1,2,3).

Results and Discussion

Tubulogenesis is a powerful 3D in vitro model for testing tumor progression/angiogenesis, involving sequential cell attachment, differentiation, invasion, and morphogenesis, all of which contribute to the aggressive nature of cancer. Caveolin-1 expression increased tubulogenesis and this response increased by HGF induction ($p<0.05$). When HGF/c-Met was blocked, tubule formation was decreased ($p<0.05$). In a similar experimental setup, treatment response was evaluated and shown that miR-181a-5p expression synergizes HGF signaling blockage. In disease, modeling 3-D system, we observed that the MASLD-microenvironment (MASLD-ME) i) activated and increased spheroid formation of HSCs, ii) increased migration of HCC and CRC, and iii) activated metabolism-related pathways. Bioinformatic analysis using patient data and validation analysis with WB, RT-PCR, showed that this model successfully mimicked MASLD-ME on the gaining aggressive phenotype of HCC and CRC cells. Additionally, the quality and amount of RNA and protein samples were suitable for high-throughput analysis including transcriptome and proteomic analysis.

Conclusion

This study reports that 3D-spheroid models, particularly 3D-MCT models, offer a useful platform for studying tumor formation, progression, metastasis, treatment responses, and biomarker discovery. Thus the MCTs and high-throughput approaches will prove new possibilities for creating personalized therapy for oncological diseases.

Keywords: 3-D co-culture system, disease modeling,

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GALENDAYS24-P05

Olfactor Receptor Gene OR2A4/7 As A Potential Biomarker in Colorectal Cancer

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Introduction

Olfactory receptors (ORs) are responsible receptors that detect and transduce odorant signals and are a member of the G protein-coupled receptor family. Recent studies revealed that in addition to their regular physiological roles, ORs hold an important place in the development and progression of different cancer types, including breast, colon, lung, and prostate. Epithelial-mesenchymal plasticity (EMP) allows cells to transition between various states within the epithelial-mesenchymal landscape, acquiring hybrid EMP features. This study investigates the potential role of the OR gene OR2A4/7 in EMP in colorectal carcinoma (CRC).

Material and Method

First, we created OR-related meta-data obtained from different cohorts, such as TCGA, using bioinformatics tools and databases such as UALCAN, cBioPortal, and UCSC XenaBrowser. We analyzed all OR family members to determine differentially expressed ORs in CRC

tissues compared to normal colon tissue. We determined OR2A4/7 as a predictive biomarker in CRC tissues. To understand its mechanistic role, we performed bioinformatics analysis using Expression Atlas, Cancer Cell Line Encyclopaedia (CCLE), and the Human Protein Atlas to determine OR2A4/7 expression in CRC lines SW-480 and SW-620. We performed RT PCR to confirm the bioinformatics analysis. CRISPR studies were performed in the SW-620 to knock out the gene OR2A4/7. LentiCRISPR V2, and pSpCas9(BB)-2A-Puro (PX459) vector plasmids were used. CRISPR validation studies were performed at the mRNA level. The expression levels of EMT markers were checked on knock-out clones. Delta Delta CT analyses were performed using RT-qPCR results. Statistical analysis was performed using the GraphPad Prism, One Way ANOVA.

Results

The bioinformatics data of patient cohorts showing the increase in the OR2A4/7 transcript level in aggressive CRC tissues is confirmed in CRC cell lines SW 620, SW 480, HT29, and HCT116 by using CCLE data and RT-qPCR. Since, both bioinformatics and RT-qPCR studies demonstrated that OR2A4/7 mRNA expression was 11-16 times higher in SW-620 (highly metastatic, aggressive) than in SW480 (low metastatic) ($p = 0.0002$), we decided to use SW-620 cell line for gene knockout. CRISPR studies proved that LentiCRISPR v2 had better success rates than pSpCas9(BB)-2A-Puro (PX459) with inhibition for OR2A4/7, 62.6 % LCV2 gRNA1($p=0,02$), 66% LCV2 gRNA2 ($p <0,01$), 23% pX459 gRNA1 ($p <0,01$) and 56% pX459 gRNA2 ($p=0,01$), respectively. When we examined prevailing EMP markers Vimentin, Fibronectin, a-SMA, E-Cadherin, N-Cadherin, SNAIL1 and EpCAM in LCV1 and 2 knockout clones, we observed that the expression of the “FN1” and “VIM” were significantly decreased ($p =0,01$, $p=0,03$ respectively**)** in OR2A4/7 KO clones.

