Systems Approaches to Cancer Biology 2022

Poster Presentation Abstracts

Poster session 1 on Thursday, October 20

1. *Divergent nucleocytoplasmic transport via double-negative feedback facilitates escape from DCIS-state in breast epithelia*

Presenter: Paudel, Bishal
University of Virginia

How cancer cells elicit heterogeneous responses to perturbation is poorly understood. For instance, hetero-dimerized ERBB receptors give rise to hyperproliferative, disorganized multi-acini in ~35% of isogenic breast epithelial spheroids, suggesting the phenotype is incompletely penetrant. The phenotypic organization of multi-acini resembles microscopic ductal carcinoma in situ escapees (DE), providing a useful model for studying the early stages of breast tumorigenesis. To identify the transcriptional signatures that prime cells towards DE, we randomly profiled 10-cell transcriptomes of single spheroids after 24 hours of ERBB activation, and frequency matched gene expression to the DE-phenotype. We uncovered a network of nucleocytoplasmic transport (NCT) regulators that alter the DE-phenotype penetrance. Of the regulators, CSE1L (an exportin) synergistically increases the DE-frequency when induced, while its knockdown reduces the phenotype, suggesting its functional role. Furthermore, we evaluated the significance of this result in vivo by perturbing Cse1l in Erbb2-amplified mouse mammary-gland tumors, and found that activation of Erbb2 (mimicked by ERBB-inhibitor release) alters the macroscale organization of tumors, reminiscent of DE. In contrast, other identified NCT regulators, NUP37, and KPNB1 (Importin-β) antagonize the ERBB-induced DE-frequency. To reconcile these results, we constructed a mathematical model of nucleocytoplasmic transport and observed that an activation of ERBBs leads to divergent nuclear accumulation of cargoes that depend on their mechanisms of import. Specifically, we found an elevated CSE1L inhibits an accumulation of classical nuclear-localizing cargoes that require binding with importin-α/β (KPNAs, KPNB1) complexes. Using proximity labeling with BirA*-fused with CSE1L, we identified ERBB1/ERBB2/ERBB4 as CSE1L interactors. Mechanistically, hetero-dimerized ERBB receptors interact with importin-α/β complexes and re-localize to the nucleus, corroborating previous observations of receptors' nuclear translocation. Intriguingly, we observed that Doxycycline-induced knockdown of clathrin heavy chain (CLTC, involved in intracellular trafficking of receptors via endocytosis) synergizes with ERBB activation to increase the DE-phenotype penetrance. Using ChIP-Seq, we found that ERBB1 binds to the locus of microRNA, miR205, which negatively regulates the expression of importin-α, mainly KPNA1. These results indicate that ERBB receptors translocated to the nucleus reduce the DE-phenotype by inhibiting importins via miR205, while CSE1L induction relieves this inhibition by reducing the receptor's nuclear accumulation. Together, this double-negative feedback creates a bistable population, which could explain the DE-incomplete penetrance in breast epithelia with active ERBB2.

2. *Phenotypic Models of the Evolution of Cancer Susceptibility*

Presenter: Compton, Zachary
Arizona Cancer Evolution Center, Arizona State University

Zachary Compton (Arizona State University, Tempe, AZ)
Amy Boddy (UC Santa Barbara, Santa Barbara, CA)
Walker Mellon (Arizona State University, Tempe, AZ)
Carlo C. Maley (Arizona State University, Tempe, AZ)

The last decade has brought tremendous interest in understanding what explains the variation in cancer risk across species. As data on cross-species cancer prevalence becomes widely available, we expect to be able to identify explanatory variables of the variation in observed cancer prevalence across the tree of life. Comparative phylogenetic models of trait evolution are well equipped to answer how evolution has shaped such diversity in cross-species cancer risk. We sought to identify regions of the mammalian phylogeny where significant changes in the evolutionary rate of body size and longevity are correlated with similarly high rates of evolution in cancer susceptibility. We fit several models of trait evolution to a cancer prevalence dataset containing 103 mammalian species. We found that models of trait evolution where a significant proportion of the variance in cancer prevalence is explained by non-neutral forces were best at describing the variation in cancer prevalence in our dataset. We also demonstrate how shifts in the estimated ancestral cancer susceptibility trait mirror similar shifts in the evolution of body size and longevity.

3. *Controlling Resistance in Hormone Refractory Breast Cancer using adaptive therapy*

Presenter: Seyedi, Sareh

Biodesign Institute and School of Life Sciences, Arizona State University

Sareh Seyedi (Biodesign Institute and School of Life Sciences, Arizona State University)
Ruthanne Teo (Biodesign Institute and School of Life Sciences, Arizona State University)
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The most important problems in cancer therapy are death due to acquired therapeutic resistance and therapeutic toxicity. The aim of this study was to test adaptive therapy as an evolutionary strategy in preclinical models with MCF7 cancer cell lines resistant to palbociclib and fulvestrant. MCF7 resistant breast cancer cells were injected in the mammary fat pads of NSG mice and grew orthotopically. We treated these mice with gemcitabine or capecitabine either intermittently or by adjusting their doses. We also used a combination of drugs that we applied in tandem or switched the drugs in every application (ping-pong) either intermittently or by adjusting their doses. In this experiment, first, we wanted to see if gemcitabine and/or capecitabine can control the resistant MCF7 cells and, second, compare adaptive therapy with standard therapy. We adjusted the drug dosage based on the tumor burden as observed via bioluminescence imaging twice per week. Our results showed a reduction in the tumor burden of most mice treated with adaptive therapy as well as prolonged progression-free survival. Moreover, we had several weeks off-treatment or we used lower drug doses in most of the mice under adaptive therapy strategies when the tumor burden was below the threshold.We found that in the ping-pong combination, in both intermittent and dose adjustment, the survival of mice increased significantly. In tandem combination therapy, the intermittent treatment was less successful compared to dose adjustment, however it was better than standard therapy in increasing survival. In single drug adaptive therapy, there was a great increase in survival in capecitabine dose adjustment treatment compared to both intermittent and standard therapy. In addition, there were no differences between adaptive strategies and standard therapy using gemcitabine. Overall, there was a significant survival benefit in all adaptive therapy strategies besides reducing the tumor burden. We often observed that tumors continued to shrink even after we stopped therapy. This suggests that once a tumor is under control using adaptive strategies, lower drug doses could be used to increase progression-free survival.

