Oral Presentation Abstracts

Podium Talks Abstracts

Session I: Podium Talks with Patient Advocates - Tumor Immunology and microenvironment

Plasma Exosomes in Obesity-driven Diabetes Exacerbate Progression of Triple Negative Breast Cancer: Insights from Animal Models
Pablo Llevenes, Not Applicable

Abstract: Objective: This study examines how obesity and type 2 diabetes (T2D) drive metabolic changes in adipose tissue, promoting triple negative breast cancer (TNBC). We focus on plasma exosomes as mediators of gene expression changes, hypothesizing that T2D and obesity alter exosome payloads, particularly microRNAs. Exosomes reshape the tumor microenvironment, promoting epithelial to mesenchymal transition (EMT) and TNBC metastasis. Our goal is to understand mechanisms linking T2D, obesity, and TNBC metastasis to identify therapeutic targets.

Methods: Female C57BL/6J mice were subjected to a high-fat diet (HFD; 60% Kcal fat) for 12 weeks, followed by an oral glucose tolerance test. Plasma exosomes were isolated and used to treat E0771 cells, a TNBC model, for 72 hours for analysis. Gene expression changes were assessed using an EMT array kit and qPCR, with data analyzed via Qiagen‚Äôs Ingenuity Pathway Analysis tool. Cell migration was evaluated using a transwell migration assay. For in vivo analysis, mice were injected with E0771-GFP cells in the mammary fat pad, and tumors were analyzed after 28 days via cell culture and flow cytometry.

Results: In vitro, HFD-derived exosomes reprogrammed gene expression in E0771 cells, driving EMT and a pro-metastatic phenotype. In vivo, HFD exosomes increased metastatic burden in the lungs and brain.

Conclusions: HFD-derived plasma exosomes reprogram EMT gene networks in E0771 cells, promoting brain metastases. This suggests that TNBC patients with obesity-driven diabetes should be assessed for increased metastasis risk, and exosome miRNA profiling could serve as a valuable biomarker in populations with high obesity and diabetes prevalence.

Spatial Analysis of Immune-Related Adverse Events Using Novel Animal Models in Myocarditis and Neurotoxicity
Kun Han, Cancer Systems Biology Consortium (CSBC)

Abstract: Immune related adverse events (irAEs) have emerged as a significant challenge in immune checkpoint blockade (ICB) therapy. To better understand the mechanisms underlying these conditions and develop effective treatments, we established two novel animal models of myocarditis and neurotoxicity.

Our studies revealed that Type 3 T cells are critical in the pathogenesis of both irAEs. We performed spatial transcriptomic analysis on the myocarditis mouse model and identified distinct functional cardiac regions with characteristically enriched signaling pathways. Type 3 T cells , NK cells, and DCs largely infiltrated into the heart tissue, which could be eliminated by a selected rescue drug. However, there were no significant changes in three memory T cell subtypes (central memory T cells, effector memory T cells, and tissue-resident memory T cells) upon treatment, suggesting the potential role in long-term cardiac damage and potential recurrence. Additionally, we observed certain activated metabolic and epigenetic pathways in these memory T cells, implying that their collaborative role in myocarditis. We are conducting similar spatial analysis on the neurotoxicity model.

Beyond cell profiling, we applied our Spatial Single Cell Crosstalk modeling tool, S2C2, on spatial transcriptomics data from these irAEs models to delineate key immune-related pathways activated in the immune-tumor microenvironment of these models of myocarditis and neurotoxicity, aiming for a systematic understanding of the underlying mechanisms.

In conclusion, the presented systems immunology strategy based on two novel irAEs models provides a comprehensive platform for studying adverse events during cancer immunotherapy, potentially mitigating irAEs and improving the efficacy of ICB therapy.

Lay Abstract: Immune-related side effects (irAEs) have become a major issue in immune checkpoint blockade (ICB) therapy. To understand these side effects better and find effective treatments, we created two new animal models for myocarditis and neurotoxicity.

Our research showed that Type 3 T cells play a crucial role in causing both types of irAEs. We used spatial transcriptomic analysis on the myocarditis model and discovered different functional areas in the heart with specific signaling pathways. We found that Type 3 T cells, NK cells, and DCs infiltrated the heart tissue, and a selected drug could remove these cells. However, three types of memory T cells (central memory T cells, effector memory T cells, and tissue-resident memory T cells) did not change significantly with treatment, suggesting they might cause long-term heart damage and recurrence. Additionally, we saw activated metabolic and epigenetic pathways in these memory T cells, indicating their role in myocarditis. We are conducting similar analyses on the neurotoxicity model.

Beyond cell profiling, we used our Spatial Single Cell Crosstalk modeling tool (S2C2) on spatial transcriptomics data from these irAEs models to identify key immune-related pathways in the immune-tumor microenvironment of these myocarditis and neurotoxicity models, aiming for a comprehensive understanding of the mechanisms involved.

In conclusion, our system immunology approach using these two new irAEs models offers a thorough platform for studying side effects during cancer immunotherapy, which could help reduce irAEs and improve the effectiveness of ICB therapy.

Apoptotic cells promote circulating tumor cell survival and metastasis

Cassidy Hagan, Metastasis Research Network (MetNet)

Abstract: During tumor progression and especially following cytotoxic therapy, cell death of both tumor and stromal cells is widespread. Despite clinical observations that high levels of apoptotic cells correlate with poorer patient outcomes, the physiological effects of dying cells on tumor progression remain incompletely understood. We found that circulating apoptotic cells robustly enhance tumor cell metastasis to the lungs. Using intravenous metastasis models, we observed that the presence of apoptotic cells, but not cells dying by other mechanisms, supports circulating tumor cell (CTC) survival following arrest in the lung vasculature. Apoptotic cells promote CTC survival by recruiting platelets to the forming metastatic niche. During apoptosis, membrane phosphatidylserine flips to the outer leaflet; this exposure of phosphatidylserine on the cell surface increases the activity of the coagulation initiator Tissue Factor. By activating the coagulation cascade in this way, apoptotic cells support the formation of platelet clots that protect proximal CTCs. Inhibiting the ability of apoptotic cells to induce coagulation by knocking out Tissue Factor, blocking phosphatidylserine, or administering the anticoagulant heparin abrogated the pro-metastatic effect of apoptotic cells. This work demonstrates a previously unappreciated role for apoptotic cells in facilitating metastasis by establishing CTC-supportive emboli, and suggests points of intervention that may reduce the pro-metastatic effect of apoptotic cells.

Lay Abstract: To fight cancer, most therapies work by causing tumor cells to die. However when cells die they do not simply disappear from the body, but rather the body perceives cell death as a wound that needs to be healed. Some studies have indicated that cell death followed by healing programs can actually promote tumor growth. Researchers have even observed that patients with dying tumor cells in their blood are more likely to develop metastasis. Metastasis, the spread of tumor cells throughout the body, is the leading cause of death in cancer patients, however, we don't fully understand how dying cells impact metastasis. Therefore we asked, do dying cells support the spread of live tumor cells throughout the body? And if so, how? To model metastasis we injected live tumor cells alone or in combination with dying tumor cells into the blood of mice. We found that when dying cells were present the mice had significantly more lung metastases. We determined that the surface of dying cells contains molecules that activate blood clotting. These clots disguise and protect tumor cells as they spread through the blood thereby allowing them to form metastases. While killing tumor cells is necessary to fight cancer, our work shows that cell death can inadvertently support metastasis, however, by blocking the features of dying cells that promote clots we may be able to counter this unintended consequence.

Claudin 7 suppresses invasion and metastasis through repression of the smooth muscle actin cytoskeleton

Junior West, Metastasis Research Network (MetNet)

Abstract: Claudin-low breast cancer is highly invasive, metastatic, and defined by down-regulation claudin genes. However, we lack a mechanistic understanding of how dysregulated claudin expression impacts the complex cell and molecular features of this subtype. Using organoids derived from independent genetically engineered mouse models (GEMMs) that reflect various breast cancer subtypes, we probed for expression of claudins. We observed a specific and conserved down-regulation of claudin 7 (Cldn7) as cells become invasive. We demonstrate that Cldn7 function is critical for suppressing invasion and metastasis by coupling Cldn7 loss-of-function and gain-of-function experiments with spontaneous in vivo metastasis assays and in vitro 3D organoid invasion assays in mouse and human models. Next, we revealed the underlying changes to gene expression that are associated with Cldn7 function using RNA-sequencing. Through this analysis we found that several genes associated with the smooth muscle actin cytoskeleton (SMA) are up-regulated in Cldn7 depleted organoids. This relationship was validated using organoids derived from GEMMs, patient derived xenograft models, and clinical samples. We further validated this relationship by making use of METABRIC and single cell breast cancer

atlas transcriptomic databases to confirm that Cldn7-low cells display up regulation of SMA related genes. Finally, we demonstrated that the up-regulation of SMA-related proteins promotes invasive cell behaviors through both small molecule inhibition and experimental up- and down-regulation of SMA related genes. Taken together, our results provide molecular mechanisms that explain how the loss of claudin expression observed in claudin-low breast cancer leads to increased invasive and metastatic cell behaviors.

Lay Abstract: Breast cancer is a complex disease that is divided into subgroups based on molecules that are present or absent in different tumor types. This study is inspired by an aggressive type of breast cancer called “claudin-low (CL) breast cancer”, which gets its name from the fact that these tumors lack the presence of critical claudin molecules. While we have known that the lack of claudin molecules correlate with this aggressive breast cancer, we know much less about how loss of these molecules actually impact the progression of this disease. In this study we used miniaturize versions of tumors, termed “organoids”, combined with genetic, cellular, and molecular biology approaches to reveal how loss of claudin molecules impact the aggressive potential of breast cancers. We found that a specific claudin, claudin 7, is lost in aggressive breast cancer cells and is responsible for stopping aggressive cancer cell behaviors. We also unveiled that loss of claudin 7 results in the production of other molecules that increase aggressive cancer cell behaviors. Taken together, these results reveal mechanisms that underlie the previously observed correlations between claudin molecules and aggressive breast cancers.

Dynamic modeling of tumor progression illuminates an occult transition that establishes metastatic potential

Joshua Ginzel, Innovative Molecular Analysis Technologies (IMAT)

Abstract: Cancer mitigation strategies seek to reduce metastatic burden by screening and treating at an early stage. However, early detection efforts have not reduced metastatic burden in all cancers, indicating that invasive adaptations occur before a detectable tumor forms. We previously developed a breast cancer mouse model for visualizing three fluorescently tagged versions of the HER2 oncogene in one mouse. Spatial transcriptomics analysis reveals cellular neighborhoods distinct to each tumor type. Exon spliced HER2 driven tumors are relatively homogenous, while truncated HER2 tumors are enriched with infiltrating immune cells and cancer associated fibroblasts. In order to understand when these adaptive cancer niches develop, thousands of observations across both the invisible and visible phases of tumorigenesis were collected through whole organ imaging. Mathematical modeling via non-linear dynamical systems describes the rate of progression from an oncogenic cell, to an occult lesion, and finally to a screen detectable tumor. The exon spliced oncogene produces large, expansile fields of cancer cells which frequently stall during progression resulting in a large reservoir of screen detectable lesions prior to invasion and metastasis. In contrast, the truncated form of HER2 does not produce proliferative fields of cells but progresses rapidly and disseminates while still an undetectable, occult lesion. This data illustrates how adaptive cellular niches are organized during an occult checkpoint which leads to non-linear tumor progression. We hope to leverage spatial analysis of this occult transition to target the adaptive spatial ecology of lethal, early disseminating cancers.

Lay Abstract: Despite significant effort and innovation in finding and treating cancers earlier, metastatic breast cancer rates have not significantly decreased. This suggests that the majority of metastasis actually begins when the primary tumor is still invisible to early screening. We used a mouse model of breast cancer that allows us to study this invisible phase of tumor development in three different types of breast tumors and collect a significant amount of data on how these tumors grow and progress. Mathematical modeling of these observations reliably describes two common clinical outcomes. The first is a proliferative cancer that makes tumors that are

detectable, and therefore easy to treat, before metastasis can occur. The second outcome is a cancer that does not grow rapidly early on but is able to spread to other organs before the tumor is screen detectable, making it much harder to successfully treat. These observations of tumor size and tumor metastasis across the life of a tumor provides experimental evidence of a checkpoint where the lethality of a cancer is determined before the cancer is clinically detectable. Pairing these findings with spatial analysis of tumor cells and the normal cells they interact with allows us to build cellular neighborhoods that define the ability of a cancer to spread. Understanding how these cellular neighborhoods are established during this checkpoint represents a promising opportunity for how and when lethal cancers can still be detected and intercepted to prevent metastasis and death.

Session IV: Podium Talks with Patient Advocates - Multiomics and Computational Cancer Research

Discrete Representation Learning for Modeling Imaging-based Spatial Transcriptomics Data

Dig Vijay Kumar Yarlagadda, Cancer Systems Biology Consortium (CSBC)

Abstract: Imaging-based spatial transcriptomics (ST) provides single transcript level spatial resolution for hundreds of genes, unlike sequencing-based ST technologies whose resolution is limited to physical capture regions (spots) on slides. Existing methods to identify patterns of interest in imaging-based ST data are built as extensions of single cell analysis methods, mostly ignoring valuable spatial information encoded in the raw imaging data. Here we present a discrete representation learning approach for modeling spatial gene expression patterns in ST datasets. By employing raw coordinates of detected transcripts and positional encoding of cell centroids as inputs, we learn discrete representations using Vector Quantized-Variational Autoencoder (VQ-VAE) to extract multi-scale structures from fluorescence in situ hybridization based ST datasets. We demonstrate the usefulness of discrete representations in terms of the quality of embedding of ST data as well as improved performance on downstream tasks for extracting biologically meaningful cellular neighborhoods and spatially variable genes. We applied the model to study mechanisms of treatment resistance in metastatic renal cell carcinoma (RCC) tumors. By analyzing patient RCC tumors with single cell sequencing and ST, we identified that RCC tumors exhibit a continuum of developmental states resembling nephrogenic developmental program, and tumors exposed to immune checkpoint blockade (ICB) therapy are enriched for nephron progenitor-like cancer (NPC) cells. To investigate mechanisms contributing to immune evasion of NPC cells, we are utilizing our model for interrogating the cellular neighborhoods of NPC cells and dissecting the longitudinal effects of ICB therapy with novel immunocompetent mouse models of RCC.

