Poster Sessions and Abstracts

Poster Session I

Poster #	Name	Title
1	Alireza Sohrabi	Ex vivo Investigation of post-radiation extracellular matrix evolution in glioblastoma
2	Andrew Chen	Highly-resolved Atlas of Human Breast Cancer enables hypothesis-driven analysis of tumor heterogeneity
3	Billy Hill	Exploring and exploiting circulating tumor cells weaknesses
4	Diana Kang	Engineering LNPs with polysarcosine lipids for mRNA delivery
5	Fernando Flores-Guzman	Novel murine model to assess Notch activation and inhibition in the tumor microenvironment
6	Ha Nguyen	DSCC: Disease Subtyping using Consensus Network and multi-omics data integration
7	Isaac Bishara	End-Stage Breast Cancers are Comprised of Tumors with Distinct Metastatic Capacities and Microenvironment Compositions
8	Jens Luebeck	AmpliconSuite: Analyzing focal amplifications in cancer genomes
9	Jorvani Cruz Villarreal	Visual Time-Temperature Indicators of Biospecimen Exposure to Thawed Conditions
10	Joseph Fernandes	Dynamic regulation of tumor cell competition determines targeted therapy resistance
11	Kevin Lybarger	Simplifying Health Information: A Corpus for Digestive Cancer Education and Novel Strategies for Reinforcement Learning
12	Magda Bujnowska	The role of AP-1 transcription factors in regulation of transcriptional plasticity in melanoma cells
13	Matthew Froid	Agent-Based Modeling of Resistance Mechanisms and Treatment Strategies for T-Cell Engaging Antibodies in Multiple Myeloma
14	Nicole Mattson	Enhancing Cancer Therapy: Biomarkers, Mechanism, and the Promise of cpd_AV2 as a Superior Integrin Inhibitor
15	Peiwen Cong	Characterization and Engineering of P53R175H-specific TCR for Cancer Adoptive Cell Therapy
16	Sanjana Rajgopal	Anti-tumor Actions of Neutrophils on Bone Metastatic Prostate Cancer

18	Seung-Hyun Ko	Mechanosensitivity of PD1 Localization on the CD8 T-cell Membrane as Interrogated Using STORM Microscopy and Monte Carlo Simulations
19	Sharvari Kemkar	Towards Cancer Digital Twins: Simulating Tumor Heterogeneity and Patient Response using Tissue level Agent-Based Modeling of Prostate Cancer
20	Sravya Prabhala	Evaluating Deep Learning Features of Chromatin-Sensitive Partial Wave Spectroscopic Microscopy for Early-Stage Lung Cancer Diagnosis
21	Srikanta Basu	CK2 signaling from ER-embedded Tollip-dependent perinuclear endosomes is an essential feature of KRAS and NRAS mutant cancers.
22	Steven Forsythe	Development of GEP-NEN Organoids for Therapeutic Screening
23	Torikul Islam	Human osteosarcoma metastasis is limited by oxidative stress-induced ferroptosis
24	Woong Young So	Yap localization mediates mechanical adaptation of human cancer cells during extravasation in vivo
25	Xucheng Hou	Close the Cancer-Immunity Cycle by integrating lipid nanoparticle-mRNA formulations and dendritic cell therapy
26	Yi Qiao	Algorithmic and informatics approaches to advance precision medicine
27	Zixian Guo	Integrating Epigenetic Regulation and Chromatin-Lamina Interactions to Unveil the Morphology of Lamin-Associated Domains
28	Vishal Srikanth	Understanding the physics behind cellular reprogramming in porous materials

Poster Session II

Poster #	Name	Title
1	Aayush Kant	The nuclear response to metastatic environmental cues driven is by chemo-mechanical chromatin reorganization
2	Alexandra Grigoropoulos	Phase behavior of protein-like synthetic heteropolymers toward targeting aberrant biomolecular condensation
3	Andre Forjaz	Replicating the Human Fallopian Tube through CODA-guided design of organoids
4	Auggie Wirasaputra	AXL as a driver of breast cancer dormancy
5	Daniel Bergman	Assimilating single-cell sequencing and imaging data into an agent-based model of the desmoplastic tumor microenvironment of pancreatic adenocarcinoma
6	Debasish Paul	mTOR-mediated transient APC/C inactivation is required for mammalian cell cycle entry
7	Eric Medina	Systems modeling reveals drug-induced transcriptional regulons linked to everolimus resistance in ER+ breast cancer
8	Geng Wang	Super-resolution multimodal nanoimaging platform for chromatin study

10	Hannah Newman	Evolutionary Enhancement of DNA-Damaging agents by Natural Killer Cells in Non-Small Cell Lung Cancer
11	Hirak Sarkar	A count-based model for delineating cell-cell interactions in spatial transcriptomics data
12	Hugo Miniere	A data assimilation framework for predicting the spatiotemporal response of high-grade gliomas to chemoradiation
14	Jingjing Liu	Systems biology-enabled combination therapies for MYC-driven Medulloblastoma
15	Khanh Ly	Fate of tumor cells post mechanical priming on substrates mimicking metastasis sites
16	Lusheng Song	Head and Neck Cancer and Microbial Infections: A Deeper Look
17	Madhan Subramanian	Extensions of the OMOP Common Data Model for Translational Research: Inclusion of Animal Models in the MeDOC Consortium
18	Michael Seen	Plasma exosomes from type 2 diabetic patients drive expression of EMT-related genes in prostate cancer models
19	Nadia Arang	Physical and functional mapping of the p53 mutation-driven interactome
20	Nehal Dwivedi	Deciphering how Tropomyosin2.1 prevents Anoikis resistance in breast cancer cells
21	Ninette Irakoze	Investigating Tumor Microenvironment Drivers of Drug-Resistant Non-Small Cell Lung Cancer
22	Parker Stevenson	Metastatic Knowledge: Transfer learning of gene regulatory signatures
23	Richa Tiwari	Mapping protein-protein interaction remodeling following chemotherapy in triple-negative breast cancer
24	Rohan Panaparambil	Targeting lipid metabolism in breast cancer metastasis
25	Ruyi Gong	Molecular specificity of nucleic acids via intrinsic stochastic fluorescence under visible light illumination
26	Sandeep Jain	Developing a Comprehensive Hematology Oncology Clinical Guideline Database
27	SJ Claire Hur	An Advanced Electroporation System for Efficient Gene Delivery in Patient-Derived Cells
28	Verima Pereira	NFAT Physical and Genetic Interaction Mapping to Predict the Impact of VEGFR2 Inhibition in HCC
29	Aditi Singh	Investigating mechanisms of cancer cell apoptosis with low frequency ultrasound

Poster Abstracts

Anti-tumor Actions of Neutrophils on Bone Metastatic Prostate Cancer
Sanjana Rajgopal, Cancer Innovation Laboratory

Abstract: The overall patient survival rate drops dramatically when localized prostate cancer (PCa) progresses to bone metastatic prostate cancer (BM-PCa). The treatment for BM-PCa is

limited to palliative therapy, and it is currently incurable. Neutrophils are the most abundant innate immune cell in the bone, and we have found that PCa enhances their recruitment and infiltration into the prostate tumor-bone microenvironment of BM-PCa patients. Additionally, we observed that bone marrow neutrophils directly induce apoptosis of PCa cells both in vitro and in vivo and that this is mediated by the inhibition of STAT5 activity, a transcription factor that promotes PCa progression and contributes to the development of resistance to standard care androgen deprivation therapy. Neutrophils specifically target and kill only cells that express STAT5, as stable knockdown of STAT5 in BM-PCa cells makes them resistant to neutrophil-mediated cytotoxicity, while the expression of STAT5 in STAT5-negative PCa cells, which are generally resistant to killing by neutrophils, sensitizes them to neutrophil-mediated killing. Furthermore, transcriptomic analyses derived from the STAT5 knockdown and STAT5 overexpressing cells suggest that STAT5 expression in PCa induces pro-inflammatory signaling and that neutrophil-mediated cytotoxicity may be exerted via STAT5-induced IL-1. Preliminary data in our laboratory show that STAT5-induced production of IL1 by PCa cells leads to their death activation of neutrophils to kill PCa cells. Further delineation of the role of neutrophils in STAT5 signaling in BM-PCa growth holds promise for enhancing neutrophil cytotoxicity in the bone which could lead to novel therapeutic options for bone metastatic prostate cancer.

Lay Abstract: Treatment of prostate cancer that has spread to the bone is extremely complicated to treat and is incurable as of now. Our lab has identified that when prostate cancer spreads to the bone, it recruits in a type of immune cell called neutrophils. We discovered that these neutrophils can directly kill prostate cancer cells, both in lab experiments and in living mouse models of prostate cancer in bone. Furthermore, we have identified that the neutrophils block the activity of a vital protein in prostate cancer cells; STAT5 (a protein aiding prostate cancer to grow and become resistant to standard hormone therapy treatments). Moreover, the neutrophils seem to specifically target and kill only the cancer cells that have STAT5. Our research suggests that STAT5 may cause the cancer cells to send out inflammatory signals that attract the neutrophils and activate them to kill. Preliminary evidence points to an inflammatory protein (pro-inflammatory cytokine); IL-1, potentially being involved in activating the neutrophils to kill the cancer cells that express STAT5. By further studying how neutrophils and STAT5 interact in prostate cancer bone metastases, we may be able to develop new therapies that boost the ability of neutrophils to kill cancer cells in the bone.

Highly-resolved Atlas of Human Breast Cancer enables hypothesis-driven analysis of tumor heterogeneity

Andrew Chen, Cancer Systems Biology Consortium (CSBC)

Abstract: Single cell transcriptomics (scRNAseq) remains the method of choice to quantify and annotate cellular heterogeneity in the tumor and its microenvironment (TME), yet most studies are under-powered to associate changes in tumor heterogeneity with phenotypes such as tumor subtype, grade, and patient age, among others. In this study, we created an integrated atlas of human breast cancer (BC), the largest resource of its kind, totaling > 700,000 cells across 129 patients, and optimized computational methods to benchmark integration performance, and robustly perform hierarchical cell type annotation. By combining single profiles, a higher-resolution annotation of immune, stromal, and epithelial cell types was achieved. Further, using this integrated atlas, generalized linear mixed effect model (GLMM)-based analysis identified significant changes in lymphoid cell type abundances associated with tumor grade, as well as significant changes in myeloid cell type abundances associated with a patient's age. These changes in TME heterogeneity were not discernible or had effects in the opposite direction when the analysis was limited to individual BC studies, highlighting the need for atlas-based mega-analysis approaches. This highly-resolved integrated scRNAseq BC atlas will be a valuable

resource for hypothesis-driven analyses of tumor heterogeneity including our own ongoing analysis of metabolic comorbidities in BC.

Lay Abstract: In cancer biology, scientists mostly rely on single cell sequencing to determine what cell types are present in a tumor and its adjacent tissues (i.e. to determine a tumor's cell type composition). However, due to the high cost of the technology, most studies only profile a limited number of patient samples and do not have enough representation of key clinical features like tumor (sub)type, tumor grade, and patient age, to rigorously assess the association of clinical features with cell type compositional changes across tumors. In this study, we rigorously integrated all publicly available datasets of breast cancer gene expression, yielding a combined resource, or "atlas", comprising more than 700,000 cells and 129 patients. By analyzing this integrated atlas, we found significant differences in the types of immune cells between high-grade (more severe) and low-grade (less severe) tumors, and also between older and younger patients. These differences were not found when we conducted the analyses separately for each individual study, demonstrating the importance of large-scale combined analyses. Our breast cancer atlas will be a valuable resource to the field to both identify new cell types and also enable further research on how different clinical features and patient health conditions may affect breast cancer.

Assimilating single-cell sequencing and imaging data into an agent-based model of the desmoplastic tumor microenvironment of pancreatic adenocarcinoma

Daniel Bergman, Cancer Systems Biology Consortium (CSBC)

Abstract: Agent-based models (ABMs) provide detailed insights into multi-scale phenomena in tumor biology, yet ABMs face challenges in accurately representing living tissue due to the complexity involved in modeling cell behavior, interactions, and signaling. Addressing this challenge will require leveraging data across multiple modalities, including bioinformatics and imaging. Recent advancements in bioinformatics enable detailed characterization of cell phenotypes and communication patterns through single cell RNA sequencing and spatial transcriptomics. Complementing this, imaging techniques can offer a comprehensive view of the main acellular component of the TME, the extracellular matrix (ECM), quantifying a diverse set of ECM properties such as stiffness, anisotropy, and porosity. Integrating these data sources, we have developed a data-driven ABM of pancreatic adenocarcinoma (PDAC), enabling exploration of ECM hypotheses, T cell trafficking, and therapeutic strategies. We demonstrate this framework by recapitulating patient heterogeneity in treatment response, correlating these outcomes with the initial TME organization. By integrating ABMs with such data, we aim to unravel tumor response heterogeneity at a patient level.

Lay Abstract: Cancer is an evolving, disease with high variability between patients. Each cancer presents with its own set of rules. While we can learn the rules of a patient's tumor from imaging and biopsy samples, these techniques are insufficient for predicting the subsequent state of the tumor and its environment. We use mathematical models built from mechanistic first principles that simulate how tissues change during cancer progression to translate these rules into actionable insights. That is, we build digital twins of patient tumors that predict the range of possible outcomes in much the same way that weather forecasters predict the path of a hurricane. In this work, we showcase these efforts applied to pancreatic cancer, a notoriously difficult-to-treat cancer due to high levels of fibrosis and low levels of tumor-attacking immune cells. Leveraging our collection of biospecimens from previously resected tumors, we analyze the composition of these tumors to bring into focus the spatial relationships of the fibrosis and limited immune compartment. We then run virtual clinical trials on cohorts of digital twins based on these patient biospecimens to predict the effect of various therapeutic interventions to identify candidate therapies for future, real patients.

Systems modeling reveals drug-induced transcriptional regulons linked to everolimus resistance in ER+ breast cancer

Eric Medina, Cancer Systems Biology Consortium (CSBC)

Abstract: Estrogen receptor positive (ER+) breast cancer represents nearly 75% of all breast tumors. Everolimus, an mTORC1 inhibitor, in combination with exemestane is approved for patients with metastatic ER+ breast cancer. However, many patients develop resistance leading to poor survival outcomes. Characterizing everolimus-resistant cells could reveal targetable molecular phenotypes and lead to new therapeutic targets. Thus, we generated ER+ breast cancer cell lines resistant or sensitive to everolimus and leveraged transcriptomic profiles before or after everolimus treatment. Using linear mixed effect models, we identified several meta-phenotypes distinct between sensitive and resistant populations. Further, we found strong activation of growth-factor signaling meta-phenotype in resistant cells, which was maintained despite everolimus treatment. Next, we adopted a transcriptional regulatory network reconstruction framework to identify drug-induced regulons responsible for the dysregulation of resistance meta-phenotypes. We identified SMARCD3, ZNF100, ARID5B and ZBTB21 as key transcription (co)-factors controlling regulons across diverse resistance meta-phenotypes, including growth-factor signaling. Interestingly, we found elevated SMARCD3 in resistant tumors from a cohort of 23 ER+ breast cancer patients treated with everolimus. To confirm the role of SMARCD3 in everolimus resistance, we repressed SMARCD3 using shRNA silencing to re-sensitize resistant cells to everolimus treatment. Further, to identify anti-cancer drugs that could invert regulon activity, we leveraged GDSC drug sensitivity information of 403 compounds and calculated regulon signature scores for 1019 cancer cell lines to re-sensitize resistant cells to everolimus. In conclusion, we used context-specific transcriptomic profiles and integrative systems-biology approaches to elucidate resistance mechanisms in response to everolimus treatment in ER+ breast cancer.

HMGB1 localization and its effect on the immune response in the lung tumor microenvironment

Glenn Simmons Jr, Cancer Systems Biology Consortium (CSBC)

Abstract: Background: Lung adenocarcinoma is the leading cause of cancer mortality. High Mobility Group Box 1 (HMGB1) is a master regulator of innate immunity that is elevated in lung cancer tissues. HMGB1 contributes to PD-L1 expression in melanoma and promotes the secretion of inhibitory cytokines TGF-α and IL-10, which have roles in the differentiation of regulatory T cells. This indicates that HMGB1 influences an immunosuppressive signaling cascade in the tumor microenvironment. However, beyond showing that HMGB1 is elevated and modulates inflammation, studies to date have not comprehensively investigated the influence of HMGB1 on the immune response to lung cancer. Interestingly, unsaturated fatty acids are known for their anti-inflammatory capacity and effects on HMGB1 function. While treatment of lung cancers with immune checkpoint inhibitors (ICI) (anti-PD-1/PD-L1) has succeeded, there are limitations. One significant limitation is the failure of patients with advanced or refractory disease to generate a solid anti-tumor immune response. Therefore, we hypothesized that high monounsaturated fatty acids (MUFAs) promote an anti-tumor immune response in the tumor microenvironment by halting HMGB1 release from cancer cells.