4. *Occult tumorigenesis establishes genetic programs of treatment resistant breast cancer*

Presenter: Snyder, Joshua
Duke University

Joshua Ginzel (Duke University, Durham, NC)
Zachary Hartman (Duke University, Durham, NC)
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The pre-diagnostic occult phase of tumorigenesis occurs over many years, if not decades. Determining how protracted latency periods shape the disease trajectory is exceptionally challenging. Dynamic adaptations to systems level programs in tumor cell behavior and the microenvironment are likely during the occult phase. Tools are needed to study this process and illuminate underlying programs in tumor cell plasticity, cell competition, and microenvironmental remodeling during the formative years of tumorigenesis that may impact treatment resistance much later. Recent observations in our Crainbow model of HER2+ breast cancer (HER2BOW) demonstrated how HER2 isoforms (WT HER2 “WT”, d16-splice-isoform “D16”, and the n-terminal truncation “95”) each have unique tumorigenic potential and associated phenotypes. Here we will show how Crainbow models can be used for systems level interrogation of the occult tumorigenic phase at the single cell level through a diversity of tissue imaging modalities – including vibratome slices, FFPE slides, whole organ imaging, and macroscopic imaging at necropsy. We use these techniques in combination with the genetic heterogeneity implicit in our model to test the hypothesis that competition of HER2 isoforms during the occult phase pre-establishes treatment resistance mechanisms in the tumor. In a series of in vivo treatment paradigms, we show that the early occult tumor programs established by d16 HER2 tumors are sensitive to front-line HER2 targeted therapy whereas p95 HER2 tumors are not. Our data demonstrate how aggressive treatment of the most proliferative tumors can select for dormant and otherwise undetected occult invasive tumors.

5. *Paclitaxel treatment partially phenocopies interferon response, and siRNA knockdown of phenocopied transcription factors slows cell line growth.*

Presenter: Calistri, Nicholas
Oregon Health & Science University

Nicholas Calistri (Oregon Health & Science University)
Laura Heiser (Oregon Health & Science University)

Background: Paclitaxel is a standard of care therapy for patients with triple negative breast cancer (TNBC); however therapeutic resistance is common and prolonged response is rare. The development of adaptive resistance is challenging to study as it often occurs concurrently with changes across a multitude of molecular pathways that are commonly activated in breast cancer.

Results: In this study we performed deep single-cell RNA sequencing (scRNA-seq) before and after paclitaxel treatment of the TNBC cell line HCC1143. Analysis of scRNA-seq profiles revealed that paclitaxel treatment resulted in cell cycle arrest and upregulation of genes related to activation of the innate immune response. Gene ontology enrichment analysis showed that paclitaxel treatment induced upregulation of pathways canonically associated with interferon response, despite evidence of interferon production. This finding is consistent with other studies which have shown that taxane therapy can activate the innate immune system via cytosolic DNA sensing pathways. We explored the relationship between paclitaxel and interferon responses by generating a second set of scRNA-seq data of HCC1143 cells treated with either Interferon-Beta or Interferon-Gamma. We found 140 genes that were uniquely upregulated during paclitaxel response, and an additional 117 genes upregulated during both paclitaxel response and either Interferon-Beta or Interferon-Gamma response. Gene ontology enrichment analysis found that the paclitaxel unique genes were related to neutrophil activation and G2/M transition, whereas the genes induced by both paclitaxel and interferon treatments were in pathways related to activation of the innate immune system in response to interferon or virus.

Chea3 transcription factor enrichment analysis identified the transcription factors most likely to mediate these responses, including the interferon response associated IRF7 and IRF9 in addition to several genes related to immediate early gene response including FOSL1, JUN, JUNB. To assess the functional importance of the most significantly upregulated transcription factors, we performed siRNA knockdown followed by treatment with either paclitaxel or vehicle control. We found that siRNA knockdown of IRF9 alone led to a significant reduction in cell number after PTX treatment as compared to control. Additionally, we found that siRNA knockdown of either ELF3 or FOSL1 resulted in significant reduction in cell number for both vehicle and paclitaxel treated cells compared to scramble siRNA control. Analysis of the 309 ER-/HER2- patients from the METABRIC cohort found that copy number amplification of ELF3 and IRF9 was relatively common (ELF3: 8.7%, FOSL1: 0.3%, IRF9: 2.6%), indicating that therapies which disrupt or specifically inhibit these transcription factors may benefit a significant number of TNBC patients . The PRECOG database, a resource listing gene association with outcome generated from meta-analysis of 18,000 human tumors, indicates that PLSCR1 and ELF3 are both significantly ($p < 0.01$) associated with poor patient prognosis in breast cancer , further supporting our findings that activation of these transcription factors offer a proliferative advantage.

Together, our analyses suggest that tumors with high expression of the transcription factors ELF3, FOSL1, and IRF9 may harbor baseline resistance to paclitaxel.