Lay Abstract: Spatial transcriptomics (ST) is an emerging technology that can profile RNA expression in intact tissues, while previous techniques break tissues into individual cells to measure gene activity. This spatial context is crucial for studying cancer, for example, in understanding how cancer cells interact with their surrounding cells and environment, which influences tumor growth and therapy resistance. Current methods for analysis of ST data mainly rely on summary statistics like cell by gene matrices, often missing important details captured in the ST data. Inspired by advances in computer vision, we created a new method using a paradigm called discrete representation learning, to better capture these details. We demonstrate the usefulness and effectiveness of our method on several tasks for extracting biologically meaningful cellular neighborhoods and spatially variable genes from the ST data. By applying this method on ST data from renal cell carcinoma tumors, we identified a population of cancer cells reexpressing

properties typically seen during early-stage embryonic development. These cells further seem to resist immune checkpoint blockade therapy, which is designed to provoke immune cells to target cancer cells. To understand the mechanisms employed by these cancer cells to avoid killing by the immune system, we are examining how these cells interact with their neighbors and testing the interactions between immune-cancer cells mediating resistance to immunotherapies in preclinical mouse models.

Unveiling genetic complexity of lung adenocarcinoma by identifying a novel EGFR mutation signature

Minjeong Kim, MeDOC

Abstract: Lung cancer is the leading cause of death in the United States. Lung adenocarcinoma (LUAD) is a subtype of non-small cell lung cancer (NSCLC), comprised of malignant alveolar type II epithelial cells. Epidermal growth factor receptor (EGFR) is a transmembrane protein with cytoplasmic kinase activity and the most common genetic alteration found in LUAD followed by other driver genes like KRAS, ALK, ROS1, RET, MET, BRAF and HER2. FDA-approved EGFR kinase domain antagonists, also known as tyrosine kinase inhibitors (TKIs), target the kinase domain of EGFR for lung cancer treatment. However, resistance to EGFR-TKIs, primarily gefitinib and erlotinib, remains a significant challenge, necessitating the identification of EGFR-TKI resistance-related gene signatures in LUAD. This study aims to characterize and validate an EGFR mutation signature in LUAD patients via analyzing four different cohorts (MSKCC, UNC, TSP and TCGA). The training dataset consisted of 192 patients and was analyzed via Significant Analysis of Microarray, with a false discovery rate of 1% or less. Differentially expressed genes were used to establish a new EGFR mutation signature, which was validated in independent datasets. A novel EGFR mutation signature consisting of 1020 genes was identified which demonstrated high predictive performance for EGFR mutation status in both training and validation sets. Furthermore, the EGFR signature was significantly associated with the LUAD Bronchioid subtype and improved 5-year survival outcomes. In sum, this project identified a novel EGFR mutation signature which elucidates the underlying mechanisms and complexity of LUAD, potentially leading to optimized treatment strategies in the future.

Lay Abstract: Lung cancer remains a formidable challenge, ranking as the second most prevalent cancer and the leading cause of cancer-related deaths in the United States. Lung adenocarcinoma (LUAD), a subtype of non-small cell lung cancer, has been the focus of intensive research efforts. This study aimed to identify and validate a unique genetic signature associated with mutations in critical genes, including EGFR, a key player in LUAD pathogenesis. The patients from four different clinical studies were analyzed to uncover a novel EGFR “fingerprint” or mutation signature that we hope will help improve patient outcomes. The first dataset included 192 patients, and using a rigorous statistical analysis, we identified genes of interested. This analysis led to the establishment of a new EGFR mutation fingerprint signature comprising 1,020 genes that we consider a “training” set of genes that we next verified in other datasets. Remarkably, the newly identified EGFR mutation signature exhibited a strong association with subtypes of LUAD and also predicted improved 5-year survival outcomes. To confirm these findings, the EGFR mutation signature was tested across independent datasets to ensure this fingerprint was a truly consistent finding. In short, the novel EGFR signature exhibited a high ability to predict EGFR mutations in both training and validation sets. Furthermore, the signature pointed to a specific subtype of LUAD called bronchioid and associated with improved survival outcomes in datasets. Results presented herein pave the way for optimized and personalized treatment strategies tailored to individual genetic profiles, ultimately improving treatment outcomes and saving lives.

Investigating Tumor Immune Suppression through Integration of PD1-PD-L1 Interactions with MDSC & T Cell Phenotypes

MOHD SAQIB, Cellular Cancer Biology Imaging Research (CCBIR)

Abstract: PD-1 or PD-L1 therapies fail in about 50% of PD-L1 positive cancer patients. High PD-L1 expression in tumors enrolls cancer patients for this therapy, but it can be deceptive as PD-1/PD-L1 interactions, not just PD-L1 levels, contribute to immune evasion in cancer. Numerous such interactions involving cancer and immune cells determine the fate of a growing or established tumor. Hence, a comprehensive analysis of interactions encompassing PD-1/PD-L1 interactions and participating immune cell types could offer a more reliable approach to assessing the immune status within tumors of cancer patients.

Using Fluorescence Lifetime Imaging - Förster Resonance Energy Transfer (FLI-FRET), we reliably quantified PD-1/PD-L1 interactions ex vivo in E0771 breast tumors from syngeneic C57BL/6 mice undergoing various treatments. By employing immunological and imaging techniques, we confirmed that both cancer cells and Myeloid Derived Suppressor Cells (MDSCs) contribute to PD-1/PD-L1 interactions in tumors. MDSCs may play a dominant role due to their higher PD-L1 expression compared to cancer cells. Surprisingly, PD-L1 expression alone does not determine the tumor growth rate. This was evidenced by inhibiting IFNAR-1, which reduced PD-L1 expression on both cancer cells and MDSCs, yet the tumor growth accelerated. This is likely due to reduced Ly6C expression on MDSCs and a decreased CD4 to CD8 T cell ratio following IFNAR-1 inhibition. In conclusion, our findings advocate quantifying PD-1/PD-L1 interactions, Ly6C expression on MDSCs, and the CD4 to CD8 T cell ratio to reliably diagnose cancer patients likely to respond to anti-PD-1 or PD-L1 therapy and predict any ongoing immunotherapy outcomes.

Lay Abstract: Immunotherapies targeting PD-1 or PD-L1 don't work for about half of cancer patients. These patients receive this therapy because their tumors have high levels of PD-L1. This could be misleading as cancer survives in the body due to interactions involving PD-1 and PD-L1, not solely because PD-L1 is expressed in tumors. These interactions suppress the immune system, preventing specialized T cells from controlling tumor growth. Immunotherapies are administered to help overcome this. Cancer cells often work together with Myeloid Derived Suppressor Cells (MDSCs) to inhibit the function of T cells. Hence, a thorough analysis of PD-/PD-L1 interactions and participating cells like T cells and MDSCs, could offer a more reliable approach to assessing the immune status within tumors.

We have developed a method using Fluorescence Lifetime Imaging - Förster Resonance Energy Transfer (FLI-FRET) to measure PD-1/PD-L1 interactions in breast tumor tissues. Using immunological tools, we discovered that MDSCs play a bigger role than cancer cells in suppressing T cells and in PD-1/PD-L1 interactions. We also found that when the ratio of CD4 to CD8 T cells drops and MDSCs have less Ly6C on their surface, the immune system becomes less effective against cancer. This was observed in rapidly growing tumors after blocking protective type 1 interferon pathways. Based on our findings, checking PD-1/PD-L1 interactions, Ly6C expression on MDSCs, and the CD4 to CD8 T cell ratio could be a more reliable way to identify patients who may benefit from anti-PD-1 or PD-L1 therapy and predict the effectiveness of ongoing immunotherapy.

3D agent-based modeling of glioblastoma subtypes and therapies

Riley Manning, Cellular Cancer Biology Imaging Research (CCBIR)

Abstract: Glioblastoma is an aggressive and highly infiltrative brain cancer with a poor survival

rate. Treatment options for patients are limited and have remained stagnant for almost two decades despite dozens of clinical trials. Advancements in genomic characterization methods allowed for the classification of three different subtypes of glioblastoma: proneural, classical, and mesenchymal. The distinct genetic alteration patterns in these subtypes lead to downstream differences in cell migration speed and immune system activation status. Despite these behavioral differences, glioblastomas are treated using a uniform standard of care regardless of tumor subtype. This work utilizes a three-dimensional agent-based model to simulate growth and development of distinct glioblastoma subtypes. Cell proliferation and migration as well as intercellular interactions are modeled for both cancer cells and cytotoxic T cells. Using data from a genetically engineered mouse model of proneural and mesenchymal glioblastomas, we are able to simulate and visualize subtype-specific outcomes. We simulate the glioblastoma standard of care protocol, newer treatment strategies such as anti-migratory therapy and adoptive T cell transfer as well as combination therapies to determine which regime is most effective at impairing tumor growth. Inhibition of cancer cell migration in combination with adoptive T cell transfer therapy significantly increases the efficiency of cytotoxic T cell-mediated cancer cell killing.

Lay Abstract: Glioblastoma is a quickly progressing form of brain cancer with a poor survival rate and few treatment options. Glioblastomas can be classified into subtypes based on distinct patterns of genetic alterations. Although different subtypes of glioblastoma have measurable differences in cell migration speed and immune response, they are treated similarly in the clinic. This work uses computational modeling to simulate glioblastoma tumor growth and development. The simulator uses a method called agent based modeling, which calculates three-dimensional cell movement and growth for each individual cell. This allows us to simulate and analyze individual cell behaviors and interactions between cells as well as overall tumor dynamics. By using data from experimental methods, we are able to simulate tumor subtypes and visualize unique growth patterns and responses to treatment. We simulate not only a standard treatment protocol for glioblastoma (resection, chemotherapy, and radiation), but also newer treatment strategies including anti-migratory therapy and adoptive T cell transfer therapy, which increases the number of cancer-fighting cytotoxic T cells in the tumor. This allows us to theoretically test treatments and combinations of treatments to determine which strategy most effectively reduces tumor growth. In this work, we find that the addition of anti-migratory therapy to adoptive T cell transfer therapy significantly increases the ability of cytotoxic T cells to find and kill cancer cells. This demonstrates the power of computational modeling and its ability to test new and innovative treatment methods.

AI-Enhanced Rapid Lifetime Determination Method for Fast Macroscopic Fluorescence Lifetime Imaging

Vikas Pandey, Innovative Molecular Analysis Technologies (IMAT)

Abstract: Fluorescence lifetime imaging (FLI) is an advanced method that allows for non-invasive, quantitative mapping of unique biosignatures, enhancing the identification of tumor margins, and monitoring treatment responses. Macroscopic-FLI (MFLI), used for a wide-field imaging (over multiple square centimeters), requires large-format time-resolved imagers with high pixel density. Over last decade, many large-format time-gated single photon avalanche diode (SPAD) array, and time-gated ICCD cameras are developed, which are central for MFLI. Still, the adoption of MFLI in surgical settings is hindered by (1) its prolonged acquisition time to collect full temporal fluorescence photon histograms, (2) repeated acquisition of instrument response function, and (3) intricate data processing pipeline involving time-consuming iterative curve fitting algorithms and/or phasor method.

Herein, we have developed an AI-enhanced rapid lifetime determination (RLD) method using the

state-of-the-art large-format SPAD array, SwissSPAD3 (SS3), containing a novel dual-gate acquisition architecture within the total exposure time of the sensor (Wayne M. et al.,2022). This AI-enhanced RLD method requires a single-shot acquisition using SS3 and generates large-format fluorescence lifetime maps in near real-time (~5fps). We validated this RLD-based approach in vitro using NIR dye/probe samples, Alexa Fluor (AF) 700 dye in different solvents, and estimated the lifetime contrast in tissue-mimicking phantoms, demonstrating its potential to enhance precision fluorescence lifetime identification. Future work will extend this methodology to HER2+ tumor models to confirm its clinical applicability. In conclusion, the AI-enhanced single-shot fluorescence lifetime estimation using the SwissSPAD3 detector might be a significant advancement in the RLD method, with promising potential for fluorescence-guided surgical applications.

Lay Abstract: Fluorescence lifetime imaging (FLI) is a technique used to study how long photon emitted by fluorescent molecules lasts after excitation. This method is valuable in medicine, particularly in cancer surgery, tumor detection, and track how well treatments work. Macroscopic-FLI (MFLI) extends this capability across large area imaging, needing advanced imagers like SPAD arrays and ICCD cameras. However, MFLI has challenges: it takes time to gather data, needs repeated calibrations, and involves complex analysis. To address these, we have developed a new method called AI-enhanced rapid lifetime determination (RLD). Using SwissSPAD3, a cutting-edge SPAD array, and AI technology, this method dramatically speeds up lifetime imaging. It captures fluorescence lifetimes in real-time (about 5 frames per second) in a single shot, making it way faster than traditional methods.

In our tests on NIR fluorescent dyes and tissue-like phantoms, AI-enhanced RLD showed highly promising in accurately measuring fluorescence lifetimes. This precision could improve how surgeons identify cancerous tissue during operations, potentially leading to more successful surgeries with fewer complications. Our future research aims to apply RLD to specific cancer types, HER2 positive tumors, to confirm its usefulness in clinical settings. If successful, this approach has potential to transform fluorescence-guided surgery, offering quicker, more reliable detection of cancerous tissues. In summary, the AI-enhanced RLD method using SwissSPAD3 shows great promise for fluorescence lifetime imaging. By making the process faster and more accurate, it could enhance cancer treatment outcomes by ensuring surgeons can better see and remove cancerous tissues during operations.

Spotlight Talks Abstracts

Concurrent Session IIA: Spotlight Talks - Translational cancer research and drug discovery

DNA Hypermethylation Impacts Tumor Suppressor Genes in BXD Preclinical Mouse Models

Samson Eugin Simon, Not Applicable

Abstract: Breast cancer (BC) is a heterogenic disease that varies in its molecular characteristics, clinical behavior, and treatment responses. Triple-negative breast cancer (TNBC) accounts for 15-20% of all BCs and is associated with aggressive clinical behaviors. Understanding the epigenetic modifications, particularly DNA methylation, in driving TNBC aggression will aid in identifying novel molecular targets. Therefore, we took advantage of two well-established genetic models to test our hypothesis that methylation of novel tumor suppressor genes will exacerbate TNBC aggression: 1) the BXD preclinical model, developed by crossing two strains, C57BL/6J ("B") and DBA/2J ("D") producing recombinant inbred lines (RILs) that have a consistent genetic background and 2) the C3(1)-Tantigen (C3Tag) genetically engineered mouse model (GEMM). We crossed C3Tag with >50 BXD strains resulting in BXD-BC progeny in which females develop spontaneous breast tumors. BXD-BC mice display significantly heritable variations in TNBC characteristics such as tumor latency, multiplicity, and survival. Through an unbiased systems genetics, a significant quantitative trait locus (QTL) was identified at Chr10 with Rassf3 as candidate gene for as a modifier of tumor latency. Rassf3 (Ras Association Domain Family Member 3) is a tumor suppressor gene. Total DNA methylation quantification correlated with Rassf3. In sum, this novel murine model allows for a unique approach to understanding how DNA methylation regulates genetic modifier expression status relating to TNBC. Findings from this study provide valuable insights into the mechanisms underlying breast cancer aggression and highlight the potential of epigenetic interventions in improving breast cancer outcomes.