Methods: To test this hypothesis we used a combination of proteomics, immunological assays, and 3-dimensional cell culture.

Results: Monounsaturated fatty acid (MUFA) availability inhibited extracellular HMGB1 release from cancer cells and inflammatory immune signaling in myeloid derived cells

Conclusions: Exploiting the MUFA/HMGB1 signaling axis could be a novel approach to improve the efficacy of therapies in populations that are not currently benefiting from current strategies for eliminating tumors.

Lay Abstract: High mobility group box protein 1 (HMGB1), a pro-inflammatory damage-associated molecular pattern protein (DAMP), plays a crucial role in modulating inflammation in the lung tumor microenvironment. It has been shown that elevated HMGB1 levels can suppress functional anti-tumor immune responses. Our lab has recently identified HMGB1 as a protein whose function is sensitive to monounsaturated fatty acid (MUFA). Using a combination of molecular assays and 3-dimensional cultures, we showed that HMGB1 was sequestered within the cancer cell instead of being released when exposed to exogenous MUFA. We hypothesized that the presence of MUFA can promote an anti-tumor immune response in an HMGB1-dependent manner. If extracellular HMGB1 is less abundant, then HMGB1-responsive signaling pathways (i.e. NF-kappa B) will be minimally activated. This has the potential to decrease maladaptive immune and inflammatory responses through innate immune cells. Taking advantage of the relationship between MUFA and HMGB1 may help increase the efficacy of cancer therapies that are currently effective in only a subset of patients.

Evolutionary Enhancement of DNA-Damaging agents by Natural Killer Cells in Non-Small Cell Lung Cancer

Hannah Newman, Cancer Systems Biology Consortium (CSBC)

Abstract: Clinical cancer therapy typically progresses through a series of first-, second-, and third-line treatments. As the cancer population evolves resistance and progresses, first line treatment is replaced by second line and so on. In this conventional sequence, resistance mechanisms are additive so that each follow-on therapy is typically less effective than prior treatments. In contrast, optimal evolution-based therapy proposes second line treatment can be more effective than initial therapy by targeting the evolutionary dynamics governing resistance - a strategy termed “evolutionary double bind.”

Here, we investigate potential double bind evolutionary dynamics in a treatment sequence beginning with DNA-damaging agents (DDA) such as radiation or chemotherapy followed by cell-based immunotherapy. We hypothesized lung cancer cells, when treated with DDA agents, upregulate DNA damage response pathways leading to increased expression of natural killer (NK) cell ligands.

Using multiple NSCLC cell lines (PC9, HCC827, H23, and H1437s), we show cells treated with DDA (radiotherapy or chemotherapy) upregulate membrane ligands recognized by NK cells including major histocompatibility complex class I chain-related protein A/B (MICA/B), poliovirus receptor (PVR), and PVRL2, as well as PD-L1. In addition, we demonstrate these cells have altered susceptibility to NK cell-mediated killing after therapy. Furthermore, computer simulations of evolutionary mathematical models can predict population dynamics of sensitive and surviving populations in sequencing these therapies.

We conclude sequential DDA followed by NK cell-based immunotherapy represents a possible in vitro evolutionary double bind that may improve treatment of non-small cell lung cancer and may be applicable to other clinical cancers.

Lay Abstract: Clinical cancer therapy typically progresses through a series of first-, second-, and third-line treatments. As cancer cells evolve resistance, first line treatment is replaced by second line and so on. Due to this sequence of therapies, each additional therapy results in a less

effective treatment then the previous due to the resulting resistant populations. In contrast, if secondary therapy is aimed at the mechanism that produces the resistant population from initial therapy, said treatment is more effective than if it was given first. This strategy is termed ”evolutionary double bind”.

Natural killer (NK) cells are lymphocytes that kill other diseased or infected cells. They recognize cells that are undergoing stress, such as DNA-damage, by recognizing different receptors and ligands on the surface of said cells.

In this study, we investigate a potential double bind using DNA-damaging agents (DDA), such as chemotherapy or radiation, followed by treatment with NK cells. We hypothesized lung cancer cells, when treated with DDA, would increase expression of NK cell ligands, making them easily targeted by NK cell therapy.

Using multiple cell lines of NSCLC, we show that cells treated with DDA upregulate NK cell ligands such as poliovirus receptor (PVR) and PVRL2. In addition, we demonstrate their altered susceptibility to dying due to NK cells. Finally, mathematical models predict the dynamics between the sensitive and surviving cells undergoing therapy.

We conclude DDA followed by NK cell therapy represents a promising evolutionary double bind that may improve treatment of NSCLC and possibly other cancer types.

A data assimilation framework for predicting the spatiotemporal response of high-grade gliomas to chemoradiation

Hugo Miniere, Cancer Systems Biology Consortium (CSBC)

Abstract: We present a novel computational platform for predicting the spatiotemporal changes of high-grade gliomas (HGG) following chemoradiation therapy post-surgery. This platform utilizes a two-species reaction-diffusion mathematical model to describe the spread, proliferation, and response to treatment in both non-enhancing and enhancing HGG regions. The model is calibrated with weekly MRI data, including anatomical, perfusion, and cellularity characteristics, collected from 21 patients undergoing adaptive radiotherapy and standard chemotherapy. We employ a data assimilation scheme (scenario 1) where MRI data from each visit are integrated into the calibration framework. This updates the model parameters, enabling patient-specific predictions of cell count and distribution until the next imaging session. These predictions are compared to those made without weekly updates (scenario 2), which represent studies without access to frequent imaging data. In scenario 2, we assess three methods for integrating parameters from the patient cohort with those calibrated individually to evaluate the utility of population-based data in predicting individual outcomes. Scenario 1 achieved median concordance correlation coefficient (CCC) values above 0.85, indicating strong local agreement. For scenario 2, calibrating parameters on the patient cohort allows for improvements in the quality of long-term treatment response predictions made during the early stages of therapy, achieving a median CCC > 0.75 for predictions made with cohort-trained parameters, versus a median CCC > 0.55 without. This suggests that linking population trends with a mechanism-based model to guide long-term predictions in cases where recurrent model updates are not readily available is a promising avenue for increasing predictive accuracy.

End-Stage Breast Cancers are Comprised of Tumors with Distinct Metastatic Capacities and Microenvironment Compositions

Isaac Bishara, Cancer Systems Biology Consortium (CSBC)

Abstract: End-stage breast cancer often presents with multiple metastatic tumors. To understand the evolutionary endpoints of these tumors, we utilized the LEGACY warm procurement protocol for sample collection. We employed single-cell RNA sequencing to profile these tumors and used PhylinSic to reconstruct their genetic phylogenies, which were consistent with the subclonal architecture seen in the whole exome sequencing. We found that some cell lineages were found primarily in a single tumor suggesting they were less likely to spread (which we interpreted as having low metastatic capacity), while others were seen in many tumors (high metastatic capacity). Similarly, some tumors were comprised predominantly of low metastatic capacity cells from a single lineage, while others were dominated by high metastatic capacity cells across multiple lineages. Analysis of single-cell transcriptomes indicated that tumors with high metastatic capacity were enriched in pathways driven by the microenvironment, such as tumor necrosis factor and complement signaling, leading us to compare the tumor microenvironment (TME) of these sites. We found that high metastatic capacity sites featured distinct TME compositions compared to low metastatic capacity sites. The low metastatic sites were distinguished by lymphocyte-rich TMEs, more differentiated macrophages exhibiting an interferon response signature, and lack of cancer inflammatory signaling. This variability in tumor phenotypes at end-stage cancer suggests that treatment strategies for late-stage metastatic breast cancer must target distinct tumor states.

Lay Abstract: Women with advanced breast cancer often have multiple tumors that have spread to different parts of the body (metastasized). To better understand these metastatic tumors, we analyzed the genetic makeup of individual cells from the tumors. We found that some groups of cancer cells were mainly contained in just one tumor site. This suggests those cells were not very good at traveling and spreading to new sites. Other groups of cancer cells were found across many different tumors in the body. This means those cells were better at spreading and metastasizing. In some tumors, most of the cells were the kind that couldn't spread very well. In other tumors, most cells were the type that could spread easily. By studying which genes were turned on in the single cells, we saw that the tumors with cancer cells that spread a lot had activated certain biological pathways related to inflammation and the environment around the tumor. When we looked at the environment surrounding the tumors, the ones with cancer cells that couldn't spread as far had more immune cells present to fight cancer. They also had specialized immune cells called macrophages exhibiting an interferon response and less cancer-related inflammation. This variety between metastatic tumors suggests that for patients with late-stage breast cancer that has spread, we may need different treatments targeting the specific types of tumors they have.

Systems biology-enabled combination therapies for MYC-driven Medulloblastoma

Jingjing Liu, Cancer Systems Biology Consortium (CSBC)

Abstract: Medulloblastoma (MB), a prevalent malignant brain tumor in children, is categorized into WNT, SHH, Group 3 (G3), and Group 4 (G4) subgroups, each with distinct clinical and molecular features. G3 MB, known for its elevated MYC expression, is associated with frequent metastasis and dismal five-year survival rates. Current MB therapies are not subgroup-specific, resulting in considerable toxicity and resistance, with severe physical and psychological impacts on young survivors. To expedite the discovery of targeted therapies for G3 MB, we have developed the SINBA (Synergy Inference by Data-driven Network-Based Bayesian Analysis) computational framework, which identifies synergistic drug combinations capable of crossing the blood-brain barrier. Through in silico analysis, we assessed over 900,000 hub gene pairs and designed a high-throughput screening of 320 drug pairs from a library of 94 small molecules. Our findings revealed 10 drugs with EC50 values below 1 μM and 19 of 32 validation drug combinations displaying synergistic effects. A combination of MEK inhibitors with Regorafenib

emerged as the most effective synergistic treatment for G3 MB. This combination was further validated for its on-target action in vitro and in vivo, showing halted tumor progression in xenograft human and mouse models, reduced target phosphorylation, and increased apoptosis. Single-cell RNA sequencing data also demonstrated significant drug target activity in tumor cell populations with progenitor-like characteristics. Our innovative approach not only presents a promising therapeutic avenue for G3 MB but also exemplifies the potential of systems biology to revolutionize treatment strategies for subgroup-specific malignancies.

Physical and functional mapping of the p53 mutation-driven interactome
Nadia Arang, Cancer Systems Biology Consortium (CSBC)

Abstract: The TP53 gene is mutated in >50% of human cancers. In contrast to other tumor suppressors, mutations in TP53 are commonly missense among a subset of hotspot mutations. Many mutations are localized to the DNA-binding domain of the protein resulting in altered structural interfaces and protein accumulation. The mechanism driving a gain-of-function phenotype, and oncogenic properties in p53-mutant cancers is not yet well-understood. Thus, we hypothesize that the accumulation of p53 and gain-of-function mediators playing an essential role in oncogenesis may yield selective therapeutic targets in p53-mutant cancer cells. Here, we profiled the protein-protein interactions (PPI) of p53 and its mutant forms using affinity-purification mass spectrometry (AP-MS) in two distinct cellular models. Across both contexts, we find unique mutation-driven interactions with novel proteins across distinct functional groups. Moreover, application of tools that integrate the usage of AlphaFold reveal the structural basis for a subset our AP-MS-derived PPIs enabling the prioritization of putative interactions. Future directions include validation of unique PPIs across different cancer models and therapeutic targeting in p53-mutant cancer types.

Lay Abstract: The TP53 gene, which plays a crucial role in preventing cancer, is found to be mutated in over half of all human cancers. Unlike other genes that suppress tumors, TP53 mutations often change a single building block in the protein, especially in certain key areas. These changes affect how the protein interacts with DNA and cause it to accumulate in cells. We don't yet fully understand how these changes make the protein act in ways that promote cancer. We believe that this accumulation and its new functions could offer targets for cancer treatments specifically aimed at cells with mutated TP53. In this study, we examined how the normal and mutated versions of the TP53 protein interact with other proteins in cells. We used a technique that identifies these interactions in detail in two different types of cells. We found that different mutations lead to unique interactions with other proteins. By using tools that can predict protein-protein interactions using computational modeling, we were able to see the structures of some of these interactions, helping us identify which ones might be important to target for future cancer therapies. Next steps will involve confirming these findings in various cancer models and exploring new treatment options for cancers with TP53 mutations.

Enhancing Cancer Therapy: Biomarkers, Mechanism, and the Promise of cpd_AV2 as a Superior Integrin Inihibitor
Nicole Mattson, Cancer Systems Biology Consortium (CSBC)

Abstract: Cancer continues to be a devastating disease, with thousands of patients succumbing to it annually. The path to developing effective therapies is often blocked by stringent approval processes, particularly concerning patient safety. To navigate these challenges, we propose targeting pathways with existing therapeutic approvals in other diseases, ensuring drug safety and tolerance. A compelling target is the integrin family of proteins, successful in treating Inflammatory Bowel Diseases and Cardiovascular Disease. Our project focuses on a novel

integrin inhibitor, cpd_AV2, comparing its efficacy against the leading integrin-targeting cancer therapeutic, Cilengitide. Although Cilengitide is well-tolerated, it has not significantly reduced cancer burden. In comparison, cpd_AV2 demonstrates rapid and potent cancer cell cytotoxicity where Cilengitide fails. Offering an opportunity to develop cpd_AV2 as a new integrin-targeted therapeutic. To elucidate cpd_AV2's superior cancer cell toxicity, we analyzed the sensitivity of hundreds of cell lines. We found that cpd_AV2 treatment leads to a population of sensitive and resistant cells, whereas Cilengitide did not show strong sensitivity profiles. Leveraging these genotypes of different cell populations, we built explainable machine learning models that identify biomarkers predicting sensitivity to cpd_AV2. These biomarkers are immediately clinically relevant, facilitating the identification of patients most likely to benefit from this therapy. Additionally, our explainable model will reveal the mechanism of action of cpd_AV2 as a therapeutic option in cancer. This project offers an innovative dual approach of biomarker identification and mechanistic discovery which positions cpd_AV2 as a promising therapeutic option in cancer.

Lay Abstract: Cancer is a serious disease that affects millions of people each year. Finding new treatments is challenging because new drugs must be proven safe for patients. To improve our chances of success, we suggest using therapeutic strategies already approved for other diseases to ensure they are safe. One promising strategy is targeting the integrin family of proteins, which has been successfully treated in diseases like Inflammatory Bowel Diseases and Cardiovascular Disease. In our study, we focus on a new drug, cpd_AV2, and compare it to the leading cancer drug, Cilengitide. Although Cilengitide is safe for patients, it hasn't been very effective in reducing cancer and therefore not approved. In comparison, cpd_AV2 shows the ability to kill cancer cells quickly, unlike Cilengitide. To understand why cpd_AV2 works better, we tested it on many different types of cancer cells. We discovered that cpd_AV2 creates two groups of cells: those that are sensitive to the drug and those that are more resistant. Cilengitide did not show this effect. By studying the genetics of these cells, we built computer models that can predict why certain cells respond to cpd_AV2. These models also help us understand how the drug works. Our research suggests that cpd_AV2 is a better option for treating cancer. It not only helps identify which patients will benefit but also reveals how the drug works.

Mapping protein-protein interaction remodeling following chemotherapy in triple-negative breast cancer.

Richa Tiwari, Cancer Systems Biology Consortium (CSBC)

Abstract: In precision medicine, machine learning models are often ‚Äúblack boxes,,Äù predicting phenotypes from genotypes without knowing the underlying mechanisms. To arm genotype-phenotype models with the insights of associated molecular mechanisms, the current study involves the generation of comprehensive maps of cellular structure/function in triple-negative breast cancer (TNBC) context in response to chemotherapy. Leveraging endogenous tagging (endo-tag) based APMS and SEC coupled mass spectrometry (SEC-MS), we aim to generate differential protein-protein interaction maps focusing on approximately 100 most frequently altered cancer-associated genes, spanning various chromatin modifier classes. Endo-tag-APMS offers high-resolution PPI network characterization, while SEC-MS provides a global view of interactome perturbations. Our SEC-MS data for the MDA-MB-468 cell line provides unique insights into the dynamics of chromatin remodeling complexes in response to paclitaxel or vorinostat treatment, impacting 50% of the proteins of interest. Concurrently, our initial endo-tag APMS-based differential PPI mapping unveils significant rewiring of interactome upon drug treatment for specific targets including HDAC3, HDAC2, BRPF1, USP7, etc. Importantly, comparative analysis indicates that SEC-MS-identified complexes could be recapitulated by endo-tag APMS, especially regarding high-confidence interactors. Ultimately, integrating large-

scale physical interactomics data with high-throughput imaging and genetic perturbation data aims to facilitate the development of interpretable machine learning models tailored for predicting therapeutic responses in cancer.

mTOR-mediated transient APC/C inactivation is required for mammalian cell cycle entry
Debasish Paul, CCR

Abstract: Mammalian cells entering the cell cycle favor glycolysis to rapidly generate ATP and produce the biosynthetic intermediates required for rapid biomass accumulation. Simultaneously, cells activate the ubiquitin ligase Anaphase-Promoting Complex/Cyclosome (APC/C)-Cdh1 to allow origin licensing and block premature DNA replication. Paradoxically, glycolysis is inhibited by the APC/CCdh1 through the degradation of key glycolytic enzymes, raising the question of how cells coordinate these mutually exclusive events to ensure proper cell division. Here we show that cells solve this paradox by transiently inactivating the APC/C during cell cycle entry which allows a transient metabolic shift favoring glycolysis. Upon mitogen stimulation, rapid mTOR-mediated phosphorylation of the APC/C adapter protein Cdh1 at the N-terminus causes it to partially dissociate from the APC/C. This partial inactivation of the APC/C leads to the accumulation of PFKFB3, a rate-limiting enzyme for glycolysis, promoting a metabolic shift towards glycolysis. Delayed accumulation of phosphatase activity later removes Cdh1 phosphorylation, restoring full APC/C activity, and shifting cells back to favoring oxidative phosphorylation. Interestingly, this regulation is absent in cancer cells, generating a window to develop better therapeutic avenues. Thus, cells coordinate the simultaneous demands of cell cycle progression and metabolism through an incoherent feedforward loop which transiently inhibits APC/C activity to generate a pulse of glycolysis and that is required for mammalian cell cycle entry.