6. *Image segmentation of irradiated tumour spheroids by Fully Convolutional Networks*

Presenter: Streller, Matthias
Hochschule für Technik und Wirtschaft Dresden

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Multicellular tumour spheroids are an established in-vitro model to quantify the effectiveness of cancer therapies. Spheroids are treated with radiotherapy and their therapeutic response over time is most frequently monitored via microscopic imaging. For analysis, it is necessary to segment the spheroids in these images, to extract their characteristics like the average diameter or circularity. While several image analysis algorithms have been developed for the automatic segmentation of spheroid images, they focus on more or less compact and circular spheroids with clearly distinguishable outer rim throughout growth. In contrast, treated spheroids are usually obscured by debris of dead cells and might be partly detached and destroyed. We train and optimize two Fully Convolutional Networks, in particular UNet and HRNet, to create an automatic segmentation which covers both cases, spheroids with and without therapy. While we successfully demonstrate the automatic segmentation for one spheroid type, we plan to extent the segmentation to other spheroid models.

7. *The signaling mechanisms of AXL-mediated resistance*

Presenter: Meyer, Aaron
University of California, Los Angeles

Marc Creixell (University of California, Los Angeles, CA)
Scott Taylor (University of California, Los Angeles, CA)
Jacqueline Gerritsen (Massachusetts Institute of Technology, Cambridge, MA)
Anu Majumder (Moffitt Cancer Center, Tampa, FL)
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Receptor tyrosine kinase (RTK) inhibitors are approved, effective lung cancer therapies but are limited by the development of resistance. A major source of resistance is so-called "bypass" signaling wherein cells switch survival dependence to another RTK. The RTK AXL is frequently observed in resistant tumors and drives further malignant phenotypes such as EMT and migration. However, a complication to clarifying the role of AXL-targeted therapies is that the functional responses that are observed in vivo are poorly reflected in vitro studies. Consequently, it is unclear which signaling and functional responses to AXL activation lead to the tumor progression effects observed in vivo and, in turn, which patients would benefit from these therapies.

RTKs simultaneously activate many pathways, preventing specific pathway-to-function assignment. To separate each pathway, we generated a mutant PC9 cell line panel with copies of AXL in which each tyrosine is mutated to phenylalanine. We measured phosphorylation responses alongside cell viability, cell death, migration, and an erlotinib-induced cell clustering effect. We developed a tailored method (Dual Data-Motif Clustering) for MS-based phosphorylation measurements that probabilistically clusters phosphosites based on both phosphorylation responses and peptide sequence. A PLSR model using these clusters accurately predicted each phenotypic response, and DDMC-derived clustering improved predictions. Our results indicated phenotypic effects were primarily driven by ABL/SRC-family kinase-induced YAP activation. We validated these clusters represent ABL/SFK using kinase-selective inhibitors.

Investigating YAP activation, we observed that it is coordinately responsive to matrix, density, treatment, and AXL activation. Patient tumors from the CPTAC consortium showed that YAP targets and pathway elements are the most correlated pathway elements with AXL abundance in patients, showing that AXL activation is indeed closely tied to YAP status in vivo. AXL activation restores the erlotinib-driven loss of YAP and blocks the treatment-induced cell clustering phenotype. We hypothesize that the ability of cancer cells to survive without cell-to-cell contacts is mediated by the AXL and YAP signaling, and the sensitivity of YAP to other environmental factors provides an explanation for the divergent importance of AXL within in vivo versus in vitro studies. We are now testing this hypothesis using a microfluidic system that can load cells into pico-liter polymer vessels to maintain cell density while blocking cell contacts. Overall, the central role of YAP activation as the downstream regulatory response driving AXL-mediated resistance provides an explanation for inconsistent observations between in vitro and in vivo model systems. It also suggests markers that may help to predict which subjects will respond to AXL-targeted therapies.

8. *Life history, the immune system, and cancer prevalence across vertebrates*

Presenter: Kapsetaki, Stefania
Arizona State University

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Life history theory claims that species with slow life histories invest more in self-maintenance, such as immune defenses, than species with fast life histories. Is this true across vertebrates? And do these immune defenses protect them from cancer? We tested whether: (1) species with slower life histories have higher concentrations of immune cells; (2) females have higher concentrations of immune cells than males; and (3) immune cell concentrations are negatively correlated with cancer or neoplasia prevalence across 201 vertebrate species. After controlling for phylogenetic relatedness and multiple testing, we found that species with slower life histories have higher concentrations of segmented neutrophils ($P < 0.0001$) but lower concentrations of lymphocytes ($P < 0.0001$). Females have higher concentrations of white blood cells than males ($P = 0.0002$), and species' immune cell concentrations are not correlated with cancer or neoplasia prevalence. These results contradict previous predictions that species with slower life histories invest more in adaptive than innate immunity, but support a large body of literature on females of several mammalian and bird species having higher levels of immune cells than males. Although species' immune cell concentrations were not correlated with cancer prevalence, future research ought to explore whether this result holds when using functional assays, with the scope of better understanding and preventing cancer susceptibility across vertebrates.