Lay Abstract: Breast cancer (BC) is the most common cause of death from cancer among women worldwide. Over two million women are diagnosed each year. BC is not one disease. It is actually several subtypes of BC with triple negative breast cancer (TNBC) displaying the worst outcomes for patients. Variation in BC is characterized by the accumulation of genetic and epigenetic modifications that changes genes and pathways that drive or suppress cancer involved in cell proliferation, differentiation, survival, and cell death. Epigenetics includes DNA methylation which plays a pivotal role in gene expression regulation, potently impacting cancer development and progression. We have created a preclinical model driven to develop TNBC but has variation in genetics to aid in identifying genetic and epigenetic variation to identify novel targets. This study explores the role of DNA methylation in BC using the BXD-BC preclinical model. By examining the cancer traits and total DNA methylation profiles of tumors in the BXD-BC model, we aim to identify specific genomic regions associated with methylation patterns using advanced computational analysis. Preliminary findings indicate that differential DNA methylation alters the expression of tumor blocking gene Rassf3, which significantly correlates with when tumors arise. These epigenetic changes may serve as biomarkers for early detection and prognosis of BC. In sum, findings suggest that reversing dysfunctional methylation may be a therapeutic strategy.

Recapitulation of Skeletal Metastasis Using an In Vitro Organ-on-a-Chip Platform

Kacey Ronaldson-Bouchard, Tissue Engineering Collaborative (TEC)

Abstract: Prostate and breast cancers often metastasize to the bone, leading to osteoblastic and osteolytic lesions, respectively. Current animal models fail to accurately mimic human skeletal metastasis. This study utilizes a novel organ-on-a-chip platform to investigate the mechanisms underlying these bone remodeling processes and identify potential therapeutic targets. We employed a microfluidic multiorgan platform incorporating bone, heart (negative control), liver, and blank hydrogel (perfusion control). Prostate cancer cell lines (LnCAP, PC3, VCaP) and the breast cancer cell line (MDA-MB-231) were evaluated for their metastatic behaviors. Our platform

successfully recapitulated the clinically expected functional skeletal remodeling behavior of metastatic prostate and breast cancers. Further functional analysis revealed osteoblastic and osteolytic bone formation and resorption, respectively, as detailed by uCT, histology and cytokine release profiles. Gene expression analysis revealed distinct profiles for osteoblastic (LnCAP) and osteolytic (PC3, MDA-MB-231) conditions. Key upregulated genes in osteoblastic conditions included RUNX2, BGLAP, and ESR1, whereas osteolytic conditions showed increased expression of MMP9, TNFSF11, and ACP5. Therapeutic intervention with drugs of known efficacy showed clinically expected behavior within our OOC platform. This organ-on-a-chip model provides a robust platform for studying skeletal metastasis, offering insights into the molecular mechanisms driving bone remodeling. Our findings highlight critical gene targets and pathways that may inform the development of targeted therapies for bone metastasis. This work underscores the potential of organ-on-a-chip technology in translational cancer research, serving as an additional tool where current animal models may not fully represent the human clinical presentation.

In situ visualization of covalent cancer drug binding in 3D mammalian tissue

Zhengyuan Pang, Innovative Molecular Analysis Technologies (IMAT)

Abstract: Covalent drugs like penicillin and aspirin represent some of the most successful therapeutics in human history. The recent decade has seen a renewed and resurgent interest in covalent drug development, including targeted inhibitors for EGFR, BTK, FGFR, and, most notably, KRAS G12C. These novel covalent drugs have truly transformed the therapeutic landscape of multiple cancers. However, due to concerns about potential irreversible off-target exposure, it is critical to comprehensively understand the in vivo on- and off-targets of covalent drugs across tissues and organs. Currently, direct visualization of small molecule drug binding in mammalian tissue is limited by insufficient imaging resolution. By strategically integrating tissue clearing and click reaction, we developed a strategy termed clearing-assisted tissue click chemistry (CATCH) for highly specific covalent drug binding mapping with cellular resolution. Our recent technical innovation has allowed us to drastically increase imaging volume and directly visualize drug binding across the whole mouse body with single-cell resolution. By mapping the binding of a BTK inhibitor Ibrutinib and an EGFR inhibitor Afatinib, we uncovered shared and distinct drug-bound structures across the body. Guided by whole-body drug characterization, we further examined the detailed drug-positive cell types of the two kinase inhibitors. The study would pave the way for covalent drug mechanism of action studies and provide a roadmap for future covalent drug development.

Lay Abstract: Whenever we take a pill, we are always curious about what the drug will do to our body. Our conventional wisdom of drug actions generally involves finding what biomolecules the drug is binding. With years of innovation, we now have a rich collection of tools to uncover the molecular targets with drug engagement. On the other hand, to ensure an anti-cancer drug can exert maximal efficacy while maintaining good safety profiles, it would be highly desirable if the drug molecule selectively engages and induces toxicity in the target cancer cells in the tumor tissue while maintaining minimal exposure to other healthy cells and tissue. However, current methods tracing drug in vivo distribution still lack the resolution to identify drug-binding events within individual cells. This calls for better tools to map and visualize drug binding in intact tissue. We tackled the challenge by utilizing advanced tissue imaging methods and highly specific chemical transformations in mammalian tissue. Our pipeline, termed CATCH, enabled visualization of small molecule drug binding with single-cell resolution in the whole mouse body. By comparing the binding of a non-small cell lung cancer drug and a leukemia drug, we uncovered shared and distinct drug binding patterns of the two drugs across different organs and tissues. Meanwhile, the findings can guide us to further examine the exact identities of drug-positive cells

in individual organs. The technical capability will bring new understandings of how drug molecules exert effects on our bodies.

Enhancing Prediction of Trastuzumab Deruxtecan Response and Resistance Mechanisms in Metastatic Breast Cancer Through Spatial Profiling and Cellular Crosstalk Analysis

Wenjuan Dong, Cancer Systems Biology Consortium (CSBC)

Abstract: Trastuzumab deruxtecan (T-DXd) is a promising antibody-drug conjugate (ADC) targeting breast cancers with varying HER2 expression levels. However, resistance remains a challenge, with non-response rates of 29.4% in HER2-overexpressing patients and 62.5% in low HER2 expression patients. Interestingly, 29.7% of HER2-non-expressing patients do respond. To better stratify metastatic breast cancer patients for T-DXd treatment and identify resistance mechanisms, we profiled 33 metastatic tissue samples before treatment using GeoMX.

Among the examined 192 regions of interest (ROIs), despite the heterogeneity in HER2 expression, responding patients exhibited significantly higher HER2 scores compared to non-responders based on their HER2 scores represented by the percentage and intensity of HER2 expression. However, HER2 scores alone only achieved 62% accuracy of predicting T-DXd response. To increase prediction accuracy, we characterized stroma and immune scores. Although stroma scores showed no significant differences, immune scores were significantly higher in responders.

Further analyses revealed that specific immune cells, including NK cells, B cells, and T cells were markedly enriched in ROIs from T-DXd responders. Conversely, neutrophils and PD-L1+ cells negatively correlated with T-DXd response. To elucidate the underlying mechanisms, we characterized the molecular features of each immune cell cluster through a spatial whole transcriptome assay on selected T-DXd responders and non-responders. Using our spatial cell-cell modeling tool, S2C2, we identified key signaling pathways in individual cell clusters and their cell-cell interaction cascades. This spatial biology strategy highlighted potential targets for combination therapies aimed at overcoming T-DXd resistance, offering new avenues to improve treatment outcomes for metastatic breast cancer patients.

Lay Abstract: Trastuzumab deruxtecan (T-DXd) is a promising new breast cancer drug targeting the HER2 protein, but it doesn't work for everyone. About 29.4% of patients with high HER2 levels and 62.5% with low HER2 don't respond, while 29.7% of patients without HER2 still respond. To understand why and identify who benefits, we studied samples from 33 patients with advanced breast cancer. We found that responders had higher HER2 levels, but this only correctly predicted responses 62% of the time. Looking at the surrounding immune cells provided more insight: responders had more helpful immune cells like dendritic cells, NK cells, B cells, cytotoxic T cells, and T helper cells, while non-responders had more suppressive immune cells. We applied an advanced technology called spatial whole transcriptome assay to study immune cells and understand their communication signals better. Then, we used our modeling tool, S2C2, to see how these cells interact. This strategy would provide new treatment combinations to help more patients overcome resistance to the drug T-DXd.

Elucidating Oncology Drug Mechanism of Action and Polypharmacology with a Large-scale Perturbational Profile Compendium

Lucas ZhongMing Hu, Cancer Systems Biology Consortium (CSBC)

Abstract: Although major target proteins are available for many specific oncology drugs, drug

efficacy and toxicity are mediated by more than single, high-affinity drug target. Rather, the pharmacologic properties of a drug are the result of its complex polypharmacology, as mediated by poorly characterized lower-affinity targets, as well as tissue-specific secondary effectors that are largely undetectable by traditional assays. The goal of this study is to address critical unresolved questions in cancer pharmacology by characterizing the proteome-wide MoA of oncology drugs as a critical, yet highly elusive step to accurately predict their clinical efficacy and toxicity. To elucidate proteome-wide, drug-mediated changes in protein activity (MoA), we generated genome-wide drug perturbation profiles from >50 cancer cell lines representing distinct tumor subtypes, selected as high-fidelity models of patients in clinical cohorts, as well as primary patient-derived tumor cells. Here we report on the analysis of the first 23 cell lines following perturbation using >700 clinically relevant oncology drugs. The corresponding PanACEA database represents the largest resources of functionally annotated, genome-wide perturbational profiles for clinically relevant drugs. VIPER-based analysis of this resource elucidated the effect of each individual drug on the activity of ~6,500 regulatory and signaling proteins. Analyses of these data elucidated functional relationship between drugs and group drugs into functionally distinct modules, thus providing insights into both MoA and polypharmacology. We validated many of our predictions using experimental assays and structure-based simulation. Our analyses also resulted many effective drug inhibitors for cancer dependent transcription (co-)factors, previously considered undruggable, that were experimentally validated.

Lay Abstract: There are over 1.7 million new cancer patients and almost 0.6 million cancer caused death in the United States in the year of 2019, mostly due to the development of resistance to treatment. There is thus an urgent need to develop new anti-cancer therapies to address this challenge. To quantitatively inform drug development and improve cancer therapies, it is critical to understand how drug-like small molecules work in cancer cells before they reach the clinics, i.e., their full, proteome-wide mechanism of action. While most drugs are characterized only in terms of their highest-affinity binding proteins (primary targets), drug efficacy, toxicity, as well as drug resistance are mediated by additional factors, including tissue context-specific changes in drug-protein interactions and effectors, as well as lower affinity-binding proteins (secondary targets). We propose using mRNA profiling in drug perturbed cells to elucidate the proteome-wide effect of the drug on protein activity. For this purpose. we developed a new, highly efficient, low-cost RNA sequencing platform (PLATE-seq) to detect drug mediated changes in mRNA abundance in 23 cancer cell lines following treatment with >700 FDA-approved and late-stage investigational compounds (i.e., in phase II and III clinical trial). VIPER-based analyses of drug vs. vehicle control-treated cells can provide an accurate assessment of proteome-wide drug mechanism of action. Several discoveries, including inhibitors of targets considered undruggable or drug polypharmacology, have been experimentally confirmed, thus demonstrating the value and potential relevance of this dataset, as well as the robustness of the computational pipeline.

Insulin Resistance Increases TNBC Aggressiveness and Brain Metastasis via Adipocyte-derived Exosomes

Yuhan Qiu, Cancer Systems Biology Consortium (CSBC)

Abstract: Type 2 Diabetes (T2D) is a chronic disease characterized by insulin-resistant adipose tissue. Patients with triple negative breast cancer (TNBC) and comorbid T2D have higher risk of metastasis and shorter survival. By mass, adipocytes are the preponderant nonmalignant cell type in the tumor microenvironment (TME) of breast cancer. However, metabolism status is often ignored in clinics and mechanisms that couple T2D to TNBC outcomes are unknown. We hypothesize that exosomes, small vesicles secreted by adipocytes, drive epithelial-to-mesenchymal transition (EMT) and metastasis in TNBC.

Exosomes were purified from conditioned media of 3T3-L1 mature adipocytes, either insulin-sensitive (IS) or insulin-resistant (IR), then characterized and quantified by NanoSight. 4T1 cells, a TNBC model, were treated with exosomes in vitro (3d), then injected into memory fat pad of mice. IHC, clonogenic assay and RNA-seq were used to detect EMT differences in primary tumor and quantify distant metastases. Exosomal RNAs were extracted and profiled to identify potential mechanism.

In primary tumors, vimentin (EMT), ki67(proliferation) and CD31 (angiogenesis) were elevated in IR group vs. control and IS groups. Brain metastases showed more mesenchymal morphology and RNA-seq analysis revealed EMT pathway enrichment in IR group. miRNAs from IR exosomes increase cell migration. A highly differentially expressed miRNAs, miR-145a-3p, between IS and IR potentially regulate metastasis.

IR adipocyte exosomes modify TME, increase EMT and promote metastasis to brain, via miRNAs. We suggest metabolic diseases (e.g., T2D) reshape TME, promoting metastasis and decreasing survival. Therefore, TNBC patients with T2D should be closely monitored for metastasis, with metabolic medications considered.

Lay Abstract: Triple negative breast cancer (TNBC), accounts for 10-20% of all breast cancer, has worse prognosis and lack of targeted drug compared to hormone receptor positive breast cancer. Outcome of TNBC has been found to be linked with metabolic health in multiple epidemiology studies, which showed that among TNBC patients, patient who are insulin resistant or T2D has worse prognosis and lower survival rate. However, the reason behind these findings remains largely unknown.

Adipocytes, also known as fat cells, are the major part of breast cancer tumor microenvironment (TME) by mass and the properties of adipocytes are significantly changed in patients who are insulin resistant or diabetic in many aspects including secreted proteins, signaling molecules, hormones. Given how sneaky cancers cells are and studies mentioned before, it is plausible to propose that tumor cell will utilize this abnormality of the adipocytes to facilitate their proliferation and progression. Combining what described above and preliminary data from our lab, here we hypothesize that exosomes, which are small vesicles containing proteins and nucleic acids, secreted from insulin resistant adipocytes will change the characteristics of TNBC cells, enabling it more invasive and proliferative.

To verify this hypothesis, we used cell line and mice model. We found that exosomes from insulin resistant fat cells increase aggressive gene levels in TNBC, as well as migration in cells and metastasis in distant organs, especially in brain. Analysis also showed significant more aggressive phenotype in metastasized TNBC cells and potential target genes involved were identified.

