Invited Speaker – 01

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

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

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

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

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

Keywords: single cell analysis, cancer, chemotherapy, metastasis

Invited Speaker – 02

New Era in Life Sciences: Organoids & Spheroids

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We are witnessing history!

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

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

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

Invited Speaker – 03

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

Serdar Altınay

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

Key points

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

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

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

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

Invited Speaker – 04

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

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

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

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

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

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

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

Invited Speaker – 05

MicroRNA-based Therapeutics for Cancer

Bulent Ozpolat

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

Invited Speaker – 06

Tumor Genomic Profiling and Treatment Decision in Gastrointestinal Cancers

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

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

- Targeted Therapies: Genomic profiling can identify specific genetic mutations or alterations that are driving the growth of the cancer.
- Immunotherapy: Some genomic alterations may make tumors more susceptible to immunotherapy drugs like checkpoint inhibitors (e.g., pembrolizumab, nivolumab). Genomic profiling can help identify patients who may benefit from immunotherapy.
- Prognosis: Certain genetic mutations can provide information about the prognosis and aggressiveness of the cancer, which can guide treatment decisions and help patients and doctors understand the likely course of the disease.
- Clinical Trials: Genomic profiling may reveal opportunities for patients to participate in clinical trials testing new targeted therapies or experimental treatments based on their tumor's specific genetic profile.
- Personalized Treatment Plans: With the information obtained from genomic profiling, oncologists can develop more personalized treatment plans that take into account the unique characteristics of the patient's cancer. This can lead to more effective and less toxic treatment options.

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

Invited Speaker – 07

Precision Medicine in Pediatric Tumors: Next Generation Sequencing Experience

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

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

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

of DICER1 mutation in a case of ovarian juvenile granulosa cell tumour contributed clinically in terms of genetic predisposition. The importance of mutations in the differential diagnosis of aneurysmal bone cyst and telangiectatic osteosarcoma has been questioned.

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

Keywords: Paediatric tumours, precision medicine, next generation sequencing

Invited Speaker – 08

The Molecular Link Between Excitotoxicity and Glioblastoma

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

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

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

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

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