Lay Abstract: To understand cancer, we must first grasp the behavior of normal cells. Since cancer exploits our cellular machinery, a detailed understanding of this machinery is crucial for developing effective therapies. Cell cycle entry is a vital process in development and normal physiological functions such as immune responses and wound healing. Notably, it is also the most critical step disrupted in cancer, leading to the hallmark of “controlled proliferation”. Therefore, it is essential to comprehend the normal cell cycle entry process and its deregulation in diseases like cancer.

In this study, we have addressed a long-standing question about how cells manage contradictory signals and how core cell cycle mechanisms interact with metabolic pathways. We elucidated the signaling mechanisms and identified key players involved in this process. Interestingly, many cancer or dormant cells bypass this regulation due to heightened oncogenic signaling. This suggests that a combination of treatments, including metabolic precursor supplements, could offer a more effective strategy for tackling the disease.

Replicating the Human Fallopian Tube through CODA-guided design of organoids
Andre Forjaz, Cellular Cancer Biology Imaging Research (CCBIR)

Abstract: Fallopian tubes play a crucial role in processes ranging from fertilization to ovarian carcinogenesis, however their complex biology remains not fully understood and is not adequately represented by existing in vitro models. Traditional 2D cultures fail to mimic the complex architecture of fallopian tubes. Lack of quantitative mapping of anatomical characteristics has impeded precise in vitro replication. Well-characterized fallopian tube organoids could allow for transformative research by preserving key structural, functional, and genetic attributes.

Here, we present a novel digital pathology-based workflow. Our integrated computational-experimental approach creates a 3D reference map from serial histological sections of healthy human fallopian tubes. This allows for direct comparison with 3D mapped organoids developed in the wet lab. The 3D mapping of an entire human fallopian tube serves as a benchmark for iterative organoid optimization.

Our methodology was validated across eight distinct structural parameters, demonstrating the potential of integrating quantitative 3D pathology for precise organoids engineering. This highlights the potential of pathology-guided organoid and biomaterial modeling for better understanding of reproductive biology, ovarian cancer research, and drug screening.

Lay Abstract: Fallopian tubes are essential for processes such as fertilization. In recent years, fallopian tubes have been linked to the development of ovarian cancer. However, their complex biology is not fully understood and existing in vitro models do not adequately represent their complexity. Traditional 2D cultures fail to replicate the intricate architecture and compositions of human fallopian tubes. The absence of quantitative 3D mapping of anatomical characteristics has hindered accuracy in vitro replication in the wet lab. Well-characterized fallopian tube organoids could facilitate transformative research by preserving key structural, functional, and genetic attributes.

In this study, we introduce a novel digital pathology-based workflow. Our integrated computational-experimental approach generates a reference 3D reconstruction of an entire healthy human fallopian tube. This allows for direct comparison with 3D reconstructed organoids developed in the laboratory. The 3D mapping of an entire human fallopian tube serves as a benchmark for iterative organoid optimization.

Our methodology was validated across eight distinct structural parameters, demonstrating the potential of integrating quantitative 3D pathology for precise organoid engineering. This highlights the promise of pathology-guided organoid and biomaterial modeling in advancing the understanding of reproductive biology, ovarian cancer research, and drug screening.

Super-resolution multimodal nanoimaging platform for chromatin study

Geng Wang, Cellular Cancer Biology Imaging Research (CCBIR)

Abstract: The three-dimensional organization of the human genome (chromatin) is critically involved in the regulation of gene expression and is highly complex. To investigate chromatin conformation, four advanced imaging technologies were integrated to develop a new nanoscale imaging platform capable of interrogating spatio-temporal changes in chromatin packing from the molecular level (e.g., individual DNA strands) to whole chromatin in hundreds of live cells. These technologies include 3D chromatin scanning transmission electron microscopy (ChromSTEM), multi-label spectroscopic single-molecule localization microscopy (sSMLM), spectroscopic intrinsic-contrast photon-localization optical nanoscopy (SICLON), and partial wave spectroscopic microscopy (PWS).

Label-free PWS is a real-time live cell imaging method with high throughput that can sense chromatin structures at the nanometer scale within their packing context. Multi-label sSMLM reveals the precise location of individual molecules and unpacks the structural complexity of chromatin, though targeting highly dense chromatin polymer with volume concentrations between 0.2 and 0.8 remains challenging. We developed a 3-color chromatin SMLM and demonstrated that chromatin organizes into packing domains, with euchromatin and active transcription occurring around the borders of constitutive and facultative heterochromatin cores. SICLON

leverages the endogenous photoswitching of DNA and its spectroscopic capability to facilitate label-free DNA imaging, potentially mitigating label sparsity issues. ChromSTEM allows for detailed examination of DNA organization, providing a ground truth for the other methods.

Moreover, by integrating this imaging platform with molecular and chromatin assays such as Hi-C and single-cell sequencing, we can relate chromatin organization to its function and the regulation of gene expression.

Lay Abstract: The way DNA is organized in three dimensions inside cells is crucial for controlling how genes are expressed. Although the genetic and histone regulation of transcription can be addressed by molecular assays, decoding the role of chromatin packing requires nanoscale imaging. The spatial resolution of conventional microscopy is limited by the diffraction of light to approximately 200 nm. To overcome this limitation, we combined four advanced imaging technologies to create a new, powerful tool that can look at changes in DNA packing at different levels, from individual DNA strands to large sections of chromatin, in hundreds of live cells.

1. 3D Chromatin Scanning Transmission Electron Microscopy (3 nm resolution): This technology provides very detailed images of DNA organization.
2. Multi-Label Spectroscopic Single-Molecule Localization Microscopy (6 nm resolution): This method shows the precise location of individual molecules, helping to understand the complex structure of chromatin.
3. Spectroscopic Intrinsic-Contrast Photon-Localization Optical Nanoscopy (6 nm resolution): This technique uses the natural behavior of DNA to switch its light signals on and off, allowing for imaging without additional labels.
4. Partial Wave Spectroscopic Microscopy (20 nm sensitivity): A real-time imaging method that can sense the structure of chromatin at the nanometer scale in live cells with high throughput.

By combining this imaging platform with other techniques like Hi-C and single-cell sequencing, we can link chromatin organization to its function and how genes are regulated. This can help us understand the function of the human genome and how its dysregulation contributes to disease.

Human osteosarcoma metastasis is limited by oxidative stress-induced ferroptosis
Md Torikul Islam, Cellular Cancer Biology Imaging Research (CCBIR)

Abstract: Osteosarcoma is a metastatic bone cancer that primarily affects children and adolescents. The current clinical care of osteosarcoma patients mainly relies on surgical resection and chemotherapy. However, most patients develop metastases. Here, we tested the hypothesis that human osteosarcoma metastasis is limited by oxidative stress and/or ferroptosis. We obtained human patient-derived osteosarcoma cell lines, HOS-MNNG, LM7, and MG63.2, tagged with stably expressed luciferase and orthotopically transplanted into NOD-SCID-Il2rg-/- (NSG) mice. The primary tumors that formed in the tibia spontaneously metastasized to distant sites such as the lung, liver, and kidneys. We found that as compared to primary tumors, circulating tumor cells and nascent metastatic nodules experienced higher levels of oxidative stress as indicated by elevated reactive oxygen species (ROS) and depleted glutathione to oxidized glutathione ratio. Supplementation of antioxidants, N-acetyl L-Cysteine (NAC), or vitamin E increased spontaneous metastasis, suggesting that oxidative stress limits osteosarcoma metastasis. Next, we found that the ferroptosis inducer, RSL3 (a GPX4 inhibitor), markedly reduced viable cells in culture, and this effect was rescued by the ferroptosis inhibitor, Liproxstatin-1 or NAC. Moreover, osteosarcoma cells from blood and nascent metastatic nodules demonstrated higher levels of lipid ROS and labile iron. To determine whether ferroptosis limits

the survival of osteosarcoma cells in the blood, we pretreated osteosarcoma cells with vehicle or Liproxstatin-1 or NAC and intravenously injected into NSG mice. Pretreatment with Liproxstatin-1 or NAC, as compared to vehicle, significantly increased metastatic disease burden. Taken together, these results demonstrate that human osteosarcoma metastasis is limited by oxidative stress-induced ferroptosis.

Lay Abstract: Antioxidants are generally believed to be healthy and recommended for people who are at risk of a variety of age-related chronic diseases. It was thought that antioxidant supplementation may prevent and/or slow down cancer progression. Therefore, large clinical trials have been conducted to test if antioxidant supplementation can reduce cancer incidence and cancer-related mortality. Surprisingly, it turned out that people who were on antioxidant supplementation were dying of cancer more frequently than individuals who were on placebo control. To solve this puzzle, our laboratory conducted rigorous experiments and discovered that when melanoma cancer cells spread throughout the body, known as metastasis, they experience oxidative stress and are fated to die. Few cells adapt to survive and form secondary tumors in distant sites causing patient death. Supplementation of antioxidants helped metastasizing melanoma cells to survive in the blood and accelerated secondary tumor formation faster than the control treatment. This landmark discovery raised a key question of whether antioxidant supplementation promotes other cancers besides melanoma. I have tested this in osteosarcoma, a devastating bone cancer that mainly affects children and adolescents. I found that similar to melanoma, metastasizing osteosarcoma cells also experience high levels of oxidative stress and die in circulation. Moreover, I found that cancer cells die in the blood via a specific cell death pathway called ferroptosis. Supplementation of antioxidants such as N-acetyl L-Cysteine or vitamin E increased metastasis. Thus, my research provides compelling and clinically relevant evidence that cancer patients should not be recommended for antioxidant supplementation.

Molecular specificity of nucleic acids via intrinsic stochastic fluorescence under visible light illumination

Ruyi Gong, Cellular Cancer Biology Imaging Research (CCBIR)

Abstract: The development of super-resolution imaging techniques has extended the resolving power to around 10 - 50 nm. However, most current super-resolution imaging techniques need exogenous fluorescent dyes as imaging contrast, whose essential weakness of labeling includes imprecise spatial localization, and perturbation of the sample. The intrinsic fluorescence of DNA under visible light excitation has been reported to have similar photo-switching properties to the organic dyes used in single-molecule localization microscopy, making it potential for label free DNA super-resolution imaging. Here, we measured the fluorescence spectra of poly-G (guanine) of different lengths (5, 8, 12, 16 base-pair), 20 base-pair single-stranded DNA molecules (poly-A, G, C, T), 40 base-pair single-stranded DNA molecules of same formation but different sequences, as well as double-stranded DNA (AT chain, GC chain), under multiple wavelengths. The spectra of mentioned DNA molecules can be classified with an accuracy of more than 90%, which demonstrates the molecular specificity of the DNA polymers via its intrinsic fluorescence. Our work paves the way for developing spectroscopic intrinsic-contrast localization optical nanoscopy for DNA imaging and chromatin study.

Lay Abstract: Traditional optical imaging has the limitation on resolution to about 200 nm using visible light illumination. Advancements in super-resolution imaging have significantly improved resolution down to 10 - 50 nanometers. However, current techniques often rely on fluorescent dyes that are externally applied to the sample. This labeling process has drawbacks such as imprecise placement and disturbance to the sample's natural state. Recent research has explored using the inherent fluorescence of DNA itself, particularly under visible light excitation. This

intrinsic property shows similar light-switching capabilities to conventional organic dyes used in high-resolution microscopy. This discovery suggests the potential for label-free super-resolution imaging of DNA.

In our study, we examined the fluorescence spectra of various DNA molecules: single-stranded poly-G of different lengths (5, 8, 12, 16 base pairs), single-stranded 20 base-pair DNA sequences (poly-A, G, C, T), and 40 base-pair sequences with different compositions. We also analyzed double-stranded DNA with AT-rich and GC-rich chains, using multiple wavelengths of light. Our findings demonstrate that each type of DNA molecule has distinct fluorescence characteristics, allowing them to be identified with over 90% accuracy based on their spectra. This molecular specificity highlights the potential for using DNA's intrinsic fluorescence as a spectroscopic tool for precise imaging and studying chromatin, paving the way for future developments in optical nanoscopy for DNA analysis.

Evaluating Deep Learning Features of Chromatin-Sensitive Partial Wave Spectroscopic Microscopy for Early-Stage Lung Cancer Diagnosis
Sravya Prabhala, Cellular Cancer Biology Imaging Research (CCBIR)

Abstract: Lung cancer remains a leading cause of mortality worldwide, highlighting the urgent need for improved early-stage detection methods. This study explores the potential of Chromatin-Sensitive Partial Wave Spectroscopic Microscopy (csPWS) for non-invasive lung cancer diagnosis utilizing buccal mucosa cells. Chromatin structure plays a crucial role in gene regulation, and its alterations are linked to cancer development. Buccal epithelial cells, particularly in smokers, exhibit molecular signatures associated with lung cancer, a phenomenon referred to as field carcinogenesis or field of injury. However, conventional microscopy struggles to resolve the intricate details of chromatin packing domains, typically below 300¬†nm in diameter, due to their size limitations. csPWS, an innovative optical spectroscopic technique, overcomes this barrier by enabling the detection of chromatin structural changes within these domains. Here, we leverage deep learning to extract complex features from csPWS data, aiming to decipher the biological pathways associated with lung cancer and identify unique diagnostic markers within the clinical data, resulting in a superior diagnostic performance with AUC of 0.92. This approach holds promise for developing a highly sensitive, non-invasive method for early-stage lung cancer detection.

Lay Abstract: Early detection of lung cancer is crucial for improving patient outcomes. This study investigates a new technique called Chromatin-Sensitive Partial Wave Spectroscopic Microscopy (csPWS) for identifying lung cancer in its early stages. csPWS is a special type of microscopy that can analyze the structure of chromatin, the material that stores our genetic information, in cells from the inside of the cheek (buccal mucosa). We know that changes in chromatin structure are linked to lung cancer development, especially in smokers. Traditional microscopes cannot see these changes because they are too small. csPWS overcomes this limitation and allows us to examine these changes. We then use artificial intelligence (AI) to analyze the csPWS data in detail, helping us understand how lung cancer develops and identify unique signatures that can be used for diagnosis. This technology has the potential to be a game-changer in lung cancer screening by offering a painless and highly accurate way to detect cancer early.

DSCC: Disease Subtyping using Consensus Network and multi-omics data integration
Ha Nguyen, Informatics Technology for Cancer Research (ITCR)

Abstract: Cancer is a complex disease driven by numerous biological processes activating on multiple levels. Various genome-wide profiling techniques have been developed to capture the

dynamics of these processes at the genomics, transcriptomics, epigenomics, and proteomics levels. Integrative analysis of data from these sources offers a comprehensive view that reveals connections unattainable through single-omic observations. In this study, we introduce DSCC (Disease Subtyping using Community detection from Consensus network), a novel approach aimed at discovering disease subtypes from multi-omics data. DSCC leverages pathway knowledge and exploits local patient relationships within each data type to construct a consensus network based on patient connectivities. Through an extensive analysis utilizing real multi-omics data encompassing over 15,000 patients across 33 cancers sourced from The Cancer Genome Atlas, METABRIC, and Gene Expression Omnibus, our findings demonstrate the robustness of DSCC against noise. Moreover, it achieves remarkable performance in identifying both known patient classes and novel subtypes, characterized by significant differences in survival profiles.