Concurrent Session IIB: Spotlight Talks - Multiomics and bioinformatics in cancer research

Single cell spatial proteomic analysis and computational evaluation pipeline
Behnaz Bozorgui, Cancer Systems Biology Consortium (CSBC)

Abstract: Resolving tissue and proteomic heterogeneity is critical to decoding the structure and function of tumor-immune microenvironment (TIME). Such understanding requires profiling of tumor and immune cell proteomic features with spatial resolution at the single-cell level. Although

such spatially resolved methods and data sets are becoming increasingly available, computational methods that can extract the complex features within TIME are lacking. To address this problem, we have developed a computational pipeline we call the Spatial Proteomics Analysis and Computational Evaluation Pipeline (SPACE). SPACE is composed of analysis modules for mining highly multiplexed imaging-based data types to explore TIME composition, organization, and heterogeneity. The pipeline generates and interprets biomarker expression and positional information from multiplexed images using algorithms for image indexing, image registration, quality control, segmentation, identification and removal of non-specific signals, data normalization, automatic identification of missing data, and adjustment for left-over signals. The accurate intensity measurements at single cell level are then used to calculate the spatial features that represent cellular interactions in TIME. We demonstrate the applications of our pipeline using Cyclic Immunofluorescence (CycIF) data in small bowel adenocarcinoma. We have generated a spatially resolved single-cell proteomic atlas of TIME from SBA patients and collected proteomics data from > 600,000 cells. A hierarchical decision tree of cell markers is used to annotate identities for individual cells. Using spatially resolved proteomic data representing tumor-intrinsic processes and states, diverse immune cell types, immune checkpoints, and tumor vascularization we can evaluate various spatial features that may impact the TIME organization in fine details.

Lay Abstract: A tumor inside the body constantly interacts with its neighboring cells in tis surrounding area called Tumor micro-environment (TME). Cells in TME influence each other: Immune cells detect, and destroy cancer cells, and cancer cells shield themselves from immune system, get nutrients, and grow inside TME. Understanding the structure of TME, specifically interaction between cancer cells and immune cells may help researchers develop more efficient treatments for cancer patients, though such effort needs a high-resolution data and advanced computational techniques. Of specific importance are data types at single cell resolution in which properties of every cell are evaluated without disturbing their spatial arrangement inside TIME. Such data are highly valuable as it informs on spatial structure around every single cell. Luckily, with advancing multiplex imaging technologies that can extract information from patient's tissue samples, such data is becoming more available. However, computational methods that analyze them and evaluate cell organization and composition in TME are lacking. To address this problem, we have developed a computational pipeline we call the Spatial Proteomics Analysis and Computational Evaluation Pipeline (SPACE). SPACE is composed of analysis modules for mining highly multiplexed imaging-based data types. Here we demonstrate how we can use the pipeline to analyze and interpret multiplex imaging data and present a spatially-resolved single-cell proteomic atlas of tumor-immune microenvironment that is created using SPACE from patients with small bowel adenocarcinoma, a rare type of cancer.

Chromosome specific organizational characteristics in rhabdomyosarcoma and normal myogenic cells

Kyle MacQuarrie, Cellular Cancer Biology Imaging Research (CCBIR)

Abstract: Rhabdomyosarcoma (RMS), the most common soft-tissue pediatric sarcoma, is a tumor of skeletal muscle. RMS has been suggested to represent an arrested state of development between skeletal myoblast and myotube. Myogenesis is the process by which proliferative normal myoblasts terminally differentiate into myotubes and is characterized by widespread gene expression changes. Though RMS and normal myogenic cells share many similarities at the level of gene expression and morphology, similarities at the level of nuclear and genomic organization are less understood. We have recently demonstrated myogenic chromosome-specific differences in chromosomal positioning relative to the nuclear axis that are absent in RMS cells, but are partially rescued when RMS cells undergo induced myogenesis. Specifically, chromosome 2 exhibits differentiation-dependent positioning along the major nuclear axis, while chromosome 18

exhibits no axial preference. We hypothesized that this spatial organization difference would result in differences in other chromosomal characteristics, such as accessibility and DNA contacts. Analyses including ATAC-Seq, Hi-C, and partial wave spectroscopic (PWS) imaging were performed in cells in vitro to assess for such differences. In agreement with our hypothesis, when myogenic cells were differentiated, chromosome 2 exhibited greater changes in multiple characteristics (eg. DNA contact scaling and accessibility) compared to chromosome 18. Other characteristics, such as chromatin organization as assessed by PWS imaging, did not demonstrate any change. Our findings suggest both the possibility of using sequencing data to infer aspects of spatial organization of chromosomes in other cellular systems, and offer another avenue to understand the failure of myogenesis in rhabdomyosarcoma cells.

Lay Abstract: Rhabdomyosarcoma (RMS) is a cancer that affects children and involves muscle. Treatment hasn't changed much for many years, and many of the children with cancer spread through their body or who have their cancer return don't survive. New treatment approaches are badly needed. Since cancer cells grow out of control, one potential approach to consider is to identify new ways to stop cancer cell growth.

In normal muscle, the organization of the cell's control center, the nucleus, is very important to how the cells grow. However, that role in RMS isn't entirely clear. Our work has found that the organization in cancer and normal muscle cells is different in some ways. We aim to use those differences to understand more about how RMS cells control their growth.

Our recent research analyzing images of chromosomes – important structures in the nucleus - showed that one chromosome changes its position when muscle cells stop growing, while another chromosome does not have a preference. None of the tumor cell chromosomes we analyzed showed a preference. We hypothesized that we could find evidence of that preference in types of data other than images of the nucleus, such as in experiments that measure how tightly packed together the chromosome is. We found the chromosome with a preference shows differences in multiple types of data, while the other chromosome did not. We hope to use this to both understand more about why the tumor cells keep growing, and look for preferences in other types of cells.

Computational framework for inference of genetic ancestry from challenging human molecular data

Pascal Belleau, Informatics Technology for Cancer Research (ITCR)

Abstract: Epidemiological data have shown a link between genetic ancestry and incidence of cancer. Recent cancer genomics research highlights genetic and phenotypic differences in tumors from patients of different ancestries. To facilitate large-scale ancestry analysis in cancer research, we developed a framework for inferring genetic ancestry from various types of molecular data beyond standard germline DNA sequencing.

Inferring ancestry from cancer-derived data poses two challenges: cancer-specific ones like somatic genome alterations in particular copy number variation, and data-specific ones like uneven genomic coverage in data from RNA-seq or ATAC-seq protocols. Moreover, each individual molecular profile comes with its unique set of sequence quality, depth and other properties affecting ancestry inference. In response, we created a computational framework termed Robust Ancestry Inference using Data Synthesis (RAIDS). By simulating cancer-derived data with known ground-truth genetic ancestry, RAIDS optimizes ancestry inference for any input molecular profile, considering sequence quality and coverage depth.

RAIDS currently enables global ancestry inference from cancer-derived RNA-seq, ATAC-seq, whole genomes, and whole exome sequences. In addition, continental admixtures can also be accurately calculated from cancer-derived genomic and transcriptional data. We validated RAIDS using data from large patient cohorts, including a major subset of TCGA.

Our framework unlocks existing molecular data for ancestry-focused studies of various human diseases and phenotypes, including cancer. By facilitating large-scale ancestry inference from diverse molecular datasets, such as data from large public repositories, RAIDS can help uncover how genetic ancestry influences cancer biology and clinical outcomes.

RAIDS is available at: https://bioconductor.org/packages/RAIDS/

Lay Abstract: Mounting evidence from recent research, encompassing a variety of cancer types, points to links between biology of the disease and the patient‚Äôs ancestral genetic background. We have created a software framework to facilitate large-scale data-driven investigation in this important area via methods to learn a patient‚Äôs genetic ancestry from tumor-derived molecular data, without the need to genotype the patient‚Äôs cancer-free DNA or to know the patient‚Äôs ethnic self-identification. This framework contains computational tools designed to make hundreds of thousands of DNA and RNA profiles of tumors, from genomic data repositories, and millions of tumor specimens in biobanks, most with no matching normal genotype and no racial/ethnic annotation, available for ancestry-oriented cancer research.

Integrating signaling and transcription to study c-Myc induced stress response in cancerous cells

Reshma Kalyan Sundaram, Physical Sciences-Oncology Network (PS-ON)

Abstract: The transcription factor c-Myc (Myc) is known to regulate a multitude of genes and cellular processes. Myc is deregulated in 70% of human cancers, making it a potent oncogene. Myc deregulation through increased Myc concentration has been suggested to cause cells to experience stoichiometric stress which may result in stress-induced cellular reprogramming. We hypothesize that extracellular signals transduced through intracellular signaling pathways can contribute to increased Myc concentration by modulating Myc protein stability via phosphorylation. We also hypothesize that increased Myc concentration can alter Myc‚Äôs transcriptional function by changing Myc-DNA interactions and hence Myc regulated gene expression. To test the first hypothesis, we built an ODE-based systems model consisting of extracellular growth and mechanical signals (effected by EGFR and integrins) and intracellular signaling pathways that modulate Myc phosphorylation. Our modeling results show that in normal cellular phenotypes, Myc is primarily regulated by EGFR. However, in the Myc-upregulated cancerous phenotype, Myc stabilization is dependent on both EGFR and integrins. Therefore, we conclude that extracellular growth and mechanical signals synergistically regulate Myc protein stabilization and accumulation in cells. To test the second hypothesis, we performed bioinformatics analysis on Myc ChIP-seq and RNA-seq datasets with varied Myc expression levels. From this analysis, we identified a new DNA-binding site of Myc, and demonstrated that Myc binding to this new site varies as a function of Myc concentration. We also showed that Myc utilizes this binding site to reprogram cells by modulating several cancer hallmarks such as proliferation, apoptosis, and cell adhesion.

Organ-specific evolution of tumor microenvironments in metastatic breast cancer

Seongyeol Park, Metastasis Research Network (MetNet)

Abstract: Metastasis is a critical stage in the progression of cancer, turning it into an incurable disease. This process is specific to different organs, each with unique tumor microenvironment

(TME) profiles. Therefore, understanding organ-specific TME is crucial for gaining insights into cancer metastasis.

In our research, we utilized spatial proteomics (Multiplexed Ion Beam Imaging, MIBI) in combination with bulk RNA and DNA sequencing to explore the metastasis of triple-negative breast cancer (TNBC). We gathered approximately 400 tissue samples from 110 patients, encompassing various organs (e.g. lymph node, liver, skin, and intestine), time points (e.g. primary and recurred metastasis, and before and on immunotherapy), and responses to immunotherapy, all of which were from individuals participating in the immunotherapy clinical trial (TONIC trial). This unique and comprehensive set of data enabled us to examine the organ-specific development of the TME and its effects on immunotherapy.

Through unsupervised clustering of spatial data, we have identified five distinct TME groups within TNBC. These TME groups exhibit varying response rates to immunotherapy. We have frequently observed changes in TME group compositions between pre- and post-treatment specimens. In addition, the composition of TME groups varied among metastatic organs. Generally, metastatic tissues had more unfavorable TME groups compared to primary breast tissues, except in lymph nodes.

Our findings emphasize the dynamic nature of the TME in TNBC and broaden the understanding of site-specific metastasis. This understanding is crucial for developing effective treatment strategies for this aggressive form of breast cancer.

Lay Abstract: Metastasis, which is the spread of cancer to different parts of the body, is a crucial stage in the progression of the disease and can make it incurable. Cancer cells adopt different strategies to settle down in different organs due to the unique tissue structure and composition of normal resident cells in each organ. This results in the creation of a unique tumor microenvironment (TME) in each organ, which is a complex ecosystem of different cells surrounding a tumor. Understanding the organ-specific TME is crucial for understanding the metastasis of cancer cells. Recently, advances in spatial omics technology have enabled us to study TME using slides of tumor tissues. This technique allows us to measure the proteins or transcripts of each cell on the slides.

In our study, we used spatial proteomics to investigate how triple-negative breast cancer (TNBC) spreads to specific organs. We collected around 400 tissue samples from 110 patients, including samples from different organs (e.g. lymph node, liver, skin, and intestine), at different time points, and with various responses to immunotherapy. By analyzing the data, we identified five distinct TME groups within TNBC. We found variations in TME groups among the different organs. Generally, we found that metastatic tissues had less favorable TME groups compared to the primary breast tissues, except in lymph nodes. Our findings highlight the dynamic nature of the TME in TNBC and stress the importance of understanding how the cancer spreads to specific sites.

Elucidating genomic and three-dimensional architectures of extrachromosomal DNA

Kaiyuan Zhu, Informatics Technology for Cancer Research (ITCR)

Abstract: Extrachromosomal DNA (ecDNA) is the dominant mechanism of focal oncogene amplification in cancer, occurring in approximately 15% of early-stage cancers and 30% of late-stage cancers. EcDNAs are circular, megabase-scale, acentric DNA molecules that dynamically modulate oncogene copy-number and rewire gene-regulatory networks, promoting tumor evolution and drug resistance. Elucidating the genomic and three-dimensional (3D) architecture

of ecDNA is critical for understanding pathology and developing effective therapies. However, the highly-rearranged, repetitive and heterogeneous nature of ecDNA genome poses intrinsic computational challenges in resolving their architectures.

Here we develop two computational methods: CoRAL, for reconstructing ecDNA genomic architectures using long-reads, and ec3D, for inferring ecDNA 3D structures from Hi-C. CoRAL reconstructs most likely cyclic architectures using quadratic programming that simultaneously optimizes parsimony of reconstruction, explained copy number, and consistency of long-read mapping. It substantially improves reconstructions in extensive simulations and datasets from previously-characterized cell-lines as compared to previous short and long-read based tools. Ec3D infers the 3D structure of ecDNA through maximizing the Poisson likelihood of interactions given the distances between individual beads. Among key methodological innovations, ec3D allows representation of circular genomes and utilizes constraint optimization to resolve ecDNA structures containing multiple copies of large segments, by separating their chromatin configuration information. Ec3D provides the first description of the 3D structure of ecDNA in cell lines and hints at the role of spatial configurations in gene regulation on ecDNA. Together, CoRAL and ec3D provide a comprehensive view into ecDNA architectures and bring deeper insights into ecDNA-driven tumor biology and pathogenesis.

Lay Abstract: Cancer cells sometimes carry large circular DNA molecules called extrachromosomal DNA (ecDNA). These ecDNAs arise from, but are not part of the cell's normal chromosomes. They often carry many extra copies of cancer-causing genes (i.e., oncogenes), drive tumor growth and drug resistance. Understanding ecDNA's structure is a critical step to developing better treatments of ecDNA-driven cancers. However, ecDNA has a rearranged, repetitive, and diverse genomic sequence which makes it difficult to reconstruct.