Conclusion

In conclusion, this study provides preliminary results showing the potential role of OR2A4/7 in CRC progression via inducing EMP. Further studies will be carried out to unveil the importance of OR2A4/7 in EMP with mechanistic insights.

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Keywords: Colorectal Cancer, Olfactory Receptors, EMP, CRISPR Cas 9, Bioinformatics

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GALENDAYS24-P06

Vitamin D Administration on a Non-Alcoholic Fatty Pancreatic Disease Animal Model

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Introduction

Non-alcoholic fatty pancreatic disease (NAFPD) is defined by obesity and fat accumulation related to pancreatic steatosis and beta (β) cell lipotoxicity leading to pancreatic dysfunction. We investigated the impact of vitamin D (VitD) treatment on liver and pancreas of the NAFPD rats regarding metabolic and histopathological changes.

Material and Method

28 male Sprague Dawley rats were randomly divided into 4 separate groups: Control (C), MetS (MS), MetS + VitD (MSD), and Control + VitD Group (CD). MetS group was fed with a diet containing 17% fat, 17% fructose, and 20% fructose water for 15 weeks. VitD group was treated with 170 IU/week orally for 12 weeks. Daily feed, water consumption, weight, and fasting blood sugar were measured during the experiment. H&E, Prussian Blue, and Masson's Trichrome staining were performed on pancreatic tissue sections. IHC staining was performed by using an insulin antibody. Serum insulin levels and oxidative stress markers in liver were assessed with ELISA method. All data were analyzed with statistical methods.

Results and Discussion

The daily calorie intake, weight, fasting blood glucose, and insulin levels of the MS group are statistically higher than those of the others. The islet sizes in the MS group significantly increased compared to the others. Irregular sizes and vacuolation are observed within the acinar cells and the islets. Fibrosis is observed in the interlobular and intralobular areas, and iron accumulation was found around some pancreatic islets and within the acinar cells. In the MS group, the islets were full of β cells, but the insulin immune reaction was weak in these cells. More organized islets, more insulin- immune positive cells, and many insulin-positive cells scattered in the exocrine tissue, either individual β cells or clusters of 3-4 cells, have been found in the MSD group compared to the MS group. Altered oxidative stress markers in liver, including reduced glutathione, oxidized glutathione, and glutathione peroxidase 4 in MS group ameliorated in MSD group.

Conclusion

VitD supplementation has ameliorated insulin metabolism, islet size, fat accumulation in endocrine and exocrine tissues, and oxidative stress metabolism in liver in the NAFPD animal model developed by a high-fat and high-fructose diet.

Keywords: Non-Alcoholic Fatty Pancreatic Disease, Vitamin D, Metabolic Syndrome, High Fructose, High Fat Diet

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GALENDAYS24-P07

Effects of Exogenous Albumin on the Growth and Migration of Human Hepatoma Cells

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Introduction

Hepatocellular carcinoma (HCC) is the most common form of liver cancer, accounting for approximately 90% of liver cancer cases. Its development is influenced by environmental, lifestyle, and genetic factors. The prognosis of HCC varies significantly depending on the cancer stage and is affected by multiple factors such as tumor size, α-fetoprotein (AFP) levels, and the Glasgow Score with the ration of CRP/Albumin (ALB). Among those factors, ALB is the predominant protein found in extracellular fluids. It is exclusively produced by hepatocytes and is secreted into the bloodstream at a rate of about 13-14 grams daily. Reports indicate that reduced levels of ALB are linked to the presence of larger HCC tumors. ALB has a crucial role in the HCC process, and has been shown to influence HCC cell growth, however, the mechanism by which ALB affects HCC progression and metastasis remains unknown.

Material and Methods

The effect of ALB treatment was determined by MTT proliferation assay, wound healing assay, and western blot. Wound healing assays were conducted to evaluate the effects of ALB treatment during migration in HCC cells. MTT assay has been used to assess the proliferation of HCC cell lines after treatment of ALB. Western blot was used to observe the expression levels of AFP after ALB treatment.