9. *Siamese neural networks to quantitatively calibrate agent-based models of cancer using tumor images*

Presenter: Finley, Stacey
University of Southern California

Colin G. Cess (University of Southern California)
Stacey D. Finley (University of Southern California)

Agent-based models (ABMs) have made great advances in the study of tumor development and therapeutic response, allowing researchers to explore the spatiotemporal evolution of the tumor and its microenvironment. However, these models face serious drawbacks in the realm of parameterization - parameters are typically set individually based on various data and literature sources, rather than through a rigorous parameter estimation approach. While ABMs could be fit to time-course data, that type of data loses the spatial information that is a defining feature of ABMs. On the other hand, tumor images, while providing spatial information, represent only a single time-point, and it is exceedingly difficult to compare tumor images to ABM simulations beyond a qualitative visual comparison. Without a quantitative method of comparing the similarity of tumor images to ABM simulations, a rigorous parameter fitting is not possible. Here, we present a novel approach that applies Siamese neural networks as a form of dimensionality reduction to represent ABM simulations as a single, continuous value. This enables a quantitative comparison of tumor images and ABM simulations. We compute the similarity between ABM predictions and tumor images as a set of spatially-resolved datapoints, preserving the spatial layout of cells. This allows us to then minimize the difference between simulated and experimental images using standard parameter-fitting algorithms. We demonstrate our approach on an ABM of the tumor-immune microenvironment. More broadly, this approach can be used to analyze various types of models, accounting for complex relationships between model outputs.

10. *Using Low Tensor Rank Approximations (ULTRA) for isolating the mechanisms of dysregulated IL-10, IL-6, and IFN-Î3 signaling in breast cancer patients*

Presenter: Ramirez, Andrew
University of California, Los Angeles

Brian Orcutt-Jahns (University of California, Los Angeles, CA)
Dr. Aaron Meyer (University of California, Los Angeles, CA)

Flow- and sequencing-based single-cell techniques have enabled the detailed characterization of variation in heterogeneous cell populations. These technologies, combined with dimensionality reduction techniques, are particularly effective at identifying new cell populations with unique molecular characteristics. Several machine learning models, such as generative statistical constructs like Gaussian mixture models, have also been applied to harmonize single-cell measurements across experiments. However, it remains challenging to profile how molecular patterns of variation exist within and across cell populations upon perturbation. Patterns across multidimensional (i.e., tensor-structured) datasets, such as single-cell perturbation experiments, can be tracked using generalizations of matrix factorization methods. Building on a previously developed tool for aligning single-cell datasets, PopAlign, we recognized that the output matrices describing the population cluster abundances, centers, and variance should retain common patterns across conditions. By reducing these cluster parameters into component patterns, we can track and isolate shifts in cell subpopulations to specific conditions, clusters, and features in an unsupervised manner.

We applied our ULTRA methodology to analyze multi-parameter flow cytometry measurements of cytokine signaling response in breast cancer (BC). Breast cancer is the most common cancer diagnosed in women, accounting for more than 1 in 10 new cancer diagnoses each year. Cytokines are cell signaling proteins with crucial roles in immune system cellular communication. Both cytokine expression and immune cells' responses to stimulation are dysregulated in estrogen receptor-positive BC; uncovering the nature and sources of this dysregulation may reveal mechanisms by which BC evades immune clearance. With ULTRA, we identified patterns of cytokine response across a dataset in which peripheral blood mononuclear cells (PBMCs) from both healthy and BC patients were profiled with 14 cytokine treatments across two time points, 17 markers, and 27 cell types. We previously identified a pattern of reduced IL-6 and IL-10 responsiveness across many cell populations on the population level. We hypothesized that analysis of single cells would reveal whether this dysregulation reflects a uniform shift in cytokine responsiveness or an alteration in a subset of cells. We find that ULTRA can effectively isolate subpopulations with both unaltered and dysregulated cytokine response between healthy subjects and BC patients. The markers unique to these dysregulated cells suggest underlying mechanisms of this dysregulation.

11. *KSTAR: Overcoming Limitations of Phosphoproteomic Data to Obtain Robust Predictions of Patient-Specific Kinase Activities*

Presenter: Crowl, Sam
University of Virginia

Ben Jordan (University of Virginia, Charlottesville, VA)
Hamza Ahmed (University of Virginia, Charlottesville, VA)
Cynthia Ma (Washington University in St. Louis, St. Louis, MO)
Kristen Naegle (University of Virginia, Charlottesville, VA)

Kinase inhibitors are one of the largest classes of FDA-approved drugs for oncology, and new molecular entities targeting various kinases are continually being developed. While kinase inhibitors have been successful in improving patient outcomes for many cancers, patients often either fail to respond to therapy initially or develop resistance over the course of treatment. Current clinical diagnostic tools rely predominantly on gene or protein expression measurements, which fail to consistently capture the activity of kinases within the tumor, or phosphorylation of the kinase activation loop, which fails to account for the complexity of kinase regulation. To improve the ability to measure kinase activity within tumor samples, we developed a quantification free, graph- and statistics-based approach, called KSTAR, that utilizes single or multi-sample phosphoproteomic datasets to predict kinase activity in a bias- and error- aware manner. We demonstrated that KSTAR expands the number of phosphorylation sites available for prediction and significantly reduces experimental and network-level bias towards well-studied kinases and substrates. When compared to other currently available kinase activity inference algorithms, we found that KSTAR exhibited higher overall accuracy and reproducibility across many different phosphoproteomic datasets, particularly for tyrosine kinases, which tend to be more influenced by study bias. Finally, we illustrate that KSTAR could provide a valuable complement to current diagnostic tools by profiling tumor biopsies from breast cancer patients with variable clinical HER2 status. Kinase activity profiling with KSTAR revealed HER2- patients that could benefit from HER2 targeted therapies, as well as HER2+ patients who were unlikely to respond. These predictions were corroborated by treatment data where available. Through robust and consistent predictions of kinase activity without using quantification, KSTAR has the potential to improve clinical outcomes through better selection of therapeutic kinase targets and by expanding our understanding of treatment response and resistance to kinase-inhibitor therapies.