We developed two computational methods to analyze ecDNA structures with increasingly-used sequencing technologies. The first method, CoRAL, reconstructs the circular genome of ecDNAs using long-read sequencing. CoRAL finds the most likely ecDNA sequence in a way that maximizes consistency with the observed sequencing data. Experimental results suggests that CoRAL can substantially improve ecDNA reconstructions compared to previous computational tools. The second method, ec3D, infers the 3D shape of ecDNAs using chromatin conformation data (Hi-C). Ec3D is the first method to determine ecDNA's 3D structure. In cancer cell lines, ec3D revealed how ecDNA's 3D folding disrupts normal chromosome organization. It also showed how the 3D shape allows distant functional elements (e.g., enhancers) and oncogenes to interact, which may lead to increased oncogene expression. Together, CoRAL and ec3D provide different views of ecDNA, from its linear sequence to its 3D shape inside the cell nucleus. We anticipate that these methods can bring new insights into how ecDNA contributes to cancer development and evolution, and enable better design of therapies that target ecDNAs.

Concurrent Session IIIA: Spotlight Talks - Niche regulation of cancer progression

Plasma exosomes from individuals with type 2 diabetes drive breast cancer progression in patient-derived organoids

Christina Ennis, Cancer Systems Biology Consortium (CSBC)

Abstract: Over 68 million American women with obesity-driven (Type 2) diabetes (T2D) and prediabetes are predisposed to more aggressive breast cancers. Despite this significant risk factor, metabolic status does not currently inform clinical management of breast cancer. We have

previously identified circulating exosomes as crucial components of intercellular communication and potent modulators of pro-oncogenic processes. To accurately model this signaling within the human breast tumor microenvironment (TME), we developed a method to generate patient-derived organoids (PDOs) from breast tumor resection samples. Novel techniques and time limited development permit native primary immune cells to be included and profiled. Here, we explored the impact of exosomal signaling on these PDOs via single-cell RNA sequencing. Intratumoral heterogeneity of the PDOs underwent notable shifts during a 3-day exosome treatment, highlighting the impact of metabolic dysregulation on the cellular architecture and transcriptional networks of breast tumors. We discovered significant upregulation of pathways associated with epithelial-to-mesenchymal transition, invasiveness, cancer stemness, and immune exhaustion in PDOs treated with T2D-derived exosomes compared to non-diabetic exosome controls, indicating enhanced tumor aggressiveness and metastatic potential. Further, analysis of ligand-receptor interactions highlighted a significant downregulation of T cell signaling pathways, leading to immune exhaustion. This immune suppression likely permits the survival of micrometastases and could undermine the efficacy of immune checkpoint therapies, such as atezolizumab, approved for triple-negative breast cancer. Together, these findings enhance our understanding of dynamic interactions within the TME and offer new insights into the significance of novel exosomal communication on tumor biology.

Lay Abstract: Over 68 million American women with obesity-driven (Type 2) diabetes (T2D) and prediabetes are at higher risk of developing more aggressive breast cancers. Despite this, doctors do not currently use a patient's metabolic health to guide cancer treatment. Our research has shown that tiny particles in the blood called exosomes play a key role in how cells communicate and can promote cancer growth. To better understand this process, we developed a way to create 3D mini-tumors, called organoids, from patient samples. These organoids include not just cancer cells but also many of the other cell types in a tumor‚Äôs environment, including immune cells and support cells. This helps us closely mimic what happens in an actual tumor and study how different conditions affect tumor behavior. We treated organoids with exosomes from either T2D or non-diabetic individuals. After three days of treatment, organoids treated with T2D exosomes showed signs of becoming more aggressive and spreading. We also discovered that these exosomes disrupted T cell signaling, which is crucial for the immune system to fight cancer. This disruption can lead to a state called immune exhaustion, where the immune system becomes less effective at attacking cancer cells. This can allow tiny cancer spreads, known as micrometastases, to grow and make breast cancer treatments, like immune checkpoint therapies such as atezolizumab, less effective. All together, our work highlights the importance of considering metabolic health in breast cancer treatment and provides new insights into how diabetes may influence cancer growth.

Neuronal substance-P drives breast cancer growth and metastasis
Ethan Seltzer, Metastasis Research Network (MetNet)

Abstract: Tumor innervation associates with worse patient outcomes in multiple cancers, suggesting that it may regulate metastasis. We observed that highly metastatic murine mammary tumors acquired more sensory innervation than less metastatic tumors. This increased sensory innervation was driven by expression of the axon guidance molecule SLIT2 in tumor vasculature. In murine models, sensory innervation was found to promote tumor growth and metastasis while in vitro studies demonstrated that sensory neurons increased the invasiveness and proliferation of cancer cells. Conditioned media from sensory neurons was sufficient to drive invasion and proliferation in vitro, indicating that sensory neurons likely drive metastasis via a secreted factor. Using three-dimensional co-cultures and in vivo models, we found that the neuropeptide substance-P (SP) promoted breast tumor growth, invasion, and metastasis. SP acts on tumoral

tachykinin receptors (TACR1) to mediate its pro-metastatic effects. Both anti-SP neutralizing antibodies and shRNA-mediated silencing to deplete TACR1 from cancer cells were sufficient to reduce cancer cell invasion and proliferation in vitro as well as tumor growth and metastasis in vivo. Our findings reveal that SP secretion by sensory neurons regulates metastatic progression in breast cancer.

Lay Abstract: Cancer patients whose tumors have more nerves tend to experience worse outcomes. This suggests that nerves in tumors may control how cancer spreads through a process called metastasis. In mouse tumors which are more likely to spread, we found more sensory nerves; the type which carry signals to the brain. This increase in nerves was due to a molecule that can attract or repel neurons, called SLIT2, which is expressed highly by cells lining blood vessels within the tumor. Sensory neurons make tumors grow larger faster and lead to increased metastasis in mice. When the neurons are grown in a dish with cancer cells, the cancer cells are able to invade and expand more readily. The liquid media that the neurons are grown in demonstrated similar effects on the cancer cells, indicating that the neurons produce something which is responsible for this phenomenon. A molecule made by the neurons, called substance-P (SP) was found to be responsible for these effects. SP binds to a receptor on cancer cells called TACR1, and when this occurs, tumors grow faster and metastasize more than tumors which lack SP or TACR1. When this interaction is blocked by antibodies or the receptor is removed, the effects are no longer seen. These experiments demonstrated that SP from sensory neurons is responsible for breast cancer metastasis.

Lymphoma Organoids Reveal T Cells Spatially Alter B Cell Receptor Signaling via Histone Modifications

Lucy Britto, Not Applicable

Abstract: Activated B cell-like diffuse large B-cell lymphoma (ABC-DLBCL) represents a highly aggressive subtype of non-Hodgkins's lymphoma and is associated with poor outcomes after treatment with standard chemoimmunotherapy. While molecular profiling has revealed promising pharmacological targets, clinical trials using targeted therapies have failed to improve outcomes for these patients. New strategies using immune-competent tissue models are crucial for improving therapeutic strategies to uncover mechanisms underlying treatment evasion or resistance in DLBCL cells. Here, synthetic hydrogel-based lymphoma organoids were employed to demonstrate how signals from the lymphoid tumor microenvironment (Ly-TME) influence B cell receptor (BCR) signaling and tri-methylation of histone 3 at lysine 9 (H3K9me3) to dampen the effects of BCR pathway inhibition. Single-cell imaging analysis revealed T cells increased DNA methyltransferase 3A expression and cytoskeleton formation in proximal DLBCL cells which was regulated by the G9Œ± H3K9me3-associated histone methyltransferase (KMT). Expansion microscopy paired with lymphoma organoids showed T cells increased the size and quantity of spatially segregated H3K9me3 clusters in neighboring ABC-DLBCL cells, suggesting reprogramming of higher-order chromatin structures and associated transcriptional states. Sensitization with an inhibitor of the G9Œ± KMT prior to BCR signaling inhibition was able to reverse T cell-mediated H3K9me3 upregulation and reduced T cell-mediated dampening of the treatment response to BCR pathway blockade. These findings emphasize the need for developing biologically relevant lymphoid tissue models to better understand how Ly-TME signaling cues direct DLBCL fate, spatially and temporally, and suggests targeting both dysregulated intracellular signaling and epigenetic cross-talk could improve treatment efficacy for patients with aggressive lymphomas.

Lay Abstract: Activated B cell-like diffuse large B-cell lymphoma (ABC-DLBCL) is an aggressive blood cancer associated with poor survival outcomes after standard therapy. While researchers

have discovered promising drug targets, new treatments have not improved patient outcomes. Scientists need new models that replicate the tissue where cancer cells grow to better understand why this cancer does not respond or stops responding to treatment. In this study, synthetic tissue models, or ‚Äúorganoids‚Äù were used to demonstrate how signals found in the tumor microenvironment led to a limited response to targeted therapy. Specifically, T cells, a different type of immune cell, were able to reprogram the organization of genetic material in cancer cells to make them more resistant to treatment. Reversing the changes caused by T cells resulted in improved effectiveness of targeted therapy. These findings suggest that the environment where cancer cells reside directly alters tumor behavior and may partly be responsible for the poor prognosis of these patients. Pairing therapies that block environmental signals with targeted therapies could improve the effectiveness of treatments for patients suffering from aggressive lymphomas.

Antidepressants Inhibit Pancreatic Ductal Adenocarcinoma Progression by Reducing Fibrosis and Perineural Invasion

Ghmkin Hassan, Physical Sciences-Oncology Network (PS-ON)

Abstract: Pancreatic ductal adenocarcinoma (PDAC) has a 5-year survival rate of 10%. Its high mortality is due to late diagnosis, metastasis, and limited treatment options. Perineural invasion (PNI), found in 70-100% of PDAC cases, is linked to increased pain, tumor recurrence, and reduced survival. Tricyclic antidepressants (TCAs) have been used in clinical practice for several decades for the treatment of depression. Our prior studies demonstrated that TCAs ameliorate hepatic fibrosis by inhibiting the lysosomal enzyme, acid ceramidase (aCDase).

Here, we investigated whether antidepressants, specifically tricyclic antidepressants (TCAs) and selective serotonin reuptake inhibitors (SSRIs), inhibit PDAC progression. Using the ERCHIVES cohort, a well-established cohort of 754,670 veteransm, and preclinical mouse models, the effects of antidepressants on PDAC incidence, tumor burden, metastasis, PNI, and fibrosis were evaluated.

In the ERCHIVES cohort, PDAC patients treated with either TCA or SSRI class antidepressants had a significantly improved overall survival. The KRas/p53 PDACs mouse model studies and RNA-seq data demonstrated that TCAs decreased aCDase enzymatic activity, myeloid infiltration and fibrosis and led to decreased tumor burden, metastasis and PNI. Pharmacological inhibation of αCMA confirmed its role in PDAC progression and response to antidepressants.

Thus, antidepressants, particularly TCAs, may inhibit PDAC progression by reducing αCMA activity, fibrosis, and PNI, thus enhancing antitumor immunity. These findings support the potential repurposing of TCAs as a therapeutic strategy in PDAC and highlights the potential of antidepressants to improve PDAC patient outcomes by modulating the tumor microenvironment.

Lay Abstract: Pancreatic ductal adenocarcinoma (PDAC) is a type of pancreatic cancer with a very low survival rate of only 10% over five years. This is because it is usually found late, spreads quickly, and has limited treatment options. One common problem in PDAC is perineural invasion (PNI), where cancer spreads along nerves, causing more pain, cancer return, and lower survival chances.

Our study looks at whether antidepressants, particularly tricyclic antidepressants (TCAs) and selective serotonin reuptake inhibitors (SSRIs), can help slow down PDAC. We used data from 754,670 veterans and lab tests with mice to see the effects of these drugs on PDAC.

The results showed that PDAC patients who took TCAs or SSRIs lived longer than those who didn't. In mouse studies, TCAs reduced the activity of a specific enzyme, acid ceramidase (aCDase), decreased immune cell buildup and tissue scarring (fibrosis), and led to smaller tumors, fewer spread sites (metastasis), and less nerve invasion.

Overall, this suggests that antidepressants, especially TCAs, might slow PDAC progression by lowering enzyme activity, reducing fibrosis, and limiting nerve invasion. These findings support the idea of using TCAs as a new treatment approach for PDAC, potentially improving outcomes for patients by changing the tumor environment.

Investigating VEGF-A induced lymphatic vessels remodeling and its implications for immune cell trafficking in pancreatic cancer using tumor-on-chip and Kaede transgenic mouse model.

Anna Kolarzyk, Tissue Engineering Collaborative (TEC)

Abstract: Pancreatic ductal adenocarcinoma (PDAC) remains one of the deadliest tumors in humans. PDAC has an immunosuppressive tumor microenvironment (TME), constituting poor immunotherapy outcomes. Lymphatic vessels (LVs) traffic leukocytes from tumor peripheries to draining lymph nodes (dLNs) for effective activation of anti-cancer immune responses. Impairment of lymphatic function results in delayed or ineffective immune activation. In this study we aim to investigate PDAC-induced LV remodeling and its consequences for immune cell migration from primary tumor through LVs. We used our innovative three-dimensional LV-on-chip technology to track phenotypic and functional changes in lymphatic endothelial cells (LECs) in co-culture with PDAC. Our studies discovered that PDAC progression tightens adherens junctions on LECs, making them less permeable via PDAC secreted vascular endothelial growth factor A (VEGF-A). We hypothesized that altered LEC morphologies may affect leukocyte transmigration through LVs in PDAC. To pursue this, we use the fluorescence Kaede mouse model to track the egress of photoconvertible leukocytes from the primary PDAC tumor site to the dLNs. This system timestamps cells in time and space with violet light. We are interested in discovering whether we can use VEGF-A treatment to boost existing immunotherapies. We found that the majority of tumor infiltrating cells are lymphocytes rather than myeloid cells and certain leukocyte populations were retained within the tumor after VEGF-A neutralization. Lastly, we will identify the function of the PDAC-egressed immune cells, and those remaining within the tumor, which will enhance our understanding of tumor immunity and LVs role in orchestrating leukocyte migration through VEGF-A signaling.

Lay Abstract: Pancreatic cancer is aggressive and immunotherapy treatments haven't been very effective. Our body's natural defense system needs to be able to reach the tumor to fight it off. Lymphatic vessels help with that, they transport immune cells from tumors to draining lymph nodes, where immune cells get activated. However, in pancreatic cancer lymphatic vessels malfunction.

In my project, I investigate how pancreatic cancer changes lymphatic vessels and how that affects immune cells. We have developed a special 3D device to study pancreatic cancer interaction with lymphatic cells and found that the cancer makes the lymphatics tighter, potentially blocking immune cells entry to the vessels. We are curios if we reverse these phenomena, we would be able to slow down the growth of pancreatic cancer. By understanding how lymphatic vessels impact immune cells activation we hope to develop better treatments for pancreatic cancer.