Results and Discussion

ALB treatment inhibited the proliferation and migration of HCC cell lines in a concentration-dependent manner. ALB also caused a reduction in AFP expression levels. The combination of VK2 and ALB did not result in additional effects.

Conclusion

Treatment of HCC cell lines with ALB demonstrated a potential inhibitory effect on both the progression and metastasis of HCC probably by decreasing AFP levels.

Keywords: Hepatocellular carcinoma (HCC), HCC growth, Albumin (ALB), α-fetoprotein (AFP), Migration

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GALENDAYS24-P08

Induction of Multiple Sclerosis Model by Oral Cuprizone Administration and Evaluation of Cognitive Functions in Wistar Albino Rats

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Introduction

Multiple sclerosis (MS) is a disease of central nervous system. Symptoms are very important in diagnosing and determining stage of MS and require personalized treatment approaches. Right diagnosis allows treatment to be managed correctly and thus to prevent waste of time and economic burden. It is recommended cognitive function tests be performed at regular intervals for cognitive dysfunction. We aimed to generate MS model with cuprizone and to investigate changes in cognitive disorders behaviorally and histopathologically.

Material and Method

Albino Wistar was induced by oral cuprizone for MS. The rats were divided into 9+3 groups. Locomotor activity, rotarod, Morris water maze were performed in rats that received cuprizone for 2,4,6,8 weeks followed by histopathological examinations of corpus callosum (CC), hippocampus.

Results and Discussion

According to the oral cuprizone model, demyelination in the CC and hippocampus was seen significantly in rats with 400 mg/kg cuprizone administration for 4 weeks. During this time,

there was no deterioration in the behavioral tests. Even if we extended the duration of cuprizone administration (8 weeks), this was not reflected in behavior, on the contrary, remyelination was observed in the CC and hippocampus. When remyelination was examined in the groups that discontinued cuprizone for 1, 2 and 3 weeks after 4 weeks of cuprizone administration, it was seen that demyelination started to decrease in both the CC and hippocampus from the 2nd week, and remyelination started to occur from the 3rd week. However, these changes were not disturbed by the cuprizon administrated for 4 weeks, and no change was observed in the remyelination groups. These findings were supported by luxol fast, myelin basic protein and TUNEL measurements.

Conclusions

The model is a suitable model for studying histological changes in the research for drugs and new drug candidate molecules in the future research. Since these changes are not reflected in behavior, it does not allow behavioral tests to be carried out.

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Olfactory Receptors as Regulators of Agressive Phenotype in Colorectal Cancer

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Introduction

Colorectal cancer (CRC) is the third most common cancer worldwide and has the second highest mortality rate among cancers. Recent studies have suggested the involvement of olfactory receptor (OR) genes in various cancer processes, including metastasis. In this study, we performed an integrative bioinformatic analysis to inspect the potential effects of OR genes in CRC, examining their expression across various groups of datasets and their association with epithelial-mesenchymal transition (EMT) marker genes.

Material and Method

In terms of their source of data, we first categorized RNA sequencing datasets into three main groups.For cell line studies, we downloaded “GSE161963”, “GSE255163” and “GSE97023” datasets from the “GEO (Gene Expression Omnibus)” database. To compare tumor tissues against normal tissue, we utilized the “TCGA-COAD” dataset from the TCGA (The Cancer

Genome Atlas)" database and the study of Simoneau et al, 2015. For the comparison of metastatic tissues against tumor tissues, we utilized "GSE100243" and "GSE50760" datasets from the GEO database. For the differential gene expression (DGE) analysis, the "edgeR" package in R was used to identify significantly differentially expressed genes (DEGs) across the groups. Functional annotation of the identified DEGs was performed using "clusterProfiler" package, alongside complementary tools such as DAVID and STRING databases, to explore the biological pathways and processes associated with the differentially expressed OR genes. The common OR genes in each three main groups are detected and cross-checked with the TCGA PANCANCER Atlas dataset, accessed through "cBioPortal" database. Additionally, we identified OR genes that were present in tumor tissues but absent in metastatic tissues.