A count-based model for delineating cell-cell interactions in spatial transcriptomics data
Hirak Sarkar, Informatics Technology for Cancer Research (ITCR)

Abstract: Motivation: Cell-cell interactions (CCIs) consist of cells exchanging signals with themselves and neighboring cells by expressing ligand and receptor molecules, and play a key role in cellular development, tissue homeostasis, and other critical biological functions. Since direct measurement of CCIs is challenging, multiple methods have been developed to infer CCIs by quantifying correlations between the gene expression of the ligands and receptors that mediate CCIs, originally from bulk RNA sequencing data and more recently from single-cell or spatially resolved transcriptomics data. Spatially resolved transcriptomics (SRT) has a particular advantage over single-cell approaches, since ligand-receptor correlations can be computed between cells or spots that are physically close in the tissue. However, the transcript counts of individual ligands and receptors in SRT data are generally low, complicating the inference of CCIs from expression correlations.

Results: We introduce Copulacci, a count-based model for inferring CCIs from SRT data. Copulacci uses a Gaussian copula to model dependencies between the expression of ligands and receptors from nearby spatial locations even when the transcript counts are low. On simulated data, Copulacci outperforms existing CCI inference methods based on the standard Spearman and Pearson correlation coefficients. Using several real SRT datasets, we show that Copulacci discovers biologically meaningful ligand-receptor interactions that are lowly expressed and undiscoverable by existing CCI inference methods.

Lay Abstract: Cells communicate with each other by sending and receiving signals through molecules called ligands and receptors. This cell-to-cell interaction is crucial for many important processes like growth and maintaining healthy tissues. Since the direct measurement of these interactions can be challenging, gene expression assays can be used to estimate the ligand and receptor expressions and therefore estimate the cell-cell interaction strengths. Initially, this was done using bulk RNA-seq data where gene expression from many cells was measured together and therefore lost the cell-specific information. Recently, a new technology known as spatial transcriptomics has enabled the collection of gene expression from single cells along with the location of the cell in a tissue. The data from this technology is better because it shows which cells are located close to each other. However, due to technology limitations, the signals from ligands and receptors in spatial transcriptomics data are usually weak.

To address this, we created a new method called Copulacci. It helps infer cell interactions from spatial data, even when the signals are weak. Copulacci uses a statistical model to understand the relationships between ligands and receptors based on their positions in the tissue. Tests with simulated data show that Copulacci is better than traditional methods. We also used Copulacci

on real data and found that it can discover important interactions that other methods miss.

AmpliconSuite: Analyzing focal amplifications in cancer genomes
Jens Luebeck, Informatics Technology for Cancer Research (ITCR)

Abstract: Focal amplifications in the cancer genome, particularly extrachromosomal DNA (ecDNA) amplifications, are a pivotal event in cancer progression across diverse cancer contexts. However, identifying and delineating these distinct events from whole-genome sequencing (WGS) data remains a challenge due to their complex profiles of copy number and structural variation. We present AmpliconSuite, a collection of tools that enables robust identification of focal amplifications from WGS data.

At the core of AmpliconSuite is the AmpliconArchitect (AA) method. AA jointly analyzes both structural variants (SVs) and copy numbers (CNs) within WGS data to identify and characterize focal amplifications. To create robust predictions of focal amplification status from AA outputs, we created AmpliconClassifier (AC), which classifies amplifications into distinct categories, including ecDNA, breakage-fusion-bridge (BFB) cycles among others. Combining these tools into a single workflow, we created AmpliconSuite-pipeline, available through GenePattern, Bioconda, Nextflow and other options.

To foster collaboration and data sharing, AmpliconSuite integrates with a platform we created called AmpliconRepository.org. This community-editable platform allows researchers to share focal amplification calls generated by AmpliconSuite publicly or privately. Notably, AmpliconRepository.org harbors ecDNA predictions on over 2,525 tumor samples from TCGA, PCAWG, and CCLE.

AmpliconSuite makes identification of focal amplifications reproducible and simple to use, and empowers users to share analyses publicly. Additionally, it introduces novel methods we recently developed, including ecContext within AC for categorizing types of ecDNA based on matching patterns of structural variation to the mechanisms of formation. Together, AmpliconSuite establishes itself as a valuable resource for researchers investigating focal amplifications in cancer.

Lay Abstract: Cancer often involves complex changes in DNA, such as extra copies of certain genes on circular DNA (ecDNA) that does not reside in chromosomes like normal DNA. These changes can drive cancer progression, but they are hard to detect using whole-genome sequencing (WGS) data. We developed a toolset called AmpliconSuite to help researchers identify these changes more easily.

AmpliconSuite includes multiple computational tools such as AmpliconArchitect (AA), which examines DNA structure and copy number in WGS data to find these specific changes. To further classify the types of DNA amplifications, we also created AmpliconClassifier (AC). AC can distinguish between different amplification types, like ecDNA and other complex DNA structures.

To make these tools accessible, we combined them into the AmpliconSuite-pipeline. This pipeline is easy to use and available through platforms for sharing scientific tools like Nextflow, GenePattern, and Bioconda.

We also launched AmpliconRepository.org, a website where researchers can share their findings. This site already includes predictions for over 2,525 tumor samples from major cancer studies like TCGA, PCAWG, and CCLE.

AmpliconSuite simplifies the process of identifying DNA amplifications and encourages data sharing within the research community. It includes new methods like ecContext, which helps categorize ecDNA based on how it forms. Overall, AmpliconSuite is a powerful resource for scientists studying changes to the genome that occur in cancer.

Developing a Comprehensive Hematology Oncology Clinical Guideline Database
Sandeep Jain, Informatics Technology for Cancer Research (ITCR)

Abstract: Introduction: Hematology and oncology clinical guidelines are critical for decision-making in cancer centers worldwide. Given their widespread use for a diverse patient population, it is vital to examine the diversity of the guidelines themselves, including their publishers and the institutions and regions they represent. As we develop a hematology-oncology learning system from extensive clinical data, guidelines will be crucial for training models. Therefore, studying the generalizability and potential biases of these guidelines is essential to avoid biased algorithms and support diverse clinical decision-making.

Methods: We utilized the HemOnc.org knowledgebase, R, and Python to extract clinical guidelines as of February 2024. Our dataset includes guideline titles, authors, affiliated institutions, publication years, and the guideline organization. Results: Currently, our database contains 1,311 guidelines from 1996 to 2024, covering 203 unique disease states and toxicities, including benign hematology conditions. These guidelines were published by over 60 organizations globally (e.g., ASCO, NCCN, ESMO, ISTH). Our database features 6,493 guideline authors and approximately 2,000 respective affiliations.

Future directions/discussion: Future enhancements to the clinical guideline database include an edit-a-thon in August. During this event, participants from around the world will help update the HemOnc.org webpage, adding more comprehensive guidelines information to further develop our database. We might attempt to create a guideline corpus from the guideline database in the future. This effort aims to better harness guidelines for practitioners and AI model training alike.

Lay Abstract: Hematology and oncology clinical guidelines guide cancer treatment worldwide. To ensure these guidelines serve diverse patients effectively, we need to study who publishes them and where they come from. As we develop a learning system using extensive clinical data, guidelines will be key in training accurate models. Understanding the generalizability and potential biases in these guidelines is essential to avoid biased algorithms and support diverse clinical decision-making.

We used the HemOnc.org knowledgebase, R, and Python to extract clinical guidelines up to February 2024. Our dataset includes titles, authors, institutions, publication years, and organizations. Our database has 1,311 guidelines from 1996 to 2024, covering 203 disease states and toxicities, including benign conditions. These guidelines come from over 60 global organizations (e.g., ASCO, NCCN, ESMO, ISTH). We have information on 6,493 authors and their >2,000 respective affiliations.

To improve our database, we are hosting an edit-a-thon in August. Participants from around the world will update the HemOnc.org webpage, adding more detailed guidelines information. We might try and collect the text from all of the guidelines we've collected and store the text within the database as well. We hope this effort will highlight both the shortcomings and benefits of our clinical guidelines and help inform a better future for patient care.

Visual Time-Temperature Indicators of Biospecimen Exposure to Thawed Conditions
Jorvani Cruz Villarreal, Innovative Molecular Analysis Technologies (IMAT)

Abstract: Many biological analytes relevant to biomedical research can become unstable when the biospecimens in which they reside are exposed to thawed conditions. Despite available guidelines for proper pre-analytical sample handling and storage (H&S), improprieties and inconsistencies can occur unwittingly, leading directly to costly false leads. To avoid using compromised samples, it is crucial to track biospecimen exposure to thawed conditions. For this purpose, we are developing inexpensive visual time-temperature indicators (TTIs) that can be used alongside biospecimens at the individual aliquot level.

The proposed TTIs are based on the kinetic control of the autocatalytic permanganate/oxalate redox reaction, which transition from an intense pink to a colorless solution. The reaction kinetics are simulated using a MATLAB script, facilitating the TTI design for specific time-temperature intervals depending on specific handling or storage needs. We have demonstrated the reaction's temperature dependency and that the reaction time at room temperature can be adjust between a few seconds and 3.5 h.

Eutectic compositions of perchlorate solutions are used to depress the system's freezing and melting point (m.p.), allowing indicators to remain active at subzero temperatures that are warmer than the storage temperature(s) indicated by biospecimen H&S guidelines. We have characterized LiClO4 (m.p. -18°C), NaClO4 (m.p. -37°C), and Mg(ClO4)2 (m.p. -67°C) at their eutectic compositions as antifreeze solutions demonstrating the reaction remains active in these below 0 °C, while maintaining kinetic control. We expect that implementation of the TTIs will help prevent the use of poor quality biospecimens and minimize false discoveries in biomedical research.

Lay Abstract: Proper handling and storage of biological samples like blood, tissue and urine is critical in medical research. If samples are not handled properly and exposed to thawed conditions prior to analysis, their stability can be compromised, producing misleading results and incorrect conclusions. Despite available sample handling and storage guidelines, inconsistencies can occur unwittingly. To avoid using compromised samples, it is crucial to track samples exposure to thawed conditions. For this purpose, we are developing inexpensive visual time-temperature indicators (TTIs) that can be implemented with individual samples to track their exposure to thawed or non-ideal frozen conditions.

The proposed indicators change color from an intense pink to colorless when exposed to thawed conditions, based on a controlled chemical reaction. If frozen properly, the reaction is halted, and the color remains unchanged. Our research has demonstrated precise control over the reaction, as well as its temperature dependency. To keep indicators active at temperatures below 0 °C but warmer than recommended storage temperature(s) (e.g, to track when required at -80 °C but stored at -20 °C), we explored the use of antifreeze solutions with different freezing and melting temperatures below 0 °C. We found that perchlorate salts can serve as antifreeze solutions, allowing for the reaction to remain active below 0 °C while maintaining control over the reaction time. The implementation of these TTIs will help prevent the use of poor quality biospecimens and minimize false discoveries in medical research.

Dynamic regulation of tumor cell competition determines targeted therapy resistance
Joseph Fernandes, Innovative Molecular Analysis Technologies (IMAT)

Abstract: Although targeted therapy can lead to tumor resistance in HER2+ breast cancer, how

tumor cell competition is regulated and leads to resistant clonal outgrowth remains unclear. Here we show that the HER2 isoform expression determines intrinsic tumor clone fitness, but the microenvironmental landscape shapes winners and losers. In HER2+ breast cancer, oncogenic isoforms exon-16 null (d16-HER2) and 95kDa c-terminal fragment (p95-HER2) are suspected drivers of treatment resistance. Preliminary studies characterizing our immune intact HER2 Crainbow in vivo model found these isoforms may determine clonal fitness and drive proliferative and invasive phenotypes. To study isoform-dependent phenotype differences, I derived cell lines from HER2 Crainbow tumors and competed them in vitro and in vivo. Initial characterization and mathematical modeling reveal that p95-HER2 as highly invasive, but slower growing than the more proliferative WT-HER2 and d16-HER2 cell lines. In vitro competition assays and immune-compromised orthotopic transplants using equal numbers of each cell line show WT-HER2 and d16-HER2 outcompete p95-HER2. However, in syngeneic orthotopic transplant, p95-HER2 outcompete WT-HER2 and d16-HER2 cells. Selective depletion of the adaptive immune compartment demonstrates that CD4+ cells are responsible for the regulation of clonal competition. These findings suggest that the adaptive immune response is regulating cellular fitness of the HER2+ tumor cells. Understanding how the immune system and HER2+ therapy bias competition in tumorigenesis may provide insight into mechanisms of acquired treatment resistance in HER2+ breast cancer patients. Future directions seek to deploy mathematical modeling approaches for controlling clone competition and preventing the evolution of treatment resistance.

Lay Abstract: Survival for HER2+ breast cancer patients has drastically improved in the past 2 decades thanks to the development of HER2-targeted therapies. However, these therapies do not work for all patients, who will progress to stage 4 metastatic disease. Therefore, it is imperative to understand why some patients respond to treatment and others do not. With the use of cutting-edge mouse models, novel cell lines, and mathematical modeling, our lab has begun to uncover biological mechanisms that may govern why some cancers respond and others do not. Our data suggests different forms of the HER2 protein exist on tumor cells that can determine how cells behave and interact with their environment. Cells expressing these different forms of HER2 compete while the tumor is in early stages of growth. Interestingly, cells with the normal form of HER2 are responsive to targeted therapy, while cells with an alternate form are not, leading the cells with the alternate form to outcompete the normal cells. We demonstrate that these alternate form HER2 cells can also interact differently with the immune system, giving them the ability to escape being killed by immune cells and allowing them to metastasize to other organs. Our overall goal is to fully understand how this alternate form of HER2 responds to therapy and interacts with neighboring cells to inform new HER2 treatments and eradicate therapy-resistant HER2+ breast cancer.

Head and Neck Cancer and Microbial Infections: A Deeper Look
Lusheng Song, Innovative Molecular Analysis Technologies (IMAT)

Abstract: Background: Head and neck cancers (HNSCC) increasingly linked to oncogenic viruses like HPV and EBV. However, infection alone rarely triggers cancers and there were growing evidence points to a poly-microbial etiology for pathogen-induced cancers. We need to track antibodies against various microbes simultaneously.

Methods: We used the Multiplexed In-Solution Protein Array (MISPA) assay to profile 232 OPSCC patients for 232 anti-microbial antibodies, encompassing proteomes of multiple variants. Subsequently, seroreactivity profiles were established in 99 HPV+ OPSCC (HPVOPC) subjects, 42 partners, and 81 controls against 94 antigens from 12 HPV types, the EBV proteome, and immunodominant antigens from 40 viruses and 26 bacteria.

Results: HPVOPC patients displayed significantly elevated antibody levels against HPV16 antigens compared to controls. The Area Under the Curve (AUC) for anti-E6, anti-E7, anti-E1, and anti-E2 were 0.979, 0.947, 0.942, and 0.928, respectively. This confirms the dominant role of HPV16 in HPVOPC. Additionally, the data suggests potential involvement of other high-risk HPV types (HPV18, 33) in a limited subset, warranting further validation. Notably, HPVOPC patients also exhibited increased antibody levels against various EBV antigens, hinting at a potential supportive role for EBV in OPSCC development.

Conclusion: Our immunoproteomic approach using MISPA not only endorses the primacy of HPV16 in HPVOPC but also suggests the possibility of co-contributors like HPV18 and 33. Moreover, the observed elevation of anti-EBV antibodies warrants investigation into EBV's potential supportive role in HPVOPC development. This study paves the way for a more comprehensive understanding of the complex interplay between microbial infections and HNSCC pathogenesis.

Lay Abstract: Traditionally, diagnosing infections and their role in diseases involves analyzing antibodies against one or a few antigens at a time. This limited approach can miss crucial details. We addressed this by developing the Multiplexed In-Solution Protein Array (MISPA) assay. MISPA is a high-throughput (>2000 samples), quantitative, and highly multiplexed (>200 antibodies) platform for anti-microbial analysis.

We employed MISPA to investigate the complex interplay between microbial and oropharyngeal squamous cell carcinoma (OPSCC). We analyzed the antibody response of OPSCC patients against a broad spectrum of antigens, including high-risk and low-risk HPV types, the entire EBV proteome, and immunodominant antigens from 40 common viruses and 26 bacteria.

MISPA pinpointed HPV 16 as the primary driver in HPV-positive OPSCC. Patients displayed significantly elevated antibody titers against HPV 16 antigens, with high sensitivity (92.9%) and specificity (95.0%). Interestingly, MISPA also revealed potential involvement of other high-risk HPV types (18 and 33) in a subset of patients. Furthermore, analysis of EBV antibodies suggested a possible supportive role for EBV in OPSCC development alongside HPV 16. These findings highlight the power of MISPA in deciphering the intricate web of microbial interactions in cancer development.

Unlike conventional methods, MISPA's ability to analyze hundreds of antibodies simultaneously provides a more comprehensive picture of the immune response. This approach is not limited to HPV-associated cancers but holds promise for elucidating the role of various pathogens in diverse diseases. By unraveling the complex interplay between infections and diseases, MISPA paves the way for more targeted diagnostics and therapeutic strategies.