In situ state of invading glioblastoma cells by single-cell and spatial transcriptimics

Toshiro Hara, Not Applicable

Abstract: Tumor cell invasion is the main characteristic of glioblastoma and one of the obstacles to successful treatments. Nonetheless, molecular features of invading glioblastoma cells remain elusive and poorly characterized, such as their gene expression states and microenvironmental influences. We developed an in vivo approach with human glioblastoma spheroid lines multiplexed for mouse intracranial xenografts. We validated this with 20 patient-derived models and identified, by scRNA-seq coupled with deconvolution analysis, in vivo models capable of infiltrating along the periphery of either neurons or blood vessels. We profiled and characterized programs, states, and clonality of invading glioblastoma cells by comparing cells from the contralateral invasive edge to their counterparts residing at the original bulk tumor. This analysis unveiled two distinct invasion expression programs, both notably enriched for the OPC-like state and depleted for the MES-like state. To validate our findings, we employed two complementary spatial transcriptomics approaches to profile both hemispheres of a glioblastoma PDX, confirming the presence of the invasion-associated programs and highlighting a unique association of astrocytes with invading cells. Finally, our functional experiments revealed that astrocytes facilitate invasion phenotypes of glioblastoma. Overall, our study elucidates the interplay between developmental cell states and glioblastoma invasion, offering critical insights that could guide the development of targeted therapeutic strategies against this devastating disease.

Lay Abstract: Glioblastoma is the most common and lethal form of intracranial tumor. It accounts for approximately 48% of the 24,500 new cases of malignant primary brain tumors diagnosed each year in children and adults in the United States. Glioblastoma can infiltrate the brain, and missing even a few cells leads to high recurrence after surgical resection. There is a major gap in our knowledge as to what is the molecular identity of such invading tumor cells. To overcome this gap in our knowledge, we use a technology called single-cell RNA sequencing to measure gene expression of individual tumor cells that invade and hide in the brain. Evidence from this approach reveals the ability of tumor cells to switch their gene expression identities, by turning them on and off, along with their location. We further find that the signals sent out from their surrounding environment can be recognized by invading tumor cells and determine their identities and functions. By controlling such environmental signals, we may be able to perturb gene expression of glioblastoma cells to disrupt their abnormal behavior. Our work hopes to have a major impact in providing new strategies to control invasiveness, which could lead to significantly increased recurrence-free survival of glioblastoma patients- things that are not possible with the current treatment.

Concurrent Session IIIB: Spotlight Talks - Genetic and epigenetic regulation of tumor progression and metastasis

RNA interference of P65 activity mediates oncogenic inflammation in TET2 mutated clonal hematopoiesis

Nana Adjoa Ben-Crentsil, Oncology Models forum and Mechanisms of Cancer therapeutics-C

Abstract: TET2 is a dioxygenase that regulates gene expression and hematopoiesis by oxidizing 5-methylcytosine residues on DNA to 5-hydroxymethylcytosine. TET2 loss-of-function mutations are common genetic events in hematological disorders and typically arise in founding clones. They are the most common mutation in Chronic Myelomonocytic leukemia and occur in a third of other myeloid malignancies. They are also the second most common mutation in clonal hematopoiesis of indeterminate potential (CHIP), a common premalignant condition in the elderly.

TET2 deficiency has been implicated in many non-hematological sequelae seen in CHIP such as atherosclerotic cardiovascular disease, chronic liver disease, and severe microbial infections. The

development of TET2-deficient hematological disorders as well as their clinical sequelae is associated with the potentiation of inflammatory circuits. However, while multiple studies have shown the response of Tet2-deficient cells to heightened inflammation, the major downstream effector responsible for this potentiation is unknown.

To address this, we used scRNA and scATAC-seq in COVID-19 patients with and without TET2 mutations reasoning that the inflammation from COVID-19 may unmask downstream effectors of TET2 deficiency. We identified MALAT1, a therapeutically tractable lncRNA, as a central effector that is both necessary and sufficient to induce most inflammatory Tet2-deficient phenotypes in vivo. We also show that increased MALAT1 expression is due to TET2-dependent loss of EGR1 repression. Finally, we describe an interaction between MALAT1 and P65 resulting in "shielding" from PP2A dephosphorylation thus preventing the resolution of inflammatory signaling. Put together, this work nominates MALAT1 as a viable therapeutic target in TET2-mutated disorders.

Lay Abstract: TET2 is an enzyme that regulates gene expression and normal blood cell production by modifying components of DNA. Mutations in the TET2 gene which result in a loss of function are common in blood disorders and typically arise in originating cells. They are the most common mutation in Chronic Myelomonocytic leukemia and occur in a third of other blood cancers. They are also the second most common mutation in clonal hematopoiesis of indeterminate potential (CHIP), a common precursor condition in the elderly.

TET2 deficiency has been implicated in many of the CHIP sequelae which occur outside the blood such as heart and liver disease, and severe infections. The development of TET2-deficient blood disorders and their clinical sequelae is associated with an increase in inflammation. However, while multiple studies have shown the response of Tet2-deficient cells to heightened inflammation, the major players responsible for this increase are unknown.

To address this, we sequenced cells in the blood of patients with COVID-19 infection, with and without TET2 mutations assuming that since COVID-19 increases inflammation, it would reveal the important players necessary for TET2-deficient features. We identified increased levels of MALAT1, an RNA that does not produce protein, with TET2 deficiency and showed this increase is required for TET2-deficient features. We also found this increase occurs because TET2 disrupts the restriction of MALAT1 production. Finally, we discovered that MALAT1 binds key components that drive inflammation and prevents them from being deactivated, resulting in increased inflammation and its associated features.

Modeling Phase Separation of a Mitosis Signaling Network in Breast Cancer
Sarah Groves, Cancer Systems Biology Consortium (CSBC)

Abstract: Mitotic signaling is often dysregulated in breast cancer, leading to high rates of chromosomal missegregation and aneuploidy. Chromosome segregation is coordinated by a signaling network centered on the Chromosome Passenger Complex (CPC). The ability of this complex to form liquid droplets or biomolecular condensates (i.e. phase separate) has been shown to be critical to proper segregation of chromatid sisters. Condensate dynamics can be modeled by the Cahn-Hilliard equation, a fourth-order differential equation that cannot be solved exactly. I have developed a multigrid numerical solver to approximate the solution to CPC condensate dynamics. This model can predict condensate behaviors seen in fluorescence microscopy during mitosis, such as the formation of several droplets along the axis between chromatids early in prophase that dissolves into a single droplet at the inner centromere by prometaphase. Understanding phase separation of CPC condensates is critical to predicting which breast cancer patient tumors will have high levels of chromosomal instability. Using readily

available clinical diagnostic data, we plan to predict chromosomal instability using a model of CPC signaling network augmented with phase separation dynamics.

Lay abstract: One key aspect of cancer is the ability of tumor cells to grow out of control. To do so, they replicate into two daughter cells through a process called mitosis. In particular, breast cancer cells make many mistakes during mitosis, which can lead to some cells having higher-than-normal numbers of chromosomes (i.e. more than 23 pairs) and others having fewer. This unequal distribution of chromosomes, called aneuploidy, helps cancer cells survive treatments like chemotherapy, so it is important to understand the mechanism by which aneuploidy is generated. Cells normally obey a stop signal, called the spindle assembly checkpoint, during mitosis in order to first fix any problems before generating two daughter cells. Sometimes, when breast cancer cells make mistakes in mitosis, the stop signal is ignored. We would like to understand why cells can ignore the stop signal, and how to prevent them from continuing to grow. We know that normally, cells that listen to the stop signal do so by making a large amount of a protein complex called CPC. CPC forms liquid droplets inside the cells, like oil droplets in water, in order to perform its function of turning on the stop signal. Understanding the formation of these droplets, or lack of formation, is important to figure out which breast cancer patients have tumors that are not progressing through mitosis properly. If we can categorize patients by whether their cancer obeys the stop signal, we may make better treatment plans for those whose cancer ignores it.

Analyzing the clonal dynamics and metastatic potential of isolated subpopulations within TNBC cells

Carolina De Santiago, Cancer Systems Biology Consortium (CSBC)

Abstract: Triple negative breast cancer (TNBC) is one of the deadliest forms of breast cancer and is characterized by high rates of metastasis and significant intra-tumor heterogeneity. Through the use of single cell RNA-sequencing and a novel genetic cell tracking technology, ClonMapper, we have identified and characterized the origins of invasive subpopulations within TNBC.

Genetic barcodes were stably integrated into HCC1806 cells to generate a population with ~1000 unique barcoded clones. Using a transwell assay, migratory cells were collected for expansion to create stable isolated replicates. Clonal abundance sequencing across all replicates showed a >8-fold decrease in the number of unique barcodes. Following cell subpopulation isolation, we observed that isolated subpopulations display faster growth rates than parental populations. Further scratch assays and transwells assays verified that the isolated subpopulations displayed elevated bidirectional migration rates as well as invasion rates. Leiden clustering of the single cell RNA-sequencing data revealed that the invasive populations are transcriptomically distinct from the parental population and gene expression analysis (GSEA) indicates that the invasive populations have increased epithelial mesenchymal transition expression through signaling pathways such as FAK-Rac and Rho-Rac. Further analysis of the isolated populations revealed two unique clusters. Using the genetic barcodes, trajectories of the most abundant clones can be tracked to determine the origin of these cells within the parental population and determine the gene signaling pathways that underlie different sets of invasive clones.

Lay Abstract: Triple negative breast cancer (TNBC) is one of the deadliest forms of breast cancer due to its high rates of metastasis as well a high rates of heterogeneity. In our study, we used

cell-tracking technologies and genetic sequencing to understand how different invasive subpopulations develop within TNBC cells.

Unique genetic labels were introduced into HCC1806 cells to create a diverse population. Migratory cells were isolated and expanded to create stable replicates for further studies. By studying these replicates, we found that these cells had faster growth rates as well has increased mobility and invasion than the original population.

Analyzing the cells on a genetic level, the invasive populations have distinct gene expression patterns when compared to the parental population. Specifically, the invasive populations have increased activity in pathways associated with epithelial mesenchymal transition which is crucial in the metastatic cascade. By tracking the genetic labels, invasive clones can be traced back to their origin within the parental population and it was shown that there are multiple sets of invasive clones.

Dynamics of chromatin reorganization in response to chemo-mechanical environmental cues

Monika Dhankhar, Metastasis Research Network (MetNet)

Abstract: Chromatin organization is pivotal in regulating gene expression, genome organization, and cellular responses. During cancer progression and metastasis, the tumor microenvironment continuously evolves, influencing chromatin organization in complex ways that are not yet fully understood. In this study, we present a meso-scale phase field model to elucidate the underlying physics of chromatin reorganization dynamics in single human cells. The interplay of energetics of chromatin-chromatin and chromatin-lamina interactions, and the kinetics of chromatin diffusion and epigenetic reactions results in formation of stable heterochromatin domains. By analyzing super-resolution images of hMSC nuclei using this model, we determine the nuclear reorganization of heterochromatin domains. We find that the interaction between diffusion fields and local stochastic processes unveils a new, slower dynamics process: the nucleation of new domains. Exploring the role of environmental cues in chromatin reorganization dynamics, we find that dynamical substrate stiffening reduces heterochromatin domains sizes and spacing while increasing their number. Furthermore, through the analysis of heterokaryon-mediated reprogramming, we observe a similar increase in number of heterochromatin domains and decrease in their size and spacing. Notably, we also find that long timescale of nucleation induces hysteresis in nuclei, persisting even after removal of environmental perturbations. Our findings underscore the pivotal role of slow nucleation dynamics of new domains in long-term spatio-temporal chromatin redistribution, highlighting its significance in oncogenesis and metastatic progression.

Lay Abstract: Our study focuses on understanding how chromatin – the material that regulates gene activity – reorganizes within individual human cells. This process is crucial for cellular functions such as growth and environmental response. As cancer progresses, the area around the cancer cells, called the tumor microenvironment, changes continuously, influencing chromatin organization in complex ways. We developed a model to explore how chromatin interacts with itself and with the nuclear lamina, the structure surrounding the nucleus. We also considered how chromatin moves and undergoes chemical modifications. These interactions lead to the formation of stable chromatin clusters known as heterochromatin domains. Using super-resolution imaging to examine human stem cell nuclei, our model helped us observe how these domains reorganize. We find that a combination of diffusion and random local processes contributes to a slower dynamic process: the formation of new heterochromatin domains. When we looked at how environmental factors influence this reorganization, we found that making the substrate stiffer

reduces the size and spacing of these domains while increasing their number. Similarly, during the reprogramming of somatic cells, we observed an increase in domain numbers and a decrease in their size and spacing. Interestingly, this slow process of domain formation can lead to long-lasting changes in the nucleus, even after the initial environmental signals are removed. These findings underscore the importance of slow nucleation dynamics in the long-term redistribution of chromatin, highlighting its significant role in cancer development and metastasis.

High-resolution characterization of age-specific changes in the HPV-negative HNSCC tumors

Lina Kroehling, Not Applicable

Abstract: It's been shown that elderly patients with head and neck squamous cell carcinomas (HNSCC) exhibit diminished survival outcomes compared to their younger counterparts. While the convergence of aging hallmarks and cancer hallmarks offers valuable insights, further work is needed to elucidate the age-specific mechanisms influencing HNSCC beyond the shared characteristics of these biological processes. To this end, we have assembled a hiqh-quality human single-cell RNA-sequencing HNSCC atlas profiling more than 230,000 cells across more than 50 patients, with ages ranging between 18 and 89, which provides a unique resource to investigate age-associated changes in the disease‚Äôs heterogeneity. To create the atlas, we integrated six publicly available single-cell RNAseq datasets from 54 HPV-negative patients. Cells were clustered, classified, and characterized by gene set enrichment analysis, both in the epithelial cell compartment and in the tumor microenvironment (TME). Differential cell type proportion analysis was performed to identify cell type compositional changes associated with age. CNV analyses were performed to identify cancer subclones and assess level of copy number variation across tumors and age. Interestingly, we identify programs that associate with age, such as the partial-EMT signature, extracellular matrix-related processes, and dysfunction in epithelial cells, fibroblasts, and T cells respectively. We also identify distinct cell populations, such as vascular endothelial cells, that are more prevalent in older patients. Further analyses are ongoing, and we plan to functionally validate the hypotheses generated, specifically the presence of differentially abundant cell populations, and age-specific ligand-receptor signaling events that lead to tumor growth.

Lay Abstract: It's been shown that elderly patients with head and neck squamous cell carcinomas (HNSCC), tumors which occur in the oral cavity, exhibit worse survival outcomes compared to their younger counterparts. While certain hypotheses exist as to why these tumors appear more aggressive in older patients, such as decreased efficacy of the immune cells, or increased oxygenation to the center of a tumor, thus facilitating its growth, more work is needed to detail the specific mechanisms of action. Single-cell RNA-sequencing (scRNAseq) is a technology that allows for the profiling of thousands of cells at one time, and is frequently used to gain gene-expression information about tumor cells and the surrounding cells (termed the tumor microenvironment) in cancers. There are a number of publicly available HNSC scRNAseq datasets, of which we have combined six, creating thus far the largest and most comprehensive atlas of the HNSC tumor microenvironment. The atlas is composed of more than 230,000 cells across more than 50 patients. We use this atlas to investigate age-associated changes, both through identification of changes in specific cell types proportions and cellular processes across age. Interestingly, we identify cellular processes that change with age, namely one that affects how the immune cells respond to the tumor cells, and another which affects the structural integrity of the tissue, both of which can influence metastasis. Further analyses are ongoing, and we plan to validate the hypotheses generated, specifically the presence of differentially abundant cell populations, and age-specific signaling events that lead to tumor growth.