12. *TGFβ Ligand Discrimination and Signaling are Rewired by TGFBR3 Coreceptor*

Presenter: Fares, Wisam
University of Virginia

Wisam Fares (University of Virginia, Charlottesville, VA)
Kevin Janes (University of Virginia, Charlottesville, VA)

Transforming growth factor ß2 (TGFß2) family signaling is often dysregulated in cancers. Signal transduction from TGFß2-family ligands is complex, because each ligand competes with other ligands in the superfamily to bind to multiple heterodimerizing receptors. Recent studies show that TGFß2 ligand-receptor architecture confers distinct signal-processing capabilities. However, these studies did not consider the role of TGFß2 coreceptors, such as TGFBR3, which bind to ligands but do not transduce signals. TGFBR3 is abundant in breast tissue but is gradually lost during breast cancer progression, and is expressed variably between cell to cell. To investigate how TGFBR3 dosage impacts signal processing from competing ligands, we combined computational modeling and experiments involving different TGFß2 ligands relevant to breast epithelial cells. To the original model architecture, we added a coreceptor species that binds ligand and exchanges it with type II receptors. We identified regimes where different type I/II receptor abundances yielded completely different responses to increases in coreceptor over the same combination of ligands. To test model predictions, we stably transduced MCF10A-5E cells (with little-to-no TGFBR3 in 2D culture) with an inducible TGFBR3 to express at near-endogenous levels observed in 3D environments. Cells were induced and treated with a combination of two TGFß2 ligands, GDF11 and TGFB1, which share type I/II receptors and bind TGFBR3. We observed a context-dependent biphasic relationship between TGFBR3 abundance and downstream effector phosphorylation. Binning TGFB1+GDF11-treated cells based on TGFBR3 expression suggested coreceptor-dependent transitions similar to those of the model, which will be refined further with TGFBR3 binding affinities to be quantified.

13. *A structure-based framework to compare inter- and intra-protein contact conservation*

Presenter: Kandoor, Alekhya Abhiram
University of Virginia, Charlottesville, VA

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Saqib Rizvi (University of Virginia, Charlottesville, VA)
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Protein domains, short protein sequences that fold and perform a specific molecular function, can be found based on their conservation by sequence, structure and function. For example, SH2 domains are 100 amino acids long and bind tyrosine phosphorylated linear peptide partners (motifs), which mediates the formation of multi-protein complexes to propagate signals in transduction cascades and regulate cell differentiation and proliferation activities. SH2 domains have duplicated through evolution such that there are 120 domains in the human proteome across a diverse range of protein families. Some of these families have highly conserved full-protein architectures, such as the SRC family kinases where the SH2 domain is sandwiched between an SH3 (polyproline binding module) and the kinase catalytic domain. Other families share sub-domain architectures, such as GRB2 which shares the SH3-SH2 architecture, but has another SH3 domain instead of a kinase domain, compared to SRC family kinases. Using available structures, we wished to understand the following about the conservation of domains and the effect on protein interactions: 1) Can we identify domain-domain binding interfaces and identify how those interactions may regulate the ligand binding interface and whether post-translational modifications or mutations regulate protein fold and function through these interfaces and 2) When larger domain architectures are present across diverse proteins, are the contacts between domains conserved along with their architectures? Here, we develop a bioinformatics pipeline to systematically map the domain-domain and domain-ligand interactions across native and mutant crystal structures available from the Protein Data Bank (PDB) for SH2-domain containing structures (359 total structures on 46 protein). Architectures that are confined to a single family include proteins with SH2 domain coexisting with FERM, SOCS_box, STAT_bind, C1_1 and PTP domains and we find >50% of contact features to remain conserved within their respective families. For domain-domain architectures that exist across multiple families, we find high conservation within the family and relatively low conservation across families. The tandem SH2 (i.e. SH2-SH2 architecture) domain interactions are greater in kinases than in phosphatases and this explains the presence of conformational difference in proteins that share a common domain architecture but differ in their function. SH2 domain on the amino terminal makes significant contact with the catalytic domains such as Pkinase_tyr and PTP in kinases and phosphatases respectively. The SH2-SH3 architecture, found in adaptor proteins, kinases, and phospholipid messenger molecules, have less than 20% overlapping features across and within families. These results suggest that we can extract within-family contacts and extend these to domains family members lacking available structures. We are intersecting these extracted domain-domain contacts with the domain-ligand interface and known PTMs and mutations to hypothesize the function of regulating protein folding and interactions within signaling networks.

14. *An ODE Model to Quantify Avidity Effects in Tandem SH2 Domain Binding*

Presenter: Portelance, Reagan
University of Virginia

The interactions between SH2 domains and phosphorylated tyrosine (pTyr) residues drives many protein-protein interactions within cells and is a key regulator of signaling pathways. A small subset of SH2-containing proteins possess two unique SH2 domains (tandem SH2 domains) with their own individual affinities and preferred binding partners, and it has been shown that the avidity effects that help drive the bivalent interactions of tandem SH2 domains is instrumental in key signaling networks. For example, in the T-cell receptor signaling network the tandem SH2 protein, ZAP-70, only gets recruited to the T-cell receptor and initiates the signaling cascade if both pTyr sites in the immune receptor tyrosine-activating motif (ITAM) are phosphorylated. This observation suggests a dramatic increase in the effective affinity due to the presence of both SH2 domains and delineates the importance of these avidity effects on cell behavior. There are ten tandem SH2-containing proteins with a large number of possible bivalent configurations in signaling, and in this work we undertake a combined modeling and experimental approach to define a set of "rules" for which interactions are controlled by avidity. We used ordinary differential equations (ODEs) to efficiently model the bivalent interactions, which allows us to establish the linker lengths and individual domain affinities that give rise to the greatest avidity dependence. We found that the ODE models successfully recapitulated known bivalent interactions that demonstrate avidity such as the phosphatase PTPN11/SHP2 binding to two sites (Y627/Y659) on the GAB1 protein. They also demonstrated that the separation of the SH2 domains on SHP2 is almost exactly optimal given the distance between the pTyr sites to achieve maximum avidity. The protein-specific models were used to scan the phosphoproteome and generate a ranked list of potential binding partners that are predicted to interact with avidity. We are currently establishing an experimental system for in vitro testing of the highest ranked bivalent interactions to establish the generalizability of the approach and help identify key signaling interactions in cells that rely on bivalent avidity interactions through tandem SH2-domain recruitment.