An Advanced Electroporation System for Efficient Gene Delivery in Patient-Derived Cells

SJ Claire Hur, Innovative Molecular Analysis Technologies (IMAT)

Abstract: Patient-derived cells are crucial in personalized medicine for precise disease modeling, efficient drug screening, and tailored therapeutic development. Traditional viral methods for gene insertion pose risks, such as viral genomic integration and mutations. Nonviral methods like electroporation also face challenges, including cytotoxicity from proprietary buffers, high voltage requirements, and inconsistent transfection efficiencies due to cell size disparities. Additionally, affinity-based cell pre-purification complicates clinical translation.

We developed an integrated electroporation system that utilizes an ultra-high throughput vortex

cell purification method. This platform efficiently purifies, permeabilizes, and delivers genetic constructs to primary cells using a microfluidic chip and microscale electrode system. The chip employs size-based vortex purification for uniform cell trapping across 144 parallel trapping chambers. Interdigitated microelectrodes generate localized electric fields of 1.5 kV/cm with low input voltages (<40 V), enhancing throughput by 3.6-fold over previous prototypes while maintaining comparable performance.

The system's rapid solution exchange allows for in situ customization of buffer compositions, improving transfection outcomes compared to conventional buffers. We demonstrated enhanced gene delivery efficiency in human mammary fibroblast primary cells using an optimized electroporation buffer, achieving up to 8-fold higher efficiency than DPBS and matching lipofection performance (>80%). Additionally, we achieved robust protein production from synthetic mRNA in HMF transfections, demonstrating the system's potential.

This novel electroporation system is being validated with circulating tumor cells from breast cancer patients to demonstrate its potential for personalized medicine.

Lay Abstract: Patient-derived cells are essential for advancing personalized medicine, which tailors treatments to individual patients. These cells help us create accurate disease models, screen drugs efficiently, and develop therapies specifically designed for each patient. Traditional methods for inserting genes into cells, known as electroporation, have several problems: they can be toxic to cells, require high voltage, and often result in inconsistent outcomes due to differences in cell sizes. Additionally, the process of preparing cells before gene insertion is complex and time-consuming, making it harder to apply these techniques in clinical settings.

To overcome these challenges, we have developed a new system that improves how we insert genes into cells. This system uses a high-throughput purification method that simplifies and speeds up the process. It consists of a microchip and tiny electrodes that trap and handle the cells efficiently. This method ensures uniformity among the cells and preserves their characteristics.

Our new system allows for quick and customizable preparation of the cells, improving the success rate of gene insertion. In tests, we showed that this system significantly improves the efficiency of gene delivery into human cells compared to traditional methods. We also achieved high levels of protein production from synthetic mRNA in these cells, demonstrating the system's effectiveness.

This innovative approach has the potential to transform personalized medicine by enabling more detailed studies of diseases and the development of highly effective, patient-specific treatments.

Understanding the physics behind cellular reprogramming in porous materials

Vishal Srikanth, Innovative Molecular Analysis Technologies (IMAT)

Abstract: Porous biomaterial scaffolds mediate efficient cellular transduction and have demonstrated utility in producing gene therapies like CAR T cell therapy. However, there is a lack of fundamental understanding about how these materials promote cell-virus interactions. In this study, we used computational modeling to simulate the motion of cells and viruses inside the pores of the scaffold to investigate the governing physical factors that enhance cell-virus collisions.

Liquid flow inside a representative elementary volume of a porous scaffold is modeled assuming

constant flow rate conditions and periodicity. The cells and viruses are suspended in the liquid medium and modeled as discrete particles advected by the flow. Three flow scenarios are modeled: (1) no-scaffold (no-flow), (2) microscale flow inside a single scaffold pore (0.5, 1, 5 micron/s), and (3) macroscale flow through a network of interconnected pores (0.5, 1, 5 micron/s). The frequency of cell-virus collisions is assumed to be the bottleneck process and the primary measure of transduction efficiency.

Our results show that virtually zero cell-virus collisions were observed in the no-scaffold scenario. The number of cell-virus collisions increased with the increase in flow velocity of the microscale flow through a single scaffold pore. The number of cell-virus collisions increased by an order of magnitude in the case of macroscale flow through a network of interconnected pores when compared to that of a single pore.

Our study finds that macroscale particle dispersion inside porous biomaterial scaffolds plays an important role in promoting cell-virus collisions, and consequently, efficient transduction.

Lay Abstract: Porous biomaterial scaffolds are useful to produce gene therapies like CAR T cell therapy since they promote high cellular transduction efficiency. However, we do not yet understand how these materials increase cell-virus interactions and lead to efficient transduction. Therefore, we used computational modeling to simulate how cells and viruses move inside the scaffold pores to understand why scaffolds increase cell-virus collisions.

We modeled a small periodically repeating portion of the porous scaffold by assuming that the liquid flows through it with a constant flow rate. We modeled the cells and viruses as discrete particles that move along with the liquid flow. We considered three flow scenarios: (1) no-scaffold (no-flow), (2) flow inside a single scaffold pore and (3) flow through a network of interconnected pores. We chose the frequency of cell-virus collisions as the primary measure of transduction efficiency since we assumed that it is the bottleneck process in transduction.

Our results show that virtually zero cell-virus collisions were observed when there was no scaffold. The number of cell-virus collisions increased with the increase in flow velocity inside a single scaffold pore. Notably, the number of cell-virus collisions increased further by an order of magnitude in the case of flow through a network of interconnected pores.

Our study finds that liquid flow inside the porous biomaterial scaffold disperses the cells and viruses, which promotes cell-virus collisions, and consequently, efficient transduction.

Fate of tumor cells post mechanical priming on substrates mimicking metastasis sites
Khanh Ly, Laboratory of Cell Biology

Abstract: Cancer is the second leading cause of death on a global scale, causing more than ten million deaths per year. Metastasis is the event where cancer cells break away from their original (primary) site to, travel through the bloodstream, and form a new tumor in other organs in the body. Despite being responsible for over 90% of cancer-related deaths, the mechanisms underpinning cancer metastasis remain unclear. The pattern of affected organs in metastasis is varied depending on tumor of origin, intrinsic cancer cell traits, physical accessibility of target organs, and composition of host-organ microenvironments. Recently, tissue biophysical properties have been shown to be as potent as genetic perturbations in driving cell fate decisions. One such factor is the mechanical phenotype of cancer cells. The mechanical phenotype encompasses the material properties of cells and extracellular matrix (ECM). ECM stiffness, or elastic modulus, is known to play a crucial role in regulating cancer cell migration, proliferation,

and differentiation. Here, we aim to investigate the fate of tumor cells post mechanical priming on soft substrates made of polydimethylsiloxane (PDMS) with elastic modulus ranged from 0.2 kPa to 8 kPa, resembling in vivo matrix of metastasis sites such as brain and liver. The mechanical phenotype, differential growth, and survival of mechanically primed cells can be characterized qualitatively and quantitatively, potentially contributing to the prediction of cancer metastasis and development of new therapeutic strategies.

Lay Abstract: Cancer is a leading cause of death worldwide, responsible for over ten million deaths each year. Metastasis, the process where cancer cells spread from their original site to other parts of the body, is known to be responsible for more than 90% of cancer-related deaths. However, the exact reasons why metastasis happens are still not well understood. The organs affected by metastasis depend on various factors, including the type of cancer, the characteristics of the cancer cells, how easily the cancer cells can reach different organs, and the microenvironment of those organs. Recent research suggests that the biophysical properties of tissues are just as important as genetic changes in influencing how cancer cells behave. One important factor is the "mechanical phenotype" of cancer cells, which includes the properties of the cells and their surrounding matrix. The stiffness of this matrix plays a key role in how cancer cells move, grow, and differentiate. This study aims to examine how cancer cells behave after being exposed to soft materials with varying elasticity that mimic the microenvironments of the brain and liver, common sites for metastasis. By investigating the growth and survival of these cells in different conditions, this research could help predict how metastasis occurs and lead to new cancer treatments.

The nuclear response to metastatic environmental cues driven is by chemo-mechanical chromatin reorganization

Aayush Kant, Metastasis Research Network (MetNet)

Abstract: Environmental cues during metastasis, such as changing tissue stiffness and mechano-osmotic forces, drive chromatin reorganization and determine the nuclear response. However, its underlying biophysical mechanisms still need to be understood. We have developed a mesoscale mathematical model of chromatin reorganization in response to these forces, grounded in first-principle non-equilibrium thermodynamics. Our model captures the emergent segregation of chromatin into heterochromatin and euchromatin phases through interchromatin energetic interactions coupled with the diffusion of nucleoplasm and epigenetic marks, particularly histone acetylation and methylation. Additionally, epigenetic regulation drives a non-conservative interconversion between these histone species.

Treating chromatin as a visco-hyperelastic polymer, the model accounts for mechanical responses to environmental cues. Our model captures the steady-state chromatin organization observed in vivo, characterized by multiple heterochromatic domains of a specific size, and demonstrates that the interplay of epigenetic kinetics and mechanical strain energy contributions regulates these domain sizes.

Model predictions explain in-vivo chromatin reorganization resulting from the modulation of epigenetic factors when cells metastasize into a chemo-mechanically distinct microenvironment. Further, during the metastatic translocation, we predict the chromatin reorganization under multiaxial mechanical loads, finding that shear loads primarily reorient heterochromatin domains without affecting overall chromatin compaction. However, uniaxial and biaxial squeezing changes the nuclear water content and increases chromatin condensation, resulting in larger compacted domains and reduced euchromatin content. These predictions are validated with experimental observations using high-resolution imaging and genomic sequencing techniques. Our model

establishes a novel framework for mechanistically understanding how metastatic cues alter gene expression and regulate cellular identity.

Lay Abstract: During cancer, diseased cells can move to different tissues with different chemical environments and mechanical stiffness. As these cells travel, they squeeze through blood vessels or experience shearing forces within the bloodstream. These environmental forces are transmitted to the nucleus, where they directly influence DNA accessibility, expression or suppression of genes, and, consequently, the cell behavior. This work explores the fundamental biophysics behind the rearrangement of chromatin - which includes DNA segments and associated histone proteins - in response to such cues. We find that this reorganization is governed broadly by two principles in tandem - (i) energetics of interactions between DNA segments, and (ii) methylation and acetylation of histones by enzymes.

Chromatin, which behaves as a flexible polymer - can form distinct regions of high packing density called heterochromatin domains. Our predictions show that these domains have a characteristic size that is determined by chemical modifications via enzymes. We also find that environmental cues can affect this balance, thus changing the size of domains and thus the DNA accessibility. Additionally, we also consider the impact of mechanical forces, such as shear and compression, and movement of water between the cell and the nucleus on the extent of chromatin compaction.

Overall, our modeling approach offers insights into how physical and chemical cues during the metastatic cascade influence overall DNA accessibility, gene expression patterns and cell behavior. Our findings can pave the way for unraveling potent cell fate determinants in metastatic cancer thus identifying innovative therapeutic targets.

Exploring and exploiting circulating tumor cells weaknesses

Billy Hill, Metastasis Research Network (MetNet)

Abstract: Preventing tumor cell (TC) metastasis is a significant challenge in cancer treatment. TCs face mechanical cues and metabolic stressors like hypoxia, acidosis, and nutrient scarcity before and during their journey in the bloodstream. These conditions lead to biophysical and metabolic adaptations retained as “mechanical memory”, enhancing TC survival and metastatic efficiency. Low levels of reactive oxygen species (ROS) under these conditions may reduce oxidative stress-induced cell death.

To test the conditions enabling survival of CTCs and early metastatic seeds, we examined which mechanical and metabolic conditions affect the metastatic behavior of MDA-MB-231 breast cancer cells. We tested whether these adaptations influence the early steps of metastasis. These first hours to days are crucial in determining metastatic efficiency. Preconditioning cells on a stiff-matrix (50 kPa) significantly increased early-stage survival compared to the soft-matrix (0.5 kPa). Metabolic deprivation prior to injection into mice strongly increased early organ colonization. Additionally, manipulating ROS levels influenced metastatic outcomes, with ROS Scavenger N-acetylcysteine promoting higher metastatic burden and ROS inducer Artesunate reducing both organ colonization and metastatic outgrowth.

In conclusion, mechanical/metabolic memory impact TC survival during early organ colonization. Pharmacological induction of ROS may reduce organ colonization and metastatic progression. These findings suggest that targeting high CTC burden pharmacologically could lead to effective interventions against metastatic spread. Understanding these early adaptations is essential for developing new therapeutic strategies to inhibit metastasis and improve patient outcomes. Our research highlights the potential for novel therapies that target these adaptations, offering a

promising approach to cancer treatment.

Lay Abstract: Stopping cancer cells from spreading is a big challenge. Before and during their journey through the bloodstream, cancer cells face tough conditions like low oxygen, high acidity, and lack of nutrients. These harsh environments force cells to adapt, which helps them survive and spread more effectively.

We studied how these tough conditions affect the spread of MDA-MB-231 breast cancer cells, focusing on the crucial early stages – the first few hours to days – that determine if the cells can form new tumors. Our findings revealed that preconditioning cells on a stiff surface (50 kPa) significantly increased their early survival compared to a soft surface (0.5 kPa). Additionally, conditioning the cells in an environment with high acidity, low oxygen and starving them of nutrients before injecting them into mice made them better at colonizing new organs. We also found that manipulating reactive oxygen species (ROS) levels influenced metastatic outcomes. Lowering ROS levels with a scavenger called N-acetylcysteine increased the metastatic spread of cancer cells, while raising ROS levels with Artesunate reduced both the colonization of new organs and the growth of new tumors.

These findings show that the adaptations cancer cells make to tough conditions are crucial for their survival and ability to grow in new organs. By targeting these adaptations with drugs, especially by changing ROS levels, we might be able to prevent cancer from spreading. This research suggests new ways to treat cancer by focusing on these early adaptations, potentially leading to better outcomes for patients,

Metastatic Knowledge: Transfer learning of gene regulatory signatures

Parker Stevenson, Metastasis Research Network (MetNet)

Abstract: Transfer learning is a facet of machine learning that allows one to evaluate the presence of a source latent space in an independent, target dataset by exploiting features shared between the datasets. Thus, transfer learning is an untapped resource for translational research; it allows one to build in silico support for findings from pre-existing, publicly available data rather than having to generate additional, costly high-throughput datasets. Our lab previously developed a transfer learning tool, projectR, that overcomes this challenge by allowing the integration of gene expression patterns learned across independent datasets and even different transcriptomic modalities. For example, projectR exploits the shared features, or genes, between two separate transcriptomics datasets to evaluate the presence of latent spaces (i.e. principal components (PCs) or clusters) that represent biological phenomena occurring in both datasets. Ultimately, projectR is a powerful tool that allows one to investigate shared latent spaces across technologies, tissue types, and even species to allow for more robust in silico interpretation of previously learned latent spaces.

Our group is interested in characterizing the molecular programs that drive metastasis from a primary tumor through robust machine learning and transfer learning computational tools. We previously surveyed invasive triple-negative breast cancer (TNBC) organoid models via longitudinal single-cell transcriptomic profiling and subsequent analysis of co-regulated gene signatures driving these processes. Here, we demonstrate the functionality of projectR by using it to further characterize our invasive gene signatures learned in TNBC across a variety of datasets, both in other model systems and transcriptomic modalities.

Lay Abstract: A projector visually displays images of a variety of subject matter; however, applying a lens filter to the projector can alter the image, creating new focal points. For example,

trading a black-and-white lens filter on a projector for a colored one allows us to see details that were previously obscured. Our lab developed projectR, which is a computational tool that likens the gene signatures that underlie biological phenomena to the lens filters of a projector. Depending on the gene signature, or lens filter, that we're investigating, different populations of our data will stand out. ProjectR does this by using a type of machine learning called transfer learning, which allows us to identify common gene signatures, and therefore shared biology, occurring in independent datasets.

Our group is specifically interested in how changes in gene expression changes drive cancer cells to become invasive and eventually metastasize. We previously identified gene signatures driving invasive behaviors in triple-negative breast cancer (TNBC) organoid models. Here, we build upon this and demonstrate how projectR can be used to better characterize these gene signatures by evaluating their usage across a variety of datasets generated from different model systems and technologies.