Results and Discussion

We identified 19 differentially expressed OR genes in cell line studies and 4 of them were common across each group which were "OR51E1", "OR51E2", "OR2A4", and "OR2A7" genes. We then utilized cBioPortal database to validate our target genes' expression levels in the largest CRC dataset. Functional annotation concerning these genes revealed that several EMT-related pathways such as the "TGF-β Signaling pathway", "Hedgehog Signaling pathway" and "WNT Signaling pathway" was upregulated when each of these OR's expression levels was increased. However, when the expression of individual EMT marker genes was inspected while OR51E1 and OR51E2 followed this trend, OR2A7 and OR2A4 showed inverse relationships with several key mesenchymal marker genes like "Vimentin", "SNAI1 and SNAI2", "Fibronectin" and "ZEB2" genes. The metastatic datasets also allowed us to highlight significant OR genes that are differentially expressed in tumor tissues but not in metastatic tissues when compared with normal tissues. Among the top 10 differentially expressed OR genes with this trend, "OR2A9P", "OR52N4", "OR10Q1", and "OR10D1" had significant expression levels to perform functional annotation analysis. Although these genes were involved in diverse processes, the enrichment score of EMT-related pathways were significantly lower (< 0.5) when compared with the first OR targets. In clinical level, when the expression of OR51E1, OR51E2, and OR10Q1 was higher, the patient's survival rate was worse, but this relationship was inversed for OR2A4, OR2A7, OR52N4, OR10AD1, and OR2A9P genes.

Conclusion

This study highlights the distinct roles of OR genes in CRC, particularly in EMT and metastasis. OR51E1 and OR51E2 may serve as therapeutic targets, while OR2A4 and OR2A7 could act as protective factors. The absence of certain OR genes in metastatic tissues suggests their potential effects as tumor suppressor genes.

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Keywords: Colorectal Cancer, Olfactory Receptors, Epithelial to Mesenchymal Transition, Bioinformatics.

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Investigation of ALCAM's Role In Glioblastoma Senescence

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1. Introduction

Glioblastoma (GBM) is a type of glioma that is the most common brain tumor in the Central Nervous System. Based on this classification, it is the highest-grade astrocytoma (grade IV), highly aggressive and invasive brain cancer. The primary treatment for GBM involves surgical resection to remove as much of the tumor as possible. This is typically followed by radiotherapy and concurrent temozolomide (TMZ) chemotherapy. Despite these multimodal approaches, the prognosis for GBM patients remains poor, with a median survival of approximately 12-16 months. Here, in this study, we wanted to investigate the role of Activated Cell Adhesion Molecule (ALCAM) in GBM senescence.

2. Material and Method

We used ALCAM targeting small interfering ribonucleic acid (siRNA) and constructed plasmid overexpressing it to manipulate the ALCAM gene level in GBM cells. Expression

levels of senescence markers and SA-βgal staining percentage were investigated upon these manipulations.

3. Results and Discussion

We found that the overexpression of ALCAM successfully increased the gene expression and protein levels in four different GBM cell lines, and siRNA resulted in significant reduction as verified by qPCR, western blot, and immunochemistry. ALCAM overexpression increased SA-beta-Gal staining and p21 gene expression level in T98G cell line. In addition, a variant of ALCAM, V4, is discovered as lncRNA, and found to be expressed in microglia.

4. Conclusion

There is a need for a more comprehensive study to understand the correct relationship. We continue to study the function of ALCAM variants in the context of brain aging and neurodegeneration.

Keywords: ALCAM, microglia, glioblastoma, senescence, neurodegeneration

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Bioinformatics Screening of Metabolic Gene Signature Associated with c-Met Over-Expression in Colorectal Adenocarcinoma to Discover Biomarkers and Target Genes

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Introduction

High c-Met expression was found to be correlated with poor prognosis by inducing early tumor invasion and metastasis. Studies have shown that increased c-Met expression promotes resistance to targeted therapies, including those targeting EGFR, BRAF, and MEK. Metabolic adaptation is found to be crucial in tumor metastasis and resistance to treatment. In this regard, enlightening c-Met-related metabolic gene signatures is very important to discover new candidates to predict tumor progression as well as therapy response in colon adenocarcinoma patients with elevated c-Met expression.