15. *Rapid assembly and extension of network models in cancer from the information in literature*

Presenter: Ahmed, Yasmine
University of Pittsburgh, Pittsburgh, PA

Yasmine Ahmed, Graduate Research Assistant (University of Pittsburgh, Pittsburgh, PA)
Natasa Miskov-Zivanov, Assistant Professor (University of Pittsburgh, Pittsburgh, PA)

Creating or extending computational models of complex systems, such as intra- and intercellular biological networks, is a time and labor-intensive task, often limited by the knowledge and experience of modelers. Automating this process would enable rapid, consistent, comprehensive, and robust analysis and understanding of complex systems. In biology, model creation is often highly dependent on human input, it requires reading hundreds of papers to extract useful information, incorporating background and common-sense knowledge of domain experts, and conducting wet lab experiments.

Mechanistic models have been used to explain how biomolecular signaling pathways regulate cell functions. Usually, modelers start with a few seed components and their interactions to build a baseline model which summarizes the modeler's knowledge about the system. Depending on the questions to be answered by the model, the baseline model is often further extended with the information extracted from literature or obtained from the domain experts. Several machine reading engines have been developed recently focusing on biomedical literature and extracting hundreds of thousands of events from thousands of papers within hours. In order to add this information to existing models, or to build new models from it, one needs methods and tools for systematic selection of most useful information from this large machine reading output.

We present CLARINET (CLARIfying NETworks), a novel methodology and a tool for automatically and efficiently expanding models by selecting most relevant and useful information extracted from literature by machine reading. CLARINET creates an Event CoLlaboration Graph (ECLG) from the events extracted by machine readers namely Extracted Event Set (EES). Each node within the ECLG represents a distinct event extracted from literature and each edge indicates a co-occurrence in the same paper of the two events corresponding to its adjacent nodes. CLARINET then uses several novel metrics for evaluating these events individually, in pairs, and in groups. These metrics are based on the frequency of occurrence and co-occurrence of events in literature, and their connectivity to the baseline model.

We tested CLARINET on three previously published biological networks of different sizes namely the naïve T cell differentiation (T-cell) model, discrete dynamic T cell large granular lymphocyte (T-LGL) leukemia model and the pancreatic cancer cell (PCC) model with different machine reading outputs that varied in size from hundreds to tens of thousands. CLARINET was able to reproduce these manually built networks with an average recall of 0.8 and average precision of 0.7, while also identifying new interactions with high confidence, all within several seconds. By automatically extending models with the information published in literature, CLARINET allows for rapid collection of the existing information in a consistent and comprehensive way, while facilitating information reuse and data reproducibility, and replacing hundreds or thousands of manual experiments, thereby reducing the time needed for the advancement of knowledge. CLARINET is parametrizable, it allows users to select different extension criteria, depending on the context, focus and goals of their models. CLARINET is highly scalable, its average runtime is at the order of ten seconds when processing several thousand interactions, and it outperforms previously proposed methods.

16. *A computational systems approach to indirectly down-regulate master regulator activities: A case study for STAT3*

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MIT Department of Biological Engineering

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A promising avenue of cancer therapy over the past two decades has been to modulate the immune response through targeting critical proteins or genes in signaling pathways. The signal transducer and activator of transcription 3 (STAT3) is one of the master regulators of immune signaling pathways. Aberrant activation of STAT3 in cancer cells promotes tumor growth and suppresses the immune response through a variety of secreted factors. Once activated, STAT3 downregulates the production of cytokines that signal an antitumor immune response by T helper cells (Th1), as well as upregulating immunosuppressive factors. These tumor stimulating factors constitute a feedforward loop, establishing consistent STAT3 activation through communication between cancer cells and immune cells in the tumor microenvironment. In addition to inducing immune suppression, hyperactivation of STAT3 plays a role in promoting metastasis via upregulation of the pro-inflammatory ligand E-selectin. Therefore, being able to control STAT3 activity could be a critical step in advancing cancer immunotherapy.

There are two main approaches to control STAT3 activity a) Targeting the STAT3 protein directly, and b) indirectly targeting signaling proteins upstream in the STAT3 pathway. Targeting the STAT3 protein directly with small molecules has had limited success due to the protein's various forms (monomeric, dimeric, aggregation) as well as several possible post-translational modifications. Silencing STAT3 expression with oligonucleotides has shown some promise, but more work is required for optimizing bioavailability, lower toxicity, and attaining ideal pharmacological properties in vivo. Indirect inhibition of STAT3 has not been as toughly explored. Transcriptional analysis of STAT3 expressing cancers cells could help identify suitable upstream targets but it has so far primarily been used to identify STAT3 as a master regulator and for finding co-expressed genes to target in combination with STAT3, as identifying perturbations in the whole biological network to indirectly access STAT3, while achieving a desired therapeutic effect, is challenging.