Combining Spatial Analysis Methods for Studies of Stromal Effects on Tumor Remission-Relapse Dynamics

Tatiana Miti, Cancer Systems Biology Consortium (CSBC)

Abstract: Stroma shapes tumor evolution, growth dynamics, invasion, and metastasis. Analyzing stroma-tumor cells' spatial interactions helps establish the radius and strength of these interactions regardless of whether they change under various treatments. We built a pipeline combining ecology and physics-based spatial analysis methods such as the Radial Distribution Function (RDF) and the Resource Selection Function, among others, to quantify the spatial extent and strength of stromal effects in the presence or absence of treatment. We strived to identify which spatial analysis methods offer the most accurate information about the stroma-tumor cell interactions as stroma topology and content changes under treatment. We tested the robustness of our pipeline by identifying and quantifying the stromal effects on tumor cell proliferation under targeted therapies in lung and breast cancer mouse models. The extracted radius and strength of stroma-proliferating tumor cells during treatment were compared against in silico-generated randoms for the same histological samples. Our preliminary results show that changes in the RDF's maximum and broadness during treatment can be used to establish the emergence of drug tolerance during treatment. At the same time, the J-function can be used robustly to identify the characteristic length of the stroma-tumor cell interactions only at the beginning of the treatment before the stroma undergoes drastic morphological changes. Our approach could be used as a fast and robust way to infer the strength of stroma-tumor cells' spatial interactions and serve as a guide for therapeutic strategies for disrupting the beneficial effects of stroma as drug resistance develops.

Lay Abstract: Stroma shapes tumor evolution, growth dynamics, invasion, and metastasis. Analyzing stroma-tumor cells' spatial interactions helps establish the radius and strength of these interactions regardless of whether they change under various treatments. We built a pipeline combining ecology and physics-based spatial analysis methods such as the Radial Distribution Function (RDF) and the Resource Selection Function, among others, to quantify the spatial extent and strength of stromal effects in the presence or absence of treatment. We strived to identify which spatial analysis methods offer the most accurate information about the stroma-tumor cell interactions as stroma topology and content changes under treatment. We tested the robustness of our pipeline by identifying and quantifying the stromal effects on tumor cell proliferation under targeted therapies in lung and breast cancer mouse models. The extracted radius and strength of stroma-proliferating tumor cells during treatment were compared against in silico-generated randoms for the same histological samples. Our preliminary results show that changes in the RDF's maximum and broadness during treatment can be used to establish the emergence of drug tolerance during treatment. At the same time, the J-function can be used robustly to identify the characteristic length of the stroma-tumor cell interactions only at the beginning of the treatment before the stroma undergoes drastic morphological changes. Our approach could be used as a fast and robust way to infer the strength of stroma-tumor cells' spatial interactions and serve as a guide for therapeutic strategies for disrupting the beneficial effects of stroma as drug resistance develops.

Concurrent Session VA: Spotlight Talks - AI/ML approaches for translational cancer research

OmicsMLRepo: Ontology-leveraged metadata harmonization to improve AI/ML-readiness of omics data in Bioconductor

Sehyun Oh, Informatics Technology for Cancer Research (ITCR)

Abstract: Efforts to establish comprehensive biological data repositories have been significant at national and institutional levels. Despite the large volume of data collected from diverse studies, the cross-study analysis across those repositories and joint modeling between omics and non-omics data remains largely limited due to the nature of non-omics metadata, such as lack of standardization, high complexity, and heterogeneity. This lack of metadata harmonization also hinders the application and development of machine learning tools, which can serve a pivotal role in managing and analyzing complex and high-dimensional multi-omics data.

To address this issue, we initiated the OmicsMLRepo project, harmonizing and standardizing metadata from Omics data resources. This process involved the manual review of metadata schema, the consolidation of similar or identical information, and the incorporation of ontologies. As a result, we have harmonized hundreds of studies on metagenomics and cancer genomics data, accessible through two R/bioconductor packages - curatedMetagenomicData and cBioPortalData. Furthermore, we developed a software package, OmicsMLRepoR, allowing users to leverage the ontologies in metadata search. Using manually harmonized metadata as a gold standard, we are developing an automated metadata harmonization tool. This ongoing project leverages various Natural Language Processing (NLP) techniques and will be applied to other Omics data resources. In summary, the OmicsMLRepo project facilitates cross-study, multi-faceted data analyses through metadata harmonization and standardization, making omics data more AI/ML-ready.

Lay Abstract: Researchers are collecting huge amounts of biological data from many different studies. However, it's very difficult to combine and analyze data across these studies because the metadata (data describing the data) is messy - it lacks standardization, is highly complex, and varies widely across studies. This hampers the use of machine learning tools that could help make sense of all this complex data.

To tackle this problem, we started the OmicsMLRepo project to standardize and harmonize the metadata from biological data resources. We manually reviewed metadata schemas, consolidated similar information, and incorporated standard terminology. Using this approach, we harmonized metadata from hundreds of metagenomics and cancer genomics studies, making the standardized metadata available through two software packages.

We also created the OmicsMLRepoR software package, which allows users to easily search the harmonized metadata using standardized terminology. While labor-intensive, this manually curated metadata serves as a gold standard for developing automated metadata harmonization tools using natural language processing techniques.

In summary, the OmicsMLRepo project is standardizing messy metadata to enable researchers to combine and analyze biological data across many studies. This harmonized, standardized metadata makes the large volume of biological data more amenable to machine learning and AI applications.

A Reliable and Adaptive Framework for High-Dimensional Fairness Aware Integration of Multiple Datasets

Yi Lian, Informatics Technology for Cancer Research (ITCR)

Abstract: In healthcare data analytics, fairness issues can arise from the heterogeneity between the groups defined by sensitive attributes such as race/ethnicity, and the imbalance in their sample sizes. If the groups are modeled jointly, the inference or prediction results are likely dominated by the majority group thus biased for the minority groups. If modeled separately, although enjoying more flexibility, the minority groups may not have enough data volume to generate accurate and reliable results. These issues can happen even if the data is a representative sample of the population therefore fairness-aware statistical analysis methods are needed. In this work, we propose a flexible multitask regression model that improves the prediction fairness for the minority groups, by integrating data from multiple sites, borrowing information from the majority group and adapting to the latent between-group heterogeneity in a data-driven manner, under high-dimensional settings. In addition, our work is in line with the notion of individual fairness in machine learning. Through extensive numerical experiments, we show that our method can improve the prediction accuracy for minority groups comparing to ordinary modeling strategies.

A self-supervised AI framework for quantitative assessment of intra-tumoral heterogeneity in GBM using MRI

Hairong Wang, Physical Sciences-Oncology Network (PS-ON)

Abstract: Intra-tumoral heterogeneity poses a significant challenge for glioblastoma (GBM) diagnosis and treatment. Histopathological and molecular analyses are limited by surgical sampling, highlighting the need for non-invasive methods to map these alterations across the entire tumor. Limited biopsy samples complicate the training of a generalizable artificial intelligence (AI) model, and patient heterogeneity further complicates the task with small sample sizes. We propose a self-supervised AI framework using extensive MRI data beyond biopsy regions to enhance AI model robustness and generalizability. Our framework has two steps. First, we train generative AI models in a self-supervised manner to translate each patient's image into one under the hypothetical condition that the patient does not have the tumor. This step captures the inherent differences in patients' brains. We developed two methods for this step to corroborate each other's results: a conditional diffusion model that translates the whole image and a vision transformer model that focuses on translating the tumoral area. Second, we classify regional biomarkers such as hallmark genes, tissue states, and cell types based on the real and generated MRI. By comparing the MRI with the tumor to the MRI without it, the model focuses on tumor-induced changes in the classification, producing more robust and generalizable results. Our study uses 283 image-localized biopsies with corresponding T1Gd and T2 MRI data from 67 GBM patients. Our model achieved high classification accuracy, surpassing state-of-the-art methods. Additionally, we generated biomarker prediction maps across entire tumors, providing a quantitative assessment of intra-tumoral heterogeneity in histopathological and molecular alterations.

Lay abstract: Glioblastoma (GBM) is an aggressive brain tumor, making it difficult to diagnose and treat because each part of the tumor can be different. Traditional methods use small samples taken during surgery, which cannot show the whole tumor. Therefore, we need new methods to

look at the entire tumor without surgery. We propose an artificial intelligence (AI) framework to analyze MRI scans that addresses these challenges. Our framework has two main steps. First, we train AI models to create images of what each patient's brain might look like if they did not have a tumor. This helps us understand the natural differences in patients' brains. We use two methods for this step to confirm each other's results. The first method, called the conditional diffusion model, changes the entire brain image to look healthy. The second method, called the vision transformer model, focuses on changing just the tumor area to look healthy. Second, we identify specific markers in the tumor, such as important genes, tissue types, and cell types, by comparing the real MRI with the generated healthy MRI. This helps the AI model focus on changes caused by the tumor, leading to more accurate and generalizable results. We tested our model using MRI data and biopsy samples from 67 GBM patients and found it to be highly accurate. Additionally, we created maps showing where these markers are located throughout the entire tumor, giving a detailed view of the tumor's structure and behavior.

Predicting the tumor microenvironment molecular composition from histopathology images to characterize immunotherapy responses in non-small cell lung cancer patients
Sushant Patkar, Informatics Technology for Cancer Research (ITCR)

Abstract: Immune checkpoint inhibitors (ICI) have become integral to non-small cell lung cancer (NSCLC) treatment. However, reliable biomarkers predictive of immunotherapy efficacy are limited. Here, we introduce HistoTME, a novel weakly supervised deep learning approach to infer the tumor microenvironment (TME) composition directly from standard Hematoxylin & Eosin (H&E)-stained pathology images of NSCLC patients. We show that HistoTME accurately predicts the expression of 30 distinct cell type-specific molecular signatures directly from whole slide images, achieving an average Pearson correlation of 0.5 with the ground truth on independent tumor cohorts. Furthermore, we find that HistoTME-predicted microenvironment signatures and their underlying interactions improve prognostication of lung cancer patients receiving immunotherapy, achieving an AUROC of 0.75[95% CI: 0.61-0.88] for predicting treatment responses following first line ICI treatment in a comprehensive, clinically annotated external cohort of 652 patients. Collectively, HistoTME presents an effective approach for interrogating the TME and predicting responses of patients to ICI, complementing existing biomarkers such as PD-L1 expression and bringing us one step closer to personalized immuno-oncology.

Lay Abstract: Immune checkpoint inhibitors (ICI) are important in treating non-small cell lung cancer (NSCLC), but we don't have many reliable ways to predict how well immunotherapy will work for each patient. Here, we introduce HistoTME, a new method that uses deep learning to figure out what the tumor microenvironment (TME) of each patient looks like directly from regular pathology images. We found that HistoTME can accurately guess the expression levels of 30 different TME-related biomarkers directly from whole slide images, matching up pretty well with the ground truth. Importantly, by leveraging HistoTME, we could better predict how lung cancer patients would respond to immunotherapy treatments without the need for expensive molecular tests or additional tissue stains. Overall, HistoTME is a useful tool for studying the tumor microenvironment from commonly available pathology slides and predicting how patients will respond to immunotherapy, adding to what we already know from other biomarkers like PD-L1 expression and moving us closer to personalized cancer treatments.

Mathematical model of AND-gated protease sensors predicts tunable improvements to signal-to-noise
Xinling Li, Synthetic Biology and Cancer Program (SynBio & Cancer)

Abstract: We developed a mass action model for binding and cleavage of single-input sensors and AND-gated sensors designed to detect a target tumor protease (MMP9) in the presence of noise (coagulation protease from blood). The model predicted higher signal over noise for two-arm AND-gated sensors than single-input sensors. To assess the results of mathematical model experimentally, we incubated a single-input sensor cleavable by GzmB and a cyclic peptide AND-gated sensor bearing two GzmB substrates with noise from media. The cyclic peptide had higher signal over noise than the single-input sensor. Therefore, the experimental results recapitulated the mathematical model results. Next, a condition with high noise was picked to interrogate whether more arms can provide the capabilities to distinguish signal and noise. From our mathematical model, AND-gated sensors with more arms had higher cooperativity and signal over noise. Therefore, increasing number of arms for the sensors was predicted to increase performance of signal detection. Our model predicted that the optimal time point for distinguishing signal and noise varied depending on the number of arms. Finally, we evaluated whether tuning system parameters could further increase signal over noise. It was predicted that two-arm AND-gated sensors were more sensitive to elevated protease concentrations in tumors than single-input sensors. Also, two-arm AND-gated sensors were predicted to be more tolerant to noise from blood proteases than single-input sensors. Collectively, our results indicate that AND-gated sensors can improve signal-to-noise ratios and our mathematical model can predict substrate kinetics that maximize detection performance.

ARCHS4 Navigator: Multimodal Feature Vectors as Descriptors of Gene Expression Data
Alexander Lachmann, Informatics Technology for Cancer Research (ITCR)

Abstract: Searching publicly available gene expression data from the Gene Expression Omnibus (GEO) is a complex challenge due to the large collection of diverse studies and the commonly inconsistent metadata provided by data submitters. While GEO offers a broad array of biological contexts, the weakly defined metadata structure and unstandardized vocabulary pose significant challenges to data findability, reusability, and integration. Manual curation of this extensive metadata is impractical, and integrating metadata with primary RNA-seq gene counts at a unified level has been elusive. The ARCHS4 Navigator project addresses these issues by leveraging our publicly available uniformly aligned gene expression data and their associated metadata. The ARCHS4 Navigator leverages large language models (LLMs) to process GEO metadata and associated publications, creating embedded vectors that characterize the metadata for each GEO sample and study. These vectors are then merged with a compressed representation of the ARCHS4 gene expression data to form a high-dimensional feature space that integrates publication text, metadata, and gene expression information. This integrated feature space facilitates complex queries for gene expression samples. Moreover, using the archs4py Python package, users of the platform can run the ARCHS4 pipeline locally, enabling them to find samples with metadata and gene expression profiles similar to their own data. The feature vectors created by the ARCHS4 Navigator platform serve as a valuable resource for various machine-learning applications. The ARCHS4 Navigator platform will be integrated within the ARCHS4 UI and will be made available as a web-service.