Targeting lipid metabolism in breast cancer metastasis

Rohan Panaparambil, Metastasis Research Network (MetNet)

Abstract: Metastasis is the leading cause of cancer mortality, particularly in breast cancer, where 5-year survival rates drop from over 90% for localized disease to 30% for metastatic disease. Despite this, the molecular processes underpinning metastasis remain poorly understood. Lipids are a major class of biomolecules which play a key role in enabling aggressive proliferation by way of supplying membrane biomass, energy, and activation of pro-growth signaling. We hypothesized that breast cancer metastases must activate lipid metabolism through the sterol regulatory element-binding protein (SREBP) pathway, which activates expression of genes necessary for lipid synthesis and uptake. To test this hypothesis, we isolated tumor clusters from primary mouse mammary tumors and embedded them in a 3D culture system that models metastatic colony formation, and studied the effect of pharmacological SREBP inhibitors on colony formation ex vivo. We found that SREBP inhibition led to reduced colony formation and induced apoptosis in clusters isolated from two GEMM models of breast cancer. Furthermore, we found that cholesterol was sufficient to rescue growth of 2D cultures treated with SREBP inhibitors. By using cyclodextrins to modulate plasma membrane cholesterol, we found that PM cholesterol depletion led to reduced colony size and sensitivity to SREBP inhibition, while PM cholesterol enrichment led to increased colony size and resistance to SREBP inhibition. Taken in sum, these results suggest that cholesterol is a limiting metabolite in breast cancer metastasis, and the SREBP pathway promotes metastatic establishment, growth, and survival by activation of genes necessary to supply the cell with cholesterol.

Lay Abstract: Metastasis is the process by which cancer cells leave a primary tumor and seed in distant organs. Once cancer cells reach the distant site, they divide rapidly, forming metastatic tumors that damage the healthy organ and are often fatal. The rapid cell division requires nutrients to sustain it, and one important class of nutrients are fats, also known as lipids. We hypothesize that metastatic cancer cells require a high amount of lipids and that they must activate a cellular switch, called, SREBP, to ramp up lipid production. We used drugs that turn off the SREBP switch and found that reduced the growth of metastases and led to death of cancer cell clusters in a 3D gel model. We then found that by adding cholesterol to the cell membrane of cancer cell clusters, they were able to form more metastatic colonies that were also larger in size, and that by removing membrane cholesterol, the clusters formed fewer, smaller colonies. These results suggest that cholesterol is a key nutrient that helps cancer cells grow and spread, thus improving our understanding of the process of metastasis. Furthermore, our results suggest that lowering cholesterol levels in cancer cells, perhaps with medication or dietary changes, may reduce the

burden of metastasis and improve patient outcomes.

Integrating Epigenetic Regulation and Chromatin-Lamina Interactions to Unveil the Morphology of Lamin-Associated Domains
Zixian Guo, Metastasis Research Network (MetNet)

Abstract: In eukaryotic nuclei, a significant portion of transcriptionally repressed chromatin is anchored to the nuclear lamina (NL), leading to the formation of lamina-associated domains (LADs). LADs are characterized by increased histone methylation level and are physically and chemically tethered to the NL by proteins like LAP2β. Here, we develop a phase-field model of chromatin organization that incorporates chromatin-chromatin interactions, chromatin-lamina affinity, and the kinetics of methylation and acetylation. Our model predicts the size and shape of peripheral heterochromatin domains and reveals that the strength of chromatin-lamina interactions drives LAD morphological regulation. Analyzing super-resolution images of hMSCs, we identify a heterogeneous, bimodal distribution of chromatin-lamina affinities. We find that soft substrate environments increase LAD thickness, linked to contractility-dependent increased nuclear localization of HDAC3, enhancing chromatin-lamina affinity and histone methylation. The model is validated for in-vitro nuclei under the alternation of various chemo-mechanical cues such as contractility inhibition and substrate stiffening. In tendinosis, a condition marked by collagen degeneration, similar increases in LAD thickness are observed, aligning with our model's predictions. Our findings emphasize the microenvironment's role in genome organization and offer insights into cellular responses to developmental cues, cancer metastasis, and degenerative diseases.

Lay Abstract: The way chromatin interacts with the cell's nuclear boundary helps control which genes are active, keeping cells functioning properly. However, how strong these interactions are and how they are influenced by the cell's environment is not well understood. In this study, we developed a model to predict the size and shape of chromatin regions that attach to the nuclear boundary in human stem cells. By analyzing high-resolution images, we found that these interactions are very uneven, with some areas showing strong attachment and others showing weak attachment. We discovered that the environment around cells significantly affects these interactions. When stem cells are grown on soft surfaces, the regions where chromatin attaches to the nuclear boundary become thicker. This change is linked to a protein called HDAC3 moving into the cell nucleus, which increases the attachment strength by interacting with another protein, LAP2β, and raising histone methylation levels. These results were confirmed by using drugs that affect cell contractility and histone methylation. Additionally, in a condition called tendinosis, where tendon tissue degenerates, we observed similar increases in chromatin attachment, reflecting the effects seen in cells on soft surfaces. Our research highlights how the cellular environment influences chromatin organization and its importance in certain diseases, like cancer metastasis, and tissue degenerated diseases.

Investigating mechanisms of cancer cell apoptosis with low frequency ultrasound
Aditi Singh, Not Applicable

Abstract: Cancer cells lack mechanosensory cytoskeletal protein known as tropomyosin 2.1 that prevents them to undergo rigidity dependent growth, in contrast to normal cells. This renders their distinct response to the mechanical cues, leading to apoptosis upon mechanical stimulation. Mechanical stretching of transformed cancer cells have shown to activate calpain-dependent apoptosis downstream of Piezo1 activation. Likewise, treatment of these cancer cells with low frequency ultrasound has shown that calcium influx through piezo1 channels activates calpain proteases in tumor cells, leading to microtubule depolymerization, increased myosin IIA-mediated

actomyosin contractility and activation of RhoA pathway; ultimately causing cell death. However, this seems only part of the model and we do not fully understand how this pathway and activation of mechanosensitive proteins is linked with the endoplasmic reticulum-mitochondrial stress pathway. Hence, we plan to target mitochondria-associated ER membranes (MAMs) and understand their role in causing apoptosis in cancer cells exhibiting variations in their mechanoresponse via differential cell killing upon mechanical stimulus, rendering the normal cells unaffected. These mechanisms when targeted aptly could further aid in cancer therapy.

Lay Abstract: Application of low frequency ultrasonic waves on cancer cells, to cause mechanical activation within the cells inciting cell death response. And understanding mechanisms behind their cell death and unaffected response of normal cells with this mechanical stimulation.

Phase behavior of protein-like synthetic heteropolymers toward targeting aberrant biomolecular condensation

Alexandra Grigoropoulos, Not Applicable

Abstract: Liquid-liquid phase separation (LLPS) of proteins in cellular environments has emerged as a highly relevant, yet elusive phenomenon. While the formation of biomolecular condensates via LLPS serves as a mechanism by which proteins and nucleic acids can be compartmentalized to achieve functions such as DNA repair and signal transduction, anomalies in LLPS-associated processes are known to result in various cancer cell pathologies. We aim to elucidate the molecular mechanism underlying this process, such that aberrant behaviors can be predicted, identified, and targeted for therapeutics. To capture the key parameters dominating this phenomenon in a synthetic model system, we design random heteropolymers (RHPs) whose segmental interactions proffer physiologically relevant lower critical solution temperatures in the absence of sequence specificity. We employ x-ray and neutron scattering techniques, coupled with computational sequence analysis to elucidate the key molecular interactions modulating the arrangement of macromolecules across length-scales. We determine that that the interchain and intrachain interactions of RHPs indeed capture the range of interactions of proteins, which retain their tertiary structure and use compositional uncertainty to assemble in the most dense and complex cytosolic environments. We map out the assembly pathway of RHPs and their biological analogs from the sequence level to the chain level in complex fluids, and ultimately to the formation and evolution of biomolecular condensates.

Novel murine model to assess Notch activation and inhibition in the tumor microenvironment

Fernando Flores-Guzman, Not Applicable

Abstract: Background: We developed an immunodeficient mouse model expressing a bright fluorescent signal upon Notch activation localized to the nucleus by crossing RAG2 -/-gammaC-/- mice to VENUS+/- mice, which expresses a bright yellow fluorescent protein (YFP) fused to histone 2B, localizing it to the nucleus.. Methods: Mice were confirmed to express one copy of the VENUS transgene by breeding heterozygotes in order to ensure an equal YFP baseline intensity. We renally implanted 1X106 neuroblastoma NGP-luciferase cells into 6 week-old mice, and harvested tumors 6 weeks later. We quantified Notch positivity (YFP) in multiple cell types by using flow cytometry (FC) and immunostaining of paraffin-embedded tumors. We used the endothelial cell markers CD31 and endomucin, the pericyte marker alpha smooth muscle actin (αSMA), macrophage marker F4/80 and granulocyte marker CD11b. To demonstrate whether YFP is sensitive to Notch inhibitors, mice received Notch1-specific inhibitor SAHM-1, or the gamma-secretase inhibitor DAPT in tumor bearing mice for 6 days. Results: In untreated mice, FC data shows that Notch is activated in 21% of tumor CD31+ endothelial cells and 38% of αSMA

pericytes. Furthermore, 34% of tumor-infiltrating macrophages (F4/80+) and 33% granulocytes (CD11b+) are YFP-(Notch)+. Immunostaining shows activated Notch signaling in areas of endothelium-pericyte interaction and confirms Notch activation in macrophages. Finally, using FC, we found that DAPT and SAHM-1 inhibited Notch activation in endothelium, (50% and 35%), in pericytes (75% and 50%), and in macrophages (47% and 41%), respectively compared to controls. In conclusion, tumor vasculature are of murine origin, and positive for Notch activation.

Lay Abstract: Blood vessels are composed of two interacting cell types. Endothelial cells form the inner lining of the vessel wall, and perivascular cells, referred to as pericytes, vascular smooth muscle cells or mural cells envelop the surface of the vascular tube. These cells create the blood vessels in tumors that provide oxygen and nutrients. Vascular endothelial growth factor (VEGF) form hypoxic tumor cells provides a signal to endothelial cells that calls for an increase in vascular function. The Notch pathway acts within the vasculature to help the endothelial cells to respond appropriately to the activating VEGF signal. We studied the detection of Notch activation in endothelium and pericytes in renal neuroblastoma of a Notch immunodeficient mouse model. In the signal-receiving cell, Notch intracellular domain (NICD) translocates to the nucleus where it is associated to the C promoter binding factor 1 (CBF1) that activates Notch gene. The histone 2B (H2B) has linked a yellow fluorescent protein (YFP) trans-gene that is produce only when Notch gene is activated. Mice were confirmed to express one copy of the YFP transgene by breeding heterozygotes in order to ensure an equal YFP-baseline intensity. We found in untreated mice, that Notch is activated in 21% of tumor endothelial cells and 38% of pericytes. To demonstrate whether YFP is sensitive to Notch inhibitor, mice received Notch1-specific inhibitor SAHM-1 or the gamma-secretase inhibitor DAPT in tumor bearing mice for 6 days. In conclusion, tumor vasculature are of murine origin, and positive for Notch activation.

Simplifying Health Information: A Corpus for Digestive Cancer Education and Novel Strategies for Reinforcement Learning

Kevin Lybarger, Not Applicable

Abstract: The reading level of health educational materials significantly impacts understandability and accessibility, especially for minoritized populations. Many current patient education resources surpass recommended reading levels. Additionally, generative AI is enabling the development of dialogue-based health interventions, where language complexity impacts effectiveness. There is an urgent need for datasets and methods to simplify health information for improved dissemination and health literacy. This need is particularly critical in cancer education, where improved prevention and screening information can reduce morbidity and mortality.

We introduce Simplified Digestive Cancer (SimpleDC), a parallel corpus of cancer education materials for text simplification research, comprising content from the American Cancer Society, Centers for Disease Control and Prevention, and National Cancer Institute. Using SimpleDC and existing resources, we explore text simplification using Large Language Models (LLMs), including fine-tuning, reinforcement learning (RL), RL from human feedback (RLHF), domain adaptation, and prompting. Experimentation includes multiple LLMs, such as Llama 2 and GPT-4. We introduce a novel RLHF reward function featuring a model for distinguishing between original and simplified texts.

Our innovative RLHF reward function outperforms existing RL text simplification rewards. Results show that RL/RLHF can augment fine-tuning, enable training on unlabeled text, and improve performance. These methods effectively adapt out-of-domain text simplification models to our target domain. RL-enhanced Llama 2 models outperformed GPT-4 in both automatic and manual evaluations.

The SimpleDC corpus provides a valuable resource to the research community, particularly in patient education. The RL/RLHF methodologies presented enable simplification model development using unlabeled text and existing simplification corpora.

Lay Abstract: The reading level of health information affects how understandable it is, especially for underrepresented and marginalized communities. Unfortunately, many health education documents and websites are too complicated for many people to easily understand. We urgently need ways to make health information easier to read and understand, including datasets and algorithms. This is particularly important for information about cancer prevention and screening, which can save lives if understood and followed properly.

Our project focuses on making cancer education materials easier to read and understand. We created a collection of simplified cancer education materials called "SimpleDC." This collection includes information from trusted sources like the American Cancer Society, the Centers for Disease Control and Prevention, and the National Cancer Institute. We also developed algorithms to train Large Language Models (LLMs), like ChatGPT, to simplify health information. These algorithms use human feedback to create models that align with human judgments.

Our new method, which uses human feedback, works better than existing methods for simplifying text. It helps the models learn from both labeled and unlabeled texts and adapt to specific types of information. The models we trained using our method performed better than the well-known GPT-4 model.

The SimpleDC collection and our new training methods are valuable tools for researchers. They can help develop models to make health information easier to understand, which is especially important for educating patients about cancer prevention and screening.

Extensions of the OMOP Common Data Model for Translational Research: Inclusion of Animal Models in the MeDOC Consortium

Madhan Subramanian, Not Applicable

Abstract: Advances in biomedical research are increasingly driven by the standardization and interoperability of data-driven discoveries. The adoption of FAIR (Findability, Accessibility, Interoperability, and Reusability) principles for scientific data management are key to this effort. Data Mapping, the process of matching fields from one database to another and Data Harmonization, the process of combining data from different sources into a unified dataset, are essential components of the FAIR data workflow. These processes will be utilized to standardize data across the MeDOC (Metabolic Dysregulation and Obesity Cancer Risk Program) Consortium. Using the Observational Medical Outcomes Partnership (OMOP) Common Data Model (CDM), we extended the CDM to support MeDOC's transdisciplinary approach to obesity-associated cancer research, which encompasses both observational human and pre-clinical translational research. MeDOC plans to incorporate individual data and biospecimens across three projects: MeDOC-Biorep, which expands the CDM to serve as virtual repository for data from both humans and mice; MeDOC-Miner, which uses Natural Language Processing (NLP) via R to extract, summarize, and analyze textual data from PubMed publications; and MeDOC-KB, which leverages information from MeDOC-Miner to generate concept relationships or associations between research targets and diseases, thus creating a knowledge base for the consortium. Through these initiatives, MeDOC aims to enhance the standardization and interoperability of biomedical data, facilitating more efficient and effective research into the links between obesity, metabolic dysregulation, and cancer risk.

Lay Abstract: Biomedical research increasingly relies on data, and following FAIR principles makes data easy to find, access, use together, and reuse. “Data Mapping” means connecting related information from different databases, while “Data Harmonization” means combining data from various sources into one cohesive set. These processes help standardize data so that it can be used more effectively. In healthcare, two main models help organize clinical data: Fast Healthcare Interoperability Resources, (FHIR) which focuses on patient-related data, and the Observational Medical Outcomes Partnership (OMOP) Common Data Model (CDM), which organizes data for human health research. The MeDOC Consortium (Metabolic Dysregulation and Obesity Cancer Risk Program) studies obesity, metabolic dysregulation, and cancer risks by combining various research approaches, including studies on humans and laboratory animals. MeDOC plans to extend the CDM to harmonize consortium data and published results from human and animal studies. This will be done through three projects. MeDOC-Biorep is a virtual repository that will collect and store data from humans and mice, and represent the data in an easy-to-use visual report. MeDOC-Miner will use computer tools to perform artificial intelligence techniques to analyze medical research papers. MeDOC-KB will use information from MeDOC-Miner to create a database of relationships between different research findings and diseases. These efforts will make data better organized and accessible, allowing scientists to make new discoveries.

The role of AP-1 transcription factors in regulation of transcriptional plasticity in melanoma cells

Magda Bujnowska, Not Applicable

Abstract: Plasticity associated with fluctuations in transcriptional programs is exploited by cancer cells, including melanomas, leading to advantageous states for tumor progression, metastasis, and therapy resistance. Despite increasing knowledge of such transcriptional states in melanoma, the molecular origin of melanoma plasticity remains to be fully understood. Recent studies across genetically diverse melanomas have shown that differentiation state of a single cell can be predicted based on the relative levels of a few key AP-1 transcription factors (TFs). Our current work builds upon these findings to determine the mechanisms through which AP-1 TFs regulate the transitions and/or maintenance of specific cell states in melanoma. We used various genetic approaches to perturb six AP-1 TFs individually or in combination in an otherwise isogenic melanoma cell line. Using highly multiplexed iterative imaging, we systematically measured the effects of each perturbation on all six AP-1 protein levels and melanoma differentiation state markers. Through dimensionality reduction analysis, we identified 20 representative cell lines with unique expression patterns of AP-1 network and analyzed them by RNA sequencing. The clustering of transcriptional profiles of these cell lines reveals distinguishable transcriptional programs. Moving forward, we plan to test the hypothesis that these changes in gene expression profiles are mediated by AP-1 TFs (with interacting partners) modulating chromatin accessibility around enhancers affecting expression of pro-melanocytic and pluripotency genes. Elucidating the mechanisms of how AP-1 TF network regulates cellular plasticity will improve our understanding of cancer heterogeneity and therapeutic resistance.