Here we take a computational approach to identify gene knockdowns that induce a similar biological response as the STAT3 knockdown. These could be upstream nodes in the network or even downstream nodes with some kind of feedback loop back to STAT3. We investigated transcriptomic profiles of shRNA gene knockdown experiments, derived from the publicly available L1000 dataset, which contains over 1 million transcriptomic signatures, and searched for perturbations that simulate the effect of the STAT3 gene knockdown in the biological system. Potential therapeutic targets are identified in regulated KEGG pathways of biological processes in shRNA STAT3 gene knockdown experiments, and Gene Set Enrichment Analysis is performed for perturbations of those candidates. Then these perturbations' pathway profiles are compared with the STAT3 gene knockdown pathway profile with a pairwise GSEA-based distance, used to quantify the similarity between two signatures in their most upregulated and downregulated pathways. Finally, we can utilize LEMBAS, a recently proposed recurrent neural network model, to expand the approach by training on the available shRNA data to simulate the effect of gene knockdowns and then mechanistically explain the observed effect in the intracellular signaling network, which leads to indirect STAT3 regulation.

17. *EGFR Inhibitor Driven Cell Death in Non-Small Cell Lung Cancer*

Presenter: Porto, Sydney
Harvey Mudd College

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Nicholas Harper (University of Massachusetts Medical School, Worcester)
Michael Lee (University of Massachusetts Medical School, Worcester)

Cancer growth is driven by recurrent oncogene or tumor suppressor gene mutations, and in the case of some non-small cell lung cancers (NSCLCs), these genetic changes include activating mutations in the epithelial growth factor receptor (EGFR). Targeted EFGR inhibitor therapies have been designed to treat these cancers. However, the response of NSCLCs to EGFR inhibition is variable and not durable, meaning patients generally relapse. The factors contributing to this variability and lack of durability are not well understood. Additionally, traditional metrics for assessing drug response are imprecise and do not allow us to distinguish between cell death and growth arrest, and this contributes to our lack of understanding. In this study, we tested the hypothesis that EGFR inhibitors are not potent killers and their ability to induce death varies across cell lines. Prior research established that the EGFR inhibitor erlotinib kills PC9 (ΔE746-A750) cells, but to a low level, and it is not known if this is a common trait among NSCLC. This study selected three other NSCLC cell lines to test, H1650 (ΔE746-A750), H1975 (L858R), and H358 (Wild type), and two EGFR inhibitors, erlotinib and icotinib. To determine if the other NSCLC cells die from EGFR inhibition, we used the FLICK assay. This fluorescence-based assay uses SYTOX to estimate the number of dead cells over the course of the 72-hour assay. If there was cell death, we identified a mechanism of death using a panel of death inhibitors and flow cytometry to look for molecular markers of death. In agreement with previous results, it was determined that PC9 cells die. However, the three other cell lines experienced only growth inhibition – not cell death – in response to the drugs. One exception to this finding was H358 which showed some cell death in response to erlotinib, but not icotinib, suggesting death is not EGFR driven. Both the inhibitor screen and flow cytometry with annexin V staining point to apoptosis as the mechanism of death in PC9. To further understand how PC9 cells die from EGFR inhibition, we conducted genetic analysis using publicly available data sets to identify essential genes impacted by EGFR inhibition. And, using the Gene Ontology Resource, we identified 9 such genes which were also related to cell death and apoptosis. In summary, EGFR inhibitors do not commonly kill NSCLCs, and PC9 cells are an exception.

18. *Removal of p53 causes the mechanism of DNA-damage induced cell death to switch from apoptotic to non-apoptotic*

Presenter: Honeywell, Megan
UMass Medical School, Worcester MA

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In healthy cells, DNA aberrations are quickly sensed and repaired by the DNA damage response (DDR). The DDR orchestrates a diverse range of processes including cell cycle arrest, senescence, DNA repair, and apoptotic cell death. Many of these crucial DDR processes are controlled by p53. In response to DNA damage p53 can activate cell cycle arrest, upregulate repair genes, or destroy damaged cells by initiating apoptotic cell death. These functions contribute to p53's role as a tumor suppressor and signaling hub. However, in the absence of p53, what outcome is predicted by this model? While we might expect that p53 removal abrogates both cell cycle arrest and apoptosis, many p53-mutated cancers are still able to execute cell death in response to DNA-damaging drugs. This suggests the presence of an additional and heretofore undescribed pathway linking the DDR to cell death.

For the first time, we have shown that removal of p53 switches the mechanism of DNA damage-induced cell death from apoptotic to non-apoptotic. This mechanism switch was found to be consistent across a large panel of genetically diverse cells and different classes of DNA-damaging chemotherapeutics. Our strategy for characterizing this novel DNA damage-induced non-apoptotic death was to perform a whole-genome CRISPR screen. Genome-wide CRISPR screens do not typically identify death regulatory genes. To overcome this limitation, we devised a new experimental and computational method for calculating the drug-induced death rate of each single-gene knockout. Our genetic screens identified more than 600 genes that specifically modulate DNA damage-induced activation of non-apoptotic death. Surprisingly, our screen also revealed that DNA damage activates a mitochondrial respiration-dependent form of cell death in the absence of p53. Understanding the preferential activation of necrotic death in p53 mutant cells will be crucial for designing effective cancer treatments. p53 is mutated or dysregulated in the vast majority of cancers, and many of these patients are treated with radiation or drugs that cause DNA damage. Characterization of the cell death pathways that can be activated in p53-mutated cancers will allow aggressive strains of cancer to be more effectively targeted.