Concurrent Session VB: Spotlight Talks - Tumor immunology and immunotherapy

Image-Guided Sonogenetic Control of Thermal-Sensitive CAR T Cells Overcomes Brain Tumor Heterogeneity and Immune Suppression
Ali Zamat, Synthetic Biology and Cancer Program (SynBio & Cancer)

Abstract: Malignant brain tumors, including glioblastoma (GBM) and breast cancer brain metastasis (BCBM), present significant clinical challenges due to their aggressive nature and poor prognosis. Immunosuppression and antigenic heterogeneity undermine the effectiveness of immunotherapies targeting a single antigen. Strategies are further hampered by dose-limiting toxicities and poor penetration of the blood-brain barrier. Here, we develop thermally sensitive CAR T cells to produce NKG2D bispecific T cell engagers (BTEs) in response to MR-guided focused ultrasound (MRgFUS) to non-invasively reduce immunosuppression, overcome tumor antigen escape, and improve survival in various preclinical models of brain cancer.

We developed thermal gene switches (TS) with low basal activity and high induction (>200-fold) under mild heating (40-42°C) and engineered αHER2 CAR T cells to control transgene expression of luciferase (TS.Fluc) or NKG2DL-targeting BTEs (TS.BTE). In brain tumor bearing mice, 10-minute remote activation of TS.Fluc αHER2 CAR T cells by MRgFUS resulted in a ~10-fold increase in transgene activation. Following transfer of TS.BTE αHER2 CAR T cells and thermal treatments in murine models of GBM, we observed a significant reduction in myeloid-derived suppressor cells (MDSCs) in the tumor by flow cytometry. Furthermore, in heterogenous BCBM models, treatment led to regression of antigen-negative tumor cells and complete clearance detected by IVIS and MRI leading to increased survival for over 100 days.

These findings demonstrate that MRgFUS-controlled CAR T cells offer a precise, non-invasive approach to effectively reduce MDSCs and enhance targeting of heterogenous brain tumors by locally secreting BTEs that drive therapeutic outcomes in models of GBM and BCBM.

Lay Abstract: Malignant brain tumors, like glioblastoma (GBM) and breast cancer brain metastasis (BCBM), are extremely challenging to treat with patients often surviving less than two years. These tumors recruit cells that lead to strong immune suppression and are comprised of a mix of different cancer cells, making it hard to target them with a single treatment. Current treatments struggle because they can't effectively reach the brain and have toxic side effects if delivered throughout the body.

To address this, we developed a new approach using CAR T cells, a type of immune cell engineered to kill cancer. We engineered these CAR T cells to be sensitive to heat and used ultrasound guided by MRI to heat them inside brain tumors. This activates the cells to produce molecules called bispecific T cell engagers that help the immune system reduce suppression and attack the tumor.

In mice with brain tumors, we found that heating the CAR T cells in the brain tumors led to a 10-fold increase in their activity. When we heated the brain tumors of mice with these CAR T cells, we saw a reduction in immune suppressor cells. Additionally, in models of heterogenous cancer, the treatment led to complete tumor clearance, significantly increasing survival by over 100 days.

These results show that our method using image-guided control of CAR T cells is a precise, non-invasive way to reduce immune suppression and effectively target different types of cancer cells in brain tumors, improving treatment outcomes in models of GBM and BCBM.

Dynamics of macrophage tumor infiltration

Kolade Adebowale, Not Applicable

Abstract: Adoptive transfer of macrophages is a clinical stage therapy against multiple tumors. Their clinical success is contingent on the ability of macrophage to migrate through the dense tumor matrix. Macrophages are phenotypically plastic in nature and exhibit a spectrum of

phenotypes that resemble features of naïve, anti-tumor and pro-tumor functions. Correspondingly, macrophages can be selectively converted ex vivo to M0, M1, and M2 using pre-defined cytokines. Most cancer clinical trials have made use of converting macrophages ex vivo to an anti-tumor phenotype (M1), and while they demonstrated efficacy in some patients, no long-term remissions have been observed so far. To be effective, the therapeutic macrophages must physically migrate to tumors. However, the trafficking of macrophages to tumors has not been rigorously studied. We hypothesized that macrophage trafficking depends on their phenotype. To test this, we developed a three-dimensional cellular assay comprising a tumor spheroid and macrophages to study and quantify macrophage transport into tumors and assessed its key features including dependence on macrophage phenotype. Cell migration, permeability, and kinetics of tumor entry were quantitatively defined and compared between the phenotypes. Our results demonstrate that compared to M0 macrophages, M1 macrophages migrate less efficiently toward the tumor spheroid and exhibit a five-fold lower tumor permeability. Live imaging data combined with unsupervised machine learning algorithms reveal that macrophage migration correlates with their shape transitions. Our studies highlight the importance of transport considerations in determining the efficacy of cell therapies and the need to develop quantitative approaches to enable their studies.

Lay Abstract: Adoptive transfer of patient-derived macrophages has been used in human clinical trials to treat a variety of tumors with no long-term remission observed. An overlooked aspect is that macrophages must physically move toward the tumor. To address this, we develop a quantitative approach to characterize the transport of macrophages into tumors and demonstrate that the clinically used M1 phenotype of macrophages exhibits a substantially lower ability to infiltrate into the tumors compared to the naïve M0 phenotype. Dynamic imaging data, combined with unsupervised machine learning, revealed that the ability of macrophages to infiltrate into the tumors correlates with their ability to undergo shape transitions. The quantitative approach presented here provides a framework to rigorously compare adoptive cell therapies.

Macrophage clusters extend multiple pseudopods to disrupt cell junctions between cohesive targets

Larry Dooling, Physical Sciences-Oncology Network (PS-ON)

Abstract: Spatial organization of tissues is critical to their function but becomes dysregulated in cancer. The spatial organization of immune cells including macrophages in tumors and any effects on their function are understudied. We showed that macrophages aggregate in vitro and in vivo when activated to phagocytose cancer cells and that phagocytosis can be modeled as a cooperative process among multiple macrophages. In further reductionist approaches here, we also show that macrophages organize into compact aggregates when activated with pro-inflammatory (M1) stimuli but disperse when activated by anti-inflammatory (M2) stimuli and that M1- and M2-activated macrophages sort away from each other in mixtures. The distinct aggregation behaviors of different macrophage states reflect underlying changes in integrin adhesion receptors and actomyosin contractility. Importantly, aggregated M1 macrophages eliminate co-cultured cancer cells more rapidly than control macrophages. By observing macrophage interactions with melanoma spheroids under treatment conditions that maximize phagocytosis, we discovered macrophages extend intrusive pseudopods or “intrudopods” between melanoma cell adhesions. Aggregates of macrophages extending multiple intrudopods might disrupt cellular adhesions more efficiently than isolated macrophages, thereby providing a key advantage of cooperative phagocytosis in solid tumors.

Lay Abstract: Macrophages are immune cells named after one of their key functions, phagocytosis or “cell eating”. They are highly abundant in many kinds of cancer, and emerging

therapies are being developed to direct them to “eat” cancer cells. Molecular and physical barriers in tumors make this a difficult and inefficient process. One such barrier is adhesion of cancer cells to one another and to connective tissue, which macrophages must somehow overcome if they are to completely surround their target cells and eat them. Therefore, my research tries to understand how macrophages literally “take a bite out of cancer”. We have discovered that groups of macrophages can work together to phagocytose cancer cells that adhere to one another, and we have identified factors that promote aggregation of macrophages. Using three-dimensional spheroids of melanoma cells, we observed that macrophages extend protrusions between cancer cells to disrupt adhesions. Groups of nearby macrophages can extend multiple protrusions that are a potential source of cooperativity for more efficient tumor phagocytosis.

Autoantibodies in high-grade serous ovarian cancer interact poorly with cytotoxicity-inducing Fc receptors

Michelle Loui, Not Applicable

Abstract: In high-grade serous ovarian cancer (HGSOC) patients, malignant epithelial cells arise from the fallopian tube and ovarian surface epitheliums. Endogenous antibodies (anti- tumor antibodies; ATAbs) target these cells and should promote recognition by the immune system. Patient-derived tumors have been found to be frequently coated in IgG, and ATAbs are present both in the tumor mass and in the fluid that builds up in the peritoneum surrounding the tumor microenvironment. They are derived from B cells that have undergone somatic hypermutation, indicating an active immune response. However, despite their widespread abundance in HGSOC, ATAbs fail to eliminate the tumor cells. We hypothesized that ATAbs are unable to eliminate tumors due to the dysregulation of immune interactions via their Fc region. Therefore, we applied a quantitative, multiplexed assay for profiling the Fc properties and immune receptor interactions of ATAbs. Our data demonstrate that ATAbs interact poorly with FcγRIIIa potent activating receptor for antibody-dependent cellular cytotoxicity (ADCC) found on natural killer cells due to fucosylation. Understanding the mechanisms of humoral immunity evasion will help with the prediction of therapeutic responses in cancer patients and uncover how immunotherapies might reactivate effective humoral immunity.

Lay Abstract: The immune system of ovarian cancer patients can produce antibodies that specifically target their tumors as part of the immune response. Previous research has demonstrated that these anti-tumor antibodies (ATAbs) commonly coat patient tumors. ATAbs are present in both the tumor mass and in the fluid surrounding the tumor microenvironment. Despite their prevalence in ovarian cancer, ATAbs fail to eradicate tumor cells, allowing the cancer to evade the immune system. We hypothesize that the inability of ATAbs to eliminate tumors is due to a defect in their Fc region, which is the part of the antibody that interacts with immune cells to initiate an immune response to attack cancer cells. Therefore, we employed a quantitative assay to measure the extent of ATAb interaction with various immune cells. Our data demonstrate that ATAbs interact poorly with FcùõæRIIIa,Äîan activating receptor for antibody-dependent cellular cytotoxicity (ADCC) found on natural killer cells,Äîdue to fucosylation. Fucosylation occurs when fucose sugars are present on the Fc region of the antibody. Uncovering the reasons behind the ATAbs' failure to clear the tumor cells will aid in developing cancer treatments that can address the defect in their Fc region.

Microenvironmental regulation of immune cell infiltrates in cutaneous melanoma

Udochi Felicia Azubuike, Metastasis Research Network (MetNet)

Abstract: Metastasis is a multistep process where cancer cells leave their organ of initiation and survive in a new organ. Worldwide, metastasis is responsible for greater than 90% of cancer

deaths, however at present, there are very limited treatment options available once disease reaches this point. Being able to decipher the mechanism through which cancer cells survive in their new environment will help find more therapies for metastasis.

In this study, we use a syngeneic zebrafish melanoma model and find that zebrafish melanoma cell lines (ZMEL1) in the brain organ use glycolysis while those in the skeletal organ use oxidative phosphorylation as their metabolic pathways. We observe variations in CD8+ T cell expression in the tumor microenvironment (TME) in both organs. Further, we find mhc1uba transcript, a non-classical MHC1 related gene that presents antigens to unconventional T cells, was highly expressed in the skeletal metastasis compared to brain metastasis.

Mhc1uba expression in the skeletal metastasis was altered in a zebrafish strain where csf1ra+ macrophages were genetically ablated. In humans, expression of MR1 (the mhc1uba ortholog) in melanoma patient cells with metastasis also showed organ specific expression, suggesting a conserved role for this protein.

Overall, our data show organ specific expression of mhc1uba and MR1 in zebrafish and human melanoma samples, suggesting the presence of unconventional T cells in metastasis to specific organs. By understanding the role of MR1 expression in metastasis, we hope to determine patient response to current immunotherapies and devise alternative treatments.

Lay Abstract: Most melanoma patients die from metastasis, which is a process where cancer cells move from the skin to different organ such as the brain or bone. Immunotherapy is a type of treatment used for metastasis but only 20% of the patients respond. Therefore, we need to find other therapies that can be effective for the nonresponsive patients and find markers that can differentiate responders from non-responders.

In this study, we used zebrafish to understand how melanoma cells that are found in the brain and skeleton survive in these environments that are different from the skin. We find that the cancer cells in the 2 organs used different pathways for their energy production. Interestingly, there was 1 type of immune cell we found in the brain, but not in the skeletal metastasis. These immune cells are responsible for recognizing the cancer cells. In conclusion, the data shows the cancer cells found in different environments use different source of energy, have different immune cells, and different ways of being recognized by the body's own immune system. This study is important as it suggests that different therapies showed be used based on the organ of metastasis.

Quantitative cell type specific immunopeptidome analysis during macrophage and tumor co-evolution reveals therapeutic MHC-I restricted peptides in glioblastoma

Yufei Cui, Cancer Systems Biology Consortium (CSBC)

Abstract: Glioblastoma is an aggressive form of adult brain cancer with poor prognosis. Despite recent progress of immune checkpoint inhibitors (ICI) in treating several types of solid tumors, ICI or other forms of immunotherapy has yet to show any efficacy in GBM, mainly because of the immunosuppressive tumor microenvironment (TME). GBM associated macrophages (GAMs) is the most abundant type of infiltrated immune cells in the TME, where they impede cytotoxic T cell functions and promotes GBM aggression. Targeting these GAMs to revert the immunosuppressive TME thus holds potential to boost immunotherapy efficacy in GBM. To uncover targetable MHC-I antigens for GAMs, we performed cell type specific immunopeptidome analysis using liquid chromatography with tandem mass spectrometry (LC-MS/MS) on primary macrophages and GBM tumor cells in a co-culture system. We characterized the systemic alteration of presented antigen repertoire on either GAMs or GBM tumor cells compared to their

respective unperturbed state. Polarization to GAM resulted in significant upregulation of presentation of proteins associated with cytokine signaling pathways on macrophages, and such alteration is also observed in human GBM samples. Additionally, we quantitively selected co-culture induced significantly altered peptides (CIVS peptides) which can serve as potential immunotherapy targets. mRNA vaccine encoding six selected CIVS peptides from GAMs and GBM tumor cells was able to control tumor growth in 40% of treated mice, demonstrating the translational potential of CIVS peptides as GBM vaccines.

Lay Abstract: Glioblastoma is a very aggressive type of brain cancer in adults with a poor outlook. Even though new treatments called immune checkpoint inhibitors (ICI) have shown promise in other cancers, they haven't worked well for GBM yet. This is mostly because GBM creates an environment that weakens the body's immune response. Macrophages, a type of immune cells, are highly prevalent in glioblastoma where they actually help the tumor growth by stopping T cells from attacking the cancer. By targeting these macrophages, we might be able to improve how well immunotherapy works against GBM. To find targets for the macrophages in GBM, we studied the changes of proteins on the surface of macrophages and glioblastoma cells when they are growing together. Through LC-MS/MS method, we quantified which proteins were at different level when the macrophages were influenced by GBM. We found that macrophages in GBM showed more proteins related to cytokine signaling pathways, and this change was also seen in human glioblastoma samples. We identified specific proteins that changed significantly in the co-culture and could be targeted for treatment. We then created a mRNA vaccine using six of these proteins. This vaccine was able to control tumor growth in 40% of treated mice, suggesting that it could potentially be used as a new treatment for glioblastoma.