Lay Abstract: Plasticity is the ability of a cell to alter the expression of its genes, which in turn determines the cell’s characteristics and behavior. Although plasticity is a normal process important for development, cancer cells exploit it to their advantage. Plasticity allows cancer cells to easily switch their gene expression profiles to adapt to their environment, which has implications on cancer progression, spread, and development of drug resistance. In our work, we focus on studying plasticity in melanoma, the deadliest type of skin cancer, known for its particularly high plasticity. Previous work from our research group has shown that a few key proteins that belong to the AP-1 family of transcription factors (TFs) are associated with

melanoma cell's behavior and development stage. AP-1 TFs are a large family of proteins that regulate expression of genes by binding to nearby DNA sequences called enhancers. To better understand how AP-1 TFs affect plasticity in melanoma, we used various genetic techniques to change the levels of six key AP-1 TFs, either one at a time or in combinations, in melanoma cells. Using imaging, we measured how these changes affect other AP-1 proteins and identified 20 unique cell lines with different AP-1 protein patterns. We are using this series of cell lines to test how differences in levels of AP-1 TFs alter melanoma plasticity by examining gene expression patterns and the accessibility of DNA around genes related to melanoma plasticity and development.

Plasma exosomes from type 2 diabetic patients drive expression of EMT-related genes in prostate cancer models

Michael Seen, Not Applicable

Abstract: Introduction: Uncontrolled diabetes is associated with poorer outcomes among prostate cancer patients. We hypothesize that differences in the miRNA payload of plasma exosomes among individuals with Type 2 diabetes (T2D) may contribute to poorer prostate cancer outcomes by promoting tumor aggressiveness.

Methods: Exosomes were isolated from platelet-free plasma using size-exclusion chromatography. NanoSight was used to measure exosome sizes and concentrations. Exosomal miRNA profiles were obtained using a commercially available PCR array. DU145 cells were treated with exosomes for 2 days. Total RNA was extracted from the exosome-treated cells. Relative expression of genes related to epithelial-to-mesenchymal transition (EMT) was measured using commercially available PCR array. RNA sequencing and Principal Component Analysis was used to measure changes in global transcription.

Results: The plasma exosome miRNA profiles of the T2D group differ significantly from that of the ND group. Treatment of DU145 cells with T2D plasma exosomes resulted in increased expression of EMT-related genes when compared with DU145 cells treated with ND plasma exosomes. Individual recombinant miRNAs represented in the profile are able to drive EMT transcription. Cells treated with ND exosomes displayed global transcription patterns similar to each other as well as to cells treated with media-only control exosomes. By contrast, DU145 cells treated with T2D plasma exosomes displayed wide variance in global transcription pattern.

Summary/Conclusion: The miRNA payload of plasma exosomes appears to be altered in T2D and has functional impact on tumor cells, thereby contributing to a poorer prostate cancer outcome among patients with co-morbid T2D

Lay Abstract: Prostate cancer patients that have uncontrolled Type 2 diabetes tend to get sicker than patients that don't. However, diabetic status is not currently considered when treating cancer patients. We hypothesize that certain components in the blood of people with Type 2 diabetes play a role in these worse cancer outcomes.

To test this hypothesis, we recruited Type 2 diabetes patients who did not have cancer from Boston Medical Center's diabetes clinic. We used blood from these patients to treat prostate cancer cells and found that the treatment made the cancer cells act more aggressively.

Next, we compared the components in the blood of Type 2 diabetic patients with the blood of non-diabetic patients. We found that Type 2 diabetic patients had certain components in their blood that are not found in the blood of non-diabetic patients. We treated prostate cancer cells with the

blood components that were only found in Type 2 diabetic patients and found that the cancer cells became more aggressive.

In summary, Type 2 diabetes patients have components in their blood that make prostate cancer cells act more aggressively. We believe that this may help to explain why prostate cancer patients who also have Type 2 diabetes tend to get sicker than patients who do not have diabetes.

Deciphering how Tropomyosin2.1 prevents Anoikis resistance in breast cancer cells
Nehal Dwivedi, Not Applicable

Abstract: Non-transformed adherent cells undergo apoptosis when detached from their extracellular matrix (ECM), demonstrating anchorage-dependent growth. This specific type of programmed cell death is termed as “anoikis”. However, cancer cells can survive even after detachment from the ECM, exhibiting anoikis resistance or anchorage-independent growth. Anoikis resistance is essential for cancer cells to acquire metastatic characteristics. Studies have reported that many cancer cells exhibit low Tropomyosin (Tpm) 2.1 expression- an actin-binding cytoskeletal protein. This project aims to understand how the lack of Tpm2.1 results in anoikis resistance in cancer cells. In this study, we have demonstrated when Tpm2.1 expression is restored in a metastatic breast cancer cell line (MDA-MB-231), the cells exhibit increased apoptosis when cultured in the absence of anchorage. The migration capacity of MDA-MB-231 cells is also impaired when Tpm2.1 expression is restored in these cells. Restoring Tpm2.1 expression in MDA-MB-231 cells causes downregulation of β-catenin, N-cadherin, and phospho-AKT levels when cultured in the absence of anchorage. Therefore, the difference in survival and migration capacity of MDA-MB-231 cells in response to Tpm2.1 expression is potentially regulated by β-catenin and AKT signaling pathways. Additionally, RNA-seq data indicates the downregulation of genes associated with various metabolic pathways (fatty acid metabolism, TCA cycle) when Tpm2.1 is restored in MDA-MB-231 cells. Further studies are focused on delineating the role of β-catenin and AKT signaling pathways in mediating anoikis resistance in Tpm2.1-deficient cancer cells. The findings of this research will provide mechanistic insights into how cancer cells modulate their signaling pathways to maintain anchorage-independent growth.

CK2 signaling from ER-embedded Tollip-dependent perinuclear endosomes is an essential feature of KRAS and NRAS mutant cancers.
Srikanta Basu, Not Applicable

Abstract: Our laboratory found that RAS induces perinuclear translocation of its effector kinases, ERK and CK2, and the signaling scaffold KSR1. KSR1 binds to ERK and CK2 and associates with recycling endosomes, forming signaling hubs termed Perinuclear signaling centers (PSCs). PSCs are present in all cancer cell lines and tissues examined, suggesting that subcellular compartmentalization of oncogenic kinases drives tumorigenesis. However, the mechanism of PSC formation, importance in tumorigenesis and identity of PSC targets are unknown. Using live cell imaging, show that the endosomal adaptor, Tollip, associates with KSR1 through the KSR1 pseudo-kinase domain and a highly conserved putative linker region in Tollip. Tollip tethers RAB11A+ endosomes containing CK2 and KSR1, but not ERK, to the perinuclear endoplasmic reticulum. KRAS and NRAS mutant tumor cells were dependent on Tollip for survival and PSC formation, whereas non-K/NRAS transformed cells and non-transformed cells were unaffected by Tollip loss. KRasG12D-induced lung tumors in Tollip KO mice rarely advanced beyond adenoma stage whereas malignant adenocarcinomas were readily observed in WT animals. Phospho-proteomic analysis of A549 cells with or without Tollip, revealed that CK2-PSCs phosphorylate a select group of proteins, many of which are involved in ribosome biogenesis and translation initiation. One such target, RIOK1, was phosphorylated on Ser22. RIOK1, a synthetic lethal gene

for mutant KRAS tumors, is an essential protein for 18S-E pre-rRNA processing. In summary, we identify Tollip as a novel regulator of localized CK2 substrate selectivity and an effector of K/NRAS-driven tumorigenesis that may represent a specific, targetable vulnerability of these cancers.

Lay Abstract: Our laboratory found that RAS induces perinuclear translocation of its effector kinases, ERK and CK2, and the signaling scaffold KSR1. KSR1 binds to ERK and CK2 and associates with recycling endosomes, forming signaling hubs termed Perinuclear signaling centers (PSCs). PSCs are present in all cancer cell lines and tissues examined, suggesting that subcellular compartmentalization of oncogenic kinases drives tumorigenesis. However, the mechanism of PSC formation, importance in tumorigenesis and identity of PSC targets are unknown. Using live cell imaging, show that the endosomal adaptor, Tollip, associates with KSR1 through the KSR1 pseudo-kinase domain and a highly conserved putative linker region in Tollip. Tollip tethers RAB11A+ endosomes containing CK2 and KSR1, but not ERK, to the perinuclear endoplasmic reticulum. KRAS and NRAS mutant tumor cells were dependent on Tollip for survival and PSC formation, whereas non-K/NRAS transformed cells and non-transformed cells were unaffected by Tollip loss. KRasG12D-induced lung tumors in Tollip KO mice rarely advanced beyond adenoma stage whereas malignant adenocarcinomas were readily observed in WT animals. Phospho-proteomic analysis of A549 cells with or without Tollip, revealed that CK2-PSCs phosphorylate a select group of proteins, many of which are involved in ribosome biogenesis and translation initiation. One such target, RIOK1, was phosphorylated on Ser22. RIOK1, a synthetic lethal gene for mutant KRAS tumors, is an essential protein for 18S-E pre-rRNA processing. In summary, we identify Tollip as a novel regulator of localized CK2 substrate selectivity and an effector of K/NRAS-driven tumorigenesis that may represent a specific, targetable vulnerability of these cancers.

Development of GEP-NEN Organoids for Therapeutic Screening
Steven Forsythe, Not Applicable

Abstract: Gastroenteropancreatic neuroendocrine tumors (GEP-NETs) are a rare subset of cancers with increasing incidence. Due to their slow growth and low mutational burden, development of new treatments has been limited. One reason behind this stagnation is the lack of applicable study models which accurately mimic the tumor. One potential solution is the utilization of patient tumor organoids (PTOs), which are capable of development directly from tumor tissue, maintain tumor characteristics, and are useable in a high throughput manner. In this study, our group developed a series of PTOs derived from 35 tumors derived from 16 patients with pancreatic, small intestinal, and gastric neuroendocrine tumors. The organoids were derived from a variety of functional and non-functional backgrounds and demonstrated stability up to passage 6 confirmed through immunohistochemistry and genomic analysis. Therapeutic targeting of the PTOs displayed both origin and grade-based response to standard of care therapies including everolimus, sunitinib, cabozantinib, and capecitabine, a temozolomide combination, along with maintaining patient tumor sensitivity and resistance. Finally, organoids demonstrated the capability to be resuscitated from cryopreservation with fidelity. This study is the first with a full focus on GEP-NET organoid development that demonstrates reliable multiple passaging allowing for further study.

Lay Abstract: Gastroenteropancreatic neuroendocrine tumors (GEP-NETs) are a rare subset of cancers with increasing incidence. The cause for this rise is unknown, but it is clear they are a rising health burden. Due to their unusual cancer characteristics, development of new treatments has been limited. One reason behind this stagnation is the lack of ways to study these cancers in the lab. One potential solution is the utilization of patient tumor organoids (PTOs), which

microtissues made directly from tumor made directly from patient tissue, are more accurate than more simple models, and are useable for large experiments. In this study, our group developed a series of PTOs derived from patients with pancreatic, small intestinal, and gastric neuroendocrine tumors. The organoids were derived from a variety of backgrounds and demonstrated long term growth and maintained tumor characteristics, ensuring they can be used many times. Treating the PTOs with a range of approved therapeutics showed the matched the expected treatment sensitivities and even matched their matched patient responses. Finally, organoids demonstrated the capability frozen and brought back to be used for future studies, allowing for multiple centers to use them. This study is the first with a full focus on GEP-NET organoid development that demonstrates reliable long term growth.

NFAT Physical and Genetic Interaction Mapping to Predict the Impact of VEGFR2 Inhibition in HCC

Verima Pereira, Not Applicable

Abstract: Hepatocellular carcinoma (HCC) is a major global health issue, ranking as the fourth leading cause of cancer-related deaths worldwide. Angiogenesis, essential for tumor growth and metastasis, is driven by the VEGFR2 pathway and ANG2. Despite their significance, the detailed molecular interactions between VEGFR2 signaling and ANG2 expression in HCC remain unclear. Nuclear Factor of Activated T Cells (NFAT) proteins, known for regulating gene expression and cellular responses, are suspected to play a pivotal role in these processes.

This research aims to elucidate the complex interactions involving NFAT proteins, VEGFR2 inhibition, and ANG2 expression in HCC. Utilizing a systems biology approach that integrates high-throughput protein interaction mapping (AP-MS) with genetic perturbation (CRISPRi) technologies, we seek to uncover novel molecular interactions and regulatory mechanisms. By thoroughly exploring NFAT's influence, this study aspires to enhance the understanding of HCC angiogenesis significantly.

The innovation of this research lies in its methodological approach, which combines advanced technological platforms to explore previously uncharted areas of NFAT's involvement in HCC angiogenesis. This approach promises to identify new therapeutic targets and strategies, ultimately transforming the management and treatment of HCC. The insights gained could pave the way for more precise and effective therapeutic interventions.

In summary, this study aims to provide groundbreaking insights into the molecular mechanisms of HCC angiogenesis. By elucidating the role of NFAT proteins in VEGFR2 inhibition and ANG2 expression, this research could revolutionize the understanding and treatment of HCC, offering potential for novel therapeutic developments.

Close the Cancer-Immunity Cycle by integrating lipid nanoparticle-mRNA formulations and dendritic cell therapy

Xucheng Hou, Not Applicable

Abstract: Effective cancer immunotherapy is usually blocked by immunosuppressive factors in the tumour microenvironment (TME), resulting in tumour promotion, metastasis, and recurrence. Here, we combine lipid nanoparticle (LNP)-mRNA formulations and dendritic cell (DC) therapy to boost the Cancer-Immunity Cycle (CIC) via progressive steps to overcome the immunosuppressive TME. Multiple types of sugar alcohol derived-LNPs are conceived to modulate the CIC. First, one type of LNPs containing CD40 ligand (CD40L) mRNA induce robust immunogenic cell death in tumoral tissues, leading to the release of tumour-associated antigens

(TAAs) and the expression of CD40L. Next, DCs engineered by another type of LNPs encapsulating CD40 mRNA are adoptively transferred, which are then activated by the CD40L molecules in tumoral tissues. This promotes the secretion of multiple cytokines and chemokines, and the upregulation of costimulatory molecules on DCs, which are crucial for reprogramming the TME and priming T cell responses. After DCs present TAAs to T cells, all the above stepwise events contribute to boosting a potent tumour-specific T cell immunity that eradicates established tumours, suppresses distal lesions, and prevents tumour rechallenge.

Algorithmic and informatics approaches to advance precision medicine

Yi Qiao, Not Applicable

Abstract: The application of advanced algorithms and high-throughput computation techniques holds promise for deepening our understanding of cancer genomics and transcriptomics as well as directly impacting clinical practice. We emphasize two primary research thrusts: the joint dissection of cancer genomic and transcriptomic heterogeneity, and the acceleration of bioinformatics algorithms for clinical applications.

Existing computational methods typically focus on either cancer genomic or transcriptomic heterogeneity independently, lacking integration. Addressing this gap, the scBayes method we developed links single-cell RNA sequencing data to genetically defined subclones reconstructed from bulk DNA sequencing, enabling the investigation of subclone specific expression phenotype. Validation with diverse data sets confirms its ability to provide novel biological insights across various single-cell sequencing techniques.

Accelerated clinical bioinformatics workflows beyond the state of the art is crucial for timely precision oncology applications. Utilizing hardware acceleration technologies like GPUs and FPGAs, we aim to reduce the analysis time of whole-genome sequencing from days to minutes. Significant speedups have been achieved, notably in redesigning data access layers and optimizing variant calling algorithms. We focus on benefiting clinical applications, such as rapid treatment selection for pediatric brain cancer patients, as well as the wider developer community by adopting an open source development model with strong emphasize on code re-usability.

Together, our methods not only deepen our understanding of cancer biology at a subclonal level but also aim to translate these insights into actionable clinical outcomes. We are committed to advancing computational tools that improve both research and patient care in precision oncology settings.

Lay Abstract: New technologies and advanced computer methods offer exciting possibilities for better understanding cancer genetics and how genes are used (transcriptomics), with direct benefits for patient care. Our research focuses on two main areas: studying the differences within tumors at the genetic and gene usage levels together, and speeding up computer programs to make them more useful for doctors.