Material and Method

We used RNA-seq data from the TCGA, PanCancer Atlas Colon Adenocarcinoma cohort via cBioPortal. We stratified tumors into four quartiles based on MET gene expression. We plotted a heatmap of the metabolically significant genes that showed meaningful changes across the quarters. We analyzed the identified metabolic gene signature in-silico using an independent

cohort.Furthermore, our previous studies tested a metabolic gene set differentially expressed by c-Met inhibitor treatment. RT-qPCR studies validated hub metabolic genes using nonmetastatic and metastatic CRC cell lines, SW480 and SW620, respectively.

Results and Discussion

As a result of our Functional Analyses, eight metabolic pathways that showed significant changes were identified. Among these pathways, the three most significant pathways, according to p-value, are 'Retinol Metabolism,' 'Arachidonic Acid Metabolism,' and 'Galactose Metabolism' pathways. As a result of our analysis in cBioPortal, we identified 50 genes involved in metabolic pathways. By analyzing the basal expression levels of these genes in metastatic (SW620) and non-metastatic (SW480) cell lines in CCLE (Cancer Cell Line Encyclopedia) and Human Protein Atlas databases and literature, we identified a signature of 12 candidate metabolic genes (HMBS, HK2, H6PD, PDPR, PFKL, PDK2, GYS1, GLUT1, PDPR, HK2, H6PD and HMBS) differentially expressed in high c-Met group. The significant difference in HMBS, HK2, H6PD, and PDRP transcript levels was confirmed with RT-qPCR ($p = 0.02$; $p = 0.004$; $p = 0.000004$; $p = 0.00003$, respectively).

Conclusion

This study demonstrates the potential of using a bioinformatics approach to determine a metabolic gene signature associated with high c-Met expression to predict prognosis and drug resistance to treatment in colorectal adenocarcinoma. The findings of this study recommended that the proposed metabolic gene panel might be considered a potential prognostic and therapeutic signature for CRC. Further validation studies are needed to validate the importance of this signature to predict metastasis and drug resistance.

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Keywords: Colon Adenocarcinoma, c-Met, Metabolic Gene Signature, Metastasis, Bioinformatics

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Influence of Preanalytical Factors on the Quality of Tissue Samples

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Introduction

Biobanks play a pivotal role in precision medicine by providing the biological samples necessary for molecular profiling and biomarker discovery. The quality of the sample is the fundamental prerequisite for any reliable data analysis, biomarker investigations, or high-throughput analyses. Recent studies show that preanalytical procedures have a significant impact on sample quality and are critical to the reliability and reproducibility of results. Although many interfering factors that influence the quality of tissue samples were defined previously, no data comparing different preanalytical procedures on tissue integrity, as well as DNA, RNA, and protein quality in a tissue-specific manner. In this study, we aimed to investigate the effects of transfer conditions and freezing methods on tissue quality in three different tissues: brain, liver, and skeletal muscle.

Material and Method

This study was designed to compare transfer conditions and cold ischemia time of the rat liver, brain, and skeletal muscle for sample quantity. Seven male Wistar rats, 6-8 weeks of age, were used for this study (Ethical approval #2021-022) We first compared the effects of three different transfer conditions (direct transfer, transfer in DMEM, and transfer in vacuum begs) and cold ischemia durations. (0-2h; 2-8h and 8-24h) on sample quality. Then, we compared the influence of three freezing methods, snap freezing, isopentane treatment, and controlled rate freezer, on the sample quality. We tested tissue integrity with histopathological analysis, including Hematoxylin Eosin (H&E) and Masson's trichrome staining. DNA and RNA quality

were analyzed using a Bioanalyzer. Furthermore, the impact of transfer conditions and time on establishing primary cell lines was tested.

Results and Discussion

We observed that each tissue type requires different transfer and freezing conditions to protect sample quality, and their stability under cold ischemia is differential. The transfer of the liver at +4oC without media was sufficient to keep sample quality. In contrast, transferring brain tissues in the DMEM and the muscle in vacuumed begs brain tissues had better sample quality in prolonged ischemia times. The freezing method did not cause any significant difference in sample quality for any of the tissue types tested.

Conclusion

Standardized procedures for sample transfer methods and time for different tissue types are needed to prevent preanalytic errors, which are critical factors in the success of precision medicine.

Keywords: Biobank, pre-analytical phase, ischemia, tissue biobanking quality, precisional medicine