Currently, most existing methods in cancer heterogeneity research look at either genetics or gene usage separately, missing how they connect. Our method, scBayes, links data from single-cell sequencing to specific genetic subclones in tumors, giving new insights on how different subclones behave.

Improving how quickly we can analyze genetic information is vital for using it in personalized medicine such as precision oncology. By using technologies like GPUs and FPGAs, we aim to cut down the time needed to analyze a whole genome from days to minutes. We've made

improvements by changing how data is handled (up to 40X faster) and by improving the software that finds genetic variations. These efforts can help doctors make fast decisions about treating children with brain cancer for example, and they are open-source so the entire developer community can benefit from our work.

In summary, our work doesn't just deepen our understanding of cancer biology at a very detailed level, it aims to turn this understanding into better care for patients. We're dedicated to making better computer tools for both research and patient treatment in the field of precision cancer medicine.

Agent-Based Modeling of Resistance Mechanisms and Treatment Strategies for T-Cell Engaging Antibodies in Multiple Myeloma

Matthew Froid, Physical Sciences-Oncology Network (PS-ON)

Abstract: T-cell engaging antibodies (TCEs) represent a novel therapeutic approach for patients with relapsed/refractory multiple myeloma (RRMM). Despite the limited number of TCEs currently approved by the FDA, resistance to these agents is already a significant concern. The resistance mechanisms are hypothesized to involve antigen loss or an exhaustive immune microenvironment, but the precise contributions of these factors to TCE therapy failure remain uncertain. Additionally, while adjuvant PD-1 inhibitors have shown synergistic effects with TCE therapy, there is no reliable biomarker to predict patient responsiveness to this combination treatment. To address these challenges, we have developed an agent-based model (ABM) of the multiple myeloma bone marrow microenvironment. This model allows us to investigate the interactions between the tumor microenvironment, the initial immune landscape, and the therapeutic effects of TCEs combined with a PD-1 inhibitor. Our ABM aims to elucidate the impact of the tumor microenvironment and the initial immune cell population on the efficacy of TCEs and identify potential resistance mechanisms. Furthermore, our model can help in exploring strategies to enhance the therapeutic outcomes, including optimizing the timing and dosage of TCEs in combination with a PD-1 inhibitor. Ultimately, this work seeks to provide insights into the factors influencing the success of TCE therapy in RRMM and guide the development of predictive biomarkers and more effective combination therapies. Our findings could significantly contribute to overcoming resistance and improving the clinical management of RRMM patients, thereby enhancing their prognosis and quality of life.

Lay Abstract: T-cell engaging antibodies (TCEs) are a new type of treatment for patients with relapsed or refractory multiple myeloma (RRMM), a blood cancer with no cure. Although only two TCEs have been approved by the FDA, patients are already becoming resistant to these treatments. This resistance might be due to the myeloma cells losing certain surface markers or the immune system becoming exhausted, but it’s not clear how these factors contribute to treatment failure. Moreover, while combining TCEs with drugs called PD-1 inhibitors have shown promise, there is no reliable way to predict which patients will benefit from this combination, or TCEs alone. To tackle these issues, we created a detailed computational model of the bone marrow environment where multiple myeloma develops. This model helps us study how multiple myeloma interacts with the immune system and how TCEs and PD-1 inhibitors work together. Our model aims to uncover how the bone marrow environment and the types of immune cells present affect the success of TCE treatments. It also seeks to identify why some patients develop resistance to TCEs. Additionally, the model can test different treatment strategies to find the best way to use TCEs and PD-1 inhibitors together, including the best timing and dosage. This knowledge could help develop better ways to predict which patients will respond well to these treatments and create more effective combination therapies. Ultimately, our work aims to improve treatment outcomes and quality of life for patients with RRMM.

Characterization and Engineering of P53R175H-specific TCR for Cancer Adoptive Cell Therapy

Peiwen Cong, Physical Sciences-Oncology Network (PS-ON)

Abstract: Adoptive cell therapy (ACT) using tumor-specific T cells has become promising immunotherapy for cancer. Naturally occurring T cell receptors (TCRs) targeting cancer neoantigens arising from driver mutations in common oncogenes, such as TP53, are emerging as important ACT modalities. However, it is not understood what parameters determine the efficacy of these TCRs in ACT, nor how to engineer more efficacious TCRs. Here, we demonstrate the structural, computational, biophysical, and functional assays in characterizing a panel of P53R175H-specific TCRs, delivering quantifications to predict their efficacy better. Furthermore, based on such new insights into TCR mechanobiology, we investigate multi-pronged design strategies in engineering more potent TCRs to enhance the anti-tumor effects for ACT.

Mechanosensitivity of PD1 Localization on the CD8 T-cell Membrane as Interrogated Using STORM Microscopy and Monte Carlo Simulations

Seung-Hyun Ko, Physical Sciences-Oncology Network (PS-ON)

Abstract: Solid tumors establish and develop within microenvironments capable of potently suppressing host immune responses against the tumor. Recent studies demonstrate that cancerous cell-secreted extracellular vesicles (EVs) contribute to tumor immunosuppression by carrying upregulated levels of the immune checkpoint molecule PD-L1. However, given the small size of EVs, it is unclear what contributes to their seemingly disproportionately potent immunosuppression observed experimentally. Our collaborators demonstrated that the adhesion molecule ICAM-1 is necessary for EVs to successfully suppress cytotoxic T-cells. Furthermore, our lab previously demonstrated that the potency of nanoparticle/cell interactions is dependent on mechanosensitive multivalent binding and clustering. Here, we utilize biophysical Monte Carlo simulations in conjunction with STORM superresolution microscopy experiments to provide novel insight into the mechanosensitivity of EV-induced PD-1 localization on the T-cell membrane. We find that various clustering patterns arise at the interaction interface in a manner dependent on cortical tension. In a stochastic model of T-cell signaling, we find that these patterns lead to significantly different levels of phosphorylated AKT, a critical transcription factor in cytotoxic T-cell activation and development. These results bolster the growing evidence supporting the importance of mechanosensitivity in cytotoxic T-cell activation, specifically providing novel insight into how PD-1 clustering may be a mechanism by which TAM EVs so potently suppress recipient T-cells. We acknowledge support from NIH through NCI PSON and NHGRI T32.

Lay Abstract: Cancers are difficult to treat in part due to their ability to suppress your body,Äôs natural defense against them, the immune system. Normally, your immune cells are able to identify and kill problematic cells, but cancer cells misuse a naturally-occuring ,ÄúDon,Äôt Kill,Äù signal called PD-L1 to trick the immune cells into letting them live. Interestingly, these PD-L1 signals can be delivered to the immune cells on cancerous particles, but we do not know why these particles are so powerful given that they are a thousand times smaller than cells. Here, we combined computational simulations with specialized microscopy techniques and found that the particles cause PD-L1,Äôs binding partner and other key proteins on the immune cell surface to cluster, or organize into specific patterns. These clustering patterns affect whether the immune cell will be able to properly kill a cancer cell. Together, these results support the hypothesis that clustering plays an important role in the cancerous particles,Äô suppression of immune cells.

Towards Cancer Digital Twins: Simulating Tumor Heterogeneity and Patient Response using Tissue level Agent-Based Modeling of Prostate Cancer

Sharvari Kemkar, Physical Sciences-Oncology Network (PS-ON)

Abstract: Tumors exhibit substantial heterogeneity, frequently undermining the efficacy of conventional treatments. We hypothesize that AI-enabled agent-based modeling can leverage patient multi-omics and integrate cancer systems models encoding cellular signaling with digital twins that can be used to study heterogeneity in a patient-customized tumor microenvironment. We will present Prostate Cancer Tissue ABM as proof-of-concept.

We have established a cellular systems biology model (CSBM) that predicts cell states of prostate cancer cells, customized to clinical patient cohorts. This cellular systems model informs agent (cell) growth in the ABM, which is seeded with a mixed population (sensitive and resistant cell types). Beyond the intrinsic genetic heterogeneity, we studied other contributors to tissue-level heterogeneity. Local sensitivity analysis on extrinsic factors, such as tissue packing densities (crowding), revealed statistically significant differences in tumor composition due to crowding. We also scanned cell-intrinsic properties like testosterone uptake rates, cell adhesion and motility.

We used the CSBM to construct an ML-based cell fate prediction model to reduce the computational expense of the ABM. As a straightforward check for global sensitivity of model parameters, we conducted Shapley value-based feature ranking with this ML model and identified most important species responsible for observed cell fates. We established the significance of these species by conducting Kaplan-Meier survival analysis.

Our results demonstrate the potential of multiscale ABMs and coupled data-driven methods in explaining complex tumor behaviors. We aim to create cancer digital twin models that can inform patient-specific precision tumor therapies and enhance clinical decision-making.

We acknowledge support from the NCI PSON.

Yap localization mediates mechanical adaptation of human cancer cells during extravasation in vivo

Woong Young So, Physical Sciences-Oncology Network (PS-ON)

Abstract: Biophysical profiling of primary tumors has revealed that individual tumor cells exhibit a highly heterogeneous continuum of mechanical phenotypes. A prevailing hypothesis is that a subset of tumor cells is “softer” to facilitate detachment and escape from the primary site, initiating metastasis. However, it is also postulated that cells must be able to deform and generate sufficient force to exit into distant sites. Here, we aimed to dissect the mechanical changes occurring during extravasation and organ colonization using zebrafish. Employing multiplexed methods of intravital microscopy and optical tweezer-based active microrheology, we obtained longitudinal images and mechanical profiles of cells during organ colonization in vivo. We determined that cells were softer upon exiting the vasculature but stiffened to values comparable to the new organ microenvironment. Additionally, we found that YAP-mediated mechanotransduction influenced global dissemination in our in vivo and in vitro models and that reducing mechanical heterogeneity could decrease extravasation. Moreover, our high-throughput analysis of mechanical phenotypes using real-time deformability cytometry revealed that the mechanics were partially regulated by the external hydrodynamic forces experienced by the cancer cells within capillary mimetics. Our findings indicate that disseminated cancer cells can continuously mutate, exhibiting a spectrum of mechano-phenotypes governed by YAP-mediated mechanosensing of hydrodynamic flow. This study underscores the role of mechanical adaptability in cancer cell extravasation and brain

metastasis.

Lay Abstract: Research has shown that individual tumor cells have different physical properties. Some cells are softer, which might help them break away from the main tumor and spread to other parts of the body. But these cells also need to be able to change shape and push through blood vessel walls to reach new sites. In this study, we looked at the physical changes that happen when cancer cells spread to new organs, using zebrafish as a model.

We used advanced imaging techniques and a method called optical tweezer-based active microrheology to observe and measure the mechanical properties of cancer cells as they spread in live zebrafish. We found that cancer cells were softer when they exited blood vessels but became stiffer to match the new tissue environment. We also discovered that a protein called YAP plays a role in how these cells spread and that reducing differences in cell stiffness could decrease their ability to spread.

Our high-throughput analysis showed that the external forces cancer cells experience in blood vessels help regulate their mechanical properties. This study highlights the importance of mechanical adaptability in cancer cell spread and uses zebrafish, recommended by the FDA for preclinical studies, to improve our understanding and treatment of metastatic cancer.

AXL as a driver of breast cancer dormancy
Auggie Wirasaputra, Synthetic Biology and Cancer Program (SynBio & Cancer)

Abstract: Metastatic breast cancer remains one of the leading causes of cancer-related deaths. Despite removal of the primary tumor, many patients experience a relapse event after a latency period ‚Äì a phenomenon known as dormancy. This poses a challenging issue as current therapeutics are unable to target and eliminate these dormant cells. AXL is such a potential future target. As a cell-surface receptor, AXL is overexpressed in many cancer types, and promotes drug resistance and cancer progression, however its role in dormancy is not yet known. In prior work, we developed a live cell lineage tracing method to study how the tumor microenvironment, namely the surrounding extracellular matrix composition, drives cellular dormancy in 3D. Using this same live cell lineage approach, we investigate the role of AXL in driving breast cancer cell dormancy in engineered 2D and 3D tunable environments with preliminary in vivo tissue staining validations.

Engineering LNPs with polysarcosine lipids for mRNA delivery
Diana Kang, Synthetic Biology and Cancer Program (SynBio & Cancer)

Abstract: Since the approval of the lipid nanoparticles (LNP)-mRNA vaccines against the SARS-CoV-2 virus, there has been an increased interest in the delivery of mRNA through LNPs. However, current LNP formulations contain PEG lipids, which can stimulate the generation of anti-PEG antibodies. The presence of these antibodies can potentially cause adverse reactions and reduce therapeutic efficacy after administration. Given the widespread deployment of the COVID-19 vaccines, the increased exposure to PEG may necessitate the evaluation of alternative LNP formulations without PEG components. In this study, we investigated a series of polysarcosine (pSar) lipids as alternatives to the PEG lipids to determine whether pSar lipids could still provide the functionality of the PEG lipids in the ALC-0315 and SM-102 LNP systems. We found that complete replacement of the PEG lipid with a pSar lipid can increase or maintain mRNA delivery efficiency and exhibit similar safety profiles in vivo. Broadening the therapeutic index of LNPs can be effective to be used not only for vaccine related purposes, but also for cancer treatment applications.

Investigating Tumor Microenvironment Drivers of Drug-Resistant Non-Small Cell Lung Cancer

Ninette Irakoze, Synthetic Biology and Cancer Program (SynBio & Cancer)

Abstract: Non-small cell lung cancer (NSCLC) is a global health challenge. Despite significant advances, patients succumb to NSCLC most often because the disease becomes multi-drug resistant (MDR). One of the mechanisms by which this disease attains MDR is due to factors from the tumor microenvironment (TME) that promote cell survival. Conventional 2D tissue culture methods does not capture the complexity of this TME, which limits our ability to understand how the TME promotes MDR in preclinical models and in patients. In this work, we develop 3D biomaterials and 3D spheroids to model cell-matrix and cell-cell interactions important to NSCLC in the TME. We culture drug-sensitive, drug-resistant, and therapeutic gene drive-modified NSCLC cells engineered by the Pritchard Lab, in 3D environments to understand how the TME regulates their relative growth and drug response rates. Preliminary results have revealed a striking phenomenon: drug-resistant cells are more difficult to eradicate in 3D matrigel compared to 3D collagen gel. These findings suggest that 3D cell-cell contacts between the drug-resistant cells may help them survive treatment. Our ongoing work is optimizing the ratio of the three PC9 cell populations within the spheroids to effectively eradicate the PC9 drug-resistant cells using the PC9 gene drive cells, pairing 3D environments and a mouse model. Our 3D environments will also include synthetic hydrogels engineered to mimic the diseased lung tissue's protein composition. This research has the potential to help develop more effective therapies for NSCLC patients and ultimately bridge the gap between preclinical and clinical results.

Ex vivo Investigation of post-radiation extracellular matrix evolution in glioblastoma

Alireza Sohrabi, Tissue Engineering Collaborative (TEC)

Abstract: Glioblastoma (GBM) is the most common and lethal type of brain cancers with median survival of 12-15 months. Patients' poor prognosis is due to recurrence of GBM tumors partially fueled by treatment resistance. While GBM resistance to chemotherapy (temozolomide, TMZ) has been investigated thoroughly, only few studies described how radiation therapy contributes to GBM treatment resistance and recurrence. Interestingly, 80% of tumors recur at the resection margins, where the highest radiation dosage is delivered. Among the plethora of interactions in GBM's complex microenvironment, extracellular matrix (ECM), the non-soluble component of the microenvironment, is underappreciated. GBM cells dynamically interact and remodel the tumor ECM. Recent studies suggest that treatment (radiation and chemotherapy) could potentially alters ECM secreted by GBM cells. We aimed to investigate the correlation between radiation-induced ECM changes in GBM and development of treatment resistance. Using the tissue-engineered scaffolds, designed in our lab (TE-pGBM), and patient-derived primary GBM cells, we first determined the radiation dosage at which cell growth is inhibited by 50% (GI50). Then, using RNA-sequencing, we investigated post-radiation transcriptomic changes of GBM cells, 1 week after administration of the cell line-specific GI50 dosage. While radiation alone changed the transcriptomic level of ECM molecules (e.g., collagen IV, fibronectin, etc.), combination of radiation with brain-like ECM (TE-pGBM) amplified these changes.We will assess ECM expression profile using proteomic mass spectroscopy and will confirm the candidates with western blotting.

Lay Abstract: Glioblastoma is the most common and lethal type of brain tumors. The patients' low survival is mainly due to the recurrence of the tumor and development of treatment resistance. Post-surgery the most common treatment course is radiation treatment. While radiation kills some GBM cells, the rest resist, grow back faster and seed recurrent tumors. We believe that radiation results in changes in expression level of GBM-secreted exrtacellular matrix (ECM) as well as ECM

architecture. We hypothesized these ECM changes facilitates radiation resistance, invasion and growth of recurrent tumors.