19. *Systematic analysis uncovers SYK dependency in NF1-LoF melanoma cells*

Presenter: Abecunas, Cara
University of Michigan, University of Virginia

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The loss of function (LoF) of NF1 is the third most frequent mutation that drives hyperactivated RAS and tumor growth in >10% of melanomas. While melanomas driven by BRAF-V600E/K/D mutations show consistent clinical response to MAPK-targeted therapies, tumors bearing NF1-LoF mutations exhibit inconsistent sensitivity to inhibitors of RAS-regulated pathways, such as MAPK and PI3K/mTOR. Thus, there is a need for the identification of more effective strategies to block tumor growth in NF1-LoF melanomas, for which efficient targeted therapies are currently unavailable. To search for actionable kinases in NF1-LoF melanomas, we performed a multiplex, compound screen targeting MAPK and PI3K/mTOR pathways. We identified MTX-216, an inhibitor of pan-PI3K and EGFR, to selectively kill NF1-LoF melanoma cells and block tumor growth in vivo. Interestingly, MTX-211, an analogue of MTX-216 with comparable potency against PI3K and EGFR, does not elicit an efficacious response in NF1-LoF melanoma cells. We thus set out to identify mechanisms that might determine the anti-tumor efficacy of MTX-216 in NF1-LoF melanomas using systems biology approaches. To identify genes associated with the unique mechanisms of MTX-216, we RNA-sequenced NF1-LoF melanoma cell lines (WM3918, MeWo, SKMEL113) treated for 24 h with either MTX-216, MTX-211, Trametinib (a MEK inhibitor), Pictilisib (a PI3K inhibitor), MTX-216/Trametinib combination, or DMSO in triplicate. Differential gene expression followed by Enrichr enrichment analysis was performed to compare and contrast genes regulated by the MTX-216 and -211 analogues. Genes downregulated uniquely by MTX-216 revealed SYK inhibition as the most significant kinase perturbation. We confirmed SYKâ€™s role in NF1-LoF melanoma by knocking down SYK. SYK siRNA reduced net growth rate in NF1-LoF cells and significantly improved Trametinib drug response. We systematically analyzed variation in gene expression across treatments, cell lines, and replicates by using principal component analysis (PCA). In PCA, MTX-216 scores revealed significantly downregulated genes associated with mitochondrial electron transport chain (ETC). MitoTracker mitochondrial dyes confirmed that MTX-216 and SYK siRNA reduced mitochondrial mass and function in NF1-LoF melanomas. In summary, the polyselectivity of MTX-216, which includes SYK inhibition and the consequential suppression of mitochondrial ETC, causes the efficacious activity of this compound in NF1-LoF melanoma. The discovery of SYK as a vulnerability in NF1-LoF melanomas represents an unconventional approach leveraging mechanistic, poly-pharmacology drug studies to identify novel cancer targets. Our results warrant further mechanistic investigation to better understand the role of SYK in NF1-LoF melanomas in future studies. Such studies may provide a path to exploit SYK dependency to selectively block NF1-LoF melanoma tumors.

20. A Systems-Based Approach Identifies Temporally Distinct Roles for the JNK and Erk MAP Kinase/AP-1 Pathways in Senescence Induction and Maintenance after DNA Damage

Presenter: Netterfield, Tatiana
MIT

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Despite advancements in immunotherapy and other molecularly-targeted therapies, treatment of patients with conventional DNA damaging agents remains a frontline therapy for the most cancers. Cell death is the primary outcome of cancer cells that undergo high levels of DNA damage. Cells that undergo low to moderate levels of DNA damage, however, have several possible fates, including: (1) programmed cell death, (2) DNA repair and continued proliferation, and (3) senescence, a type of long-term cell cycle arrest. Senescent cells, in particular, within the tumor microenvironment have been associated with poor clinical outcomes, due to the pro-tumorigenic functions of Senescence Associated Secretory Phenotype (SASP) cytokines, and potential tumor recurrence due to reentry of residual senescent cells back into the cell cycle. Understanding the molecular signaling mechanisms responsible for cancer cell fate choice after DNA damage is therefore critical to improve therapeutic response. Fate choice involves the molecular integration of information from several distinct signaling pathways that monitor both the external stimuli and the internal state of the cell. To study the signaling dynamics that contribute to cell fate choice after DNA damage at the single cell level, we treated osteosarcoma cells (U2OS) with various doses doxorubicin, a topoisomerase II inhibitor, for a short four-hour pulse under conditions where the cells primarily displayed cell death, senescence, or a mixture of these two fates. Automated quantitative microscopy was used to profile 19 phospho- and total proteins along with their cellular localization at six timepoints after treatment. The signaling responses were then correlated with phenotypic measurements of cell cycle distribution, apoptosis, and proliferation under the same treatment conditions using a tensor PLSR model as well as PCA analysis of the signaling matrix alone. These studies demonstrated time-dependent roles for the JNK and Erk MAP kinase pathways converging on the phosphorylation and nuclear accumulation of the AP-1 transcription factor c-Jun as a primary driver of DNA damage-induced cell senescence. These findings were further explored using small molecule inhibitors of JNK and MEK1, as well as through the use of a dominant negative c-Jun construct. These experiments showed that JNK/Erk signaling through AP-1 within the first 12 hours after DNA damage controls the senescence cell fate decision, despite the fact that the senescent phenotype itself takes days to appear. In contrast, JNK/Erk signaling through AP-1 at times greater than 12 hours is required for full expression of the SASP cytokines IL-6 and IL-8. Taken together, these studies demonstrate distinct time-dependent roles for JNK, Erk, and the AP-1 component c-Jun after DNA damage, with early signaling through these pathways regulating the senescence/proliferation fate decision, and late signaling through these pathways controlling the SASP.

21. *A Scalable, Open-Source Implementation of a Large-Scale Mechanistic Model for Single Cell Proliferation and Death Signaling*

Presenter: Erdem, Cemal
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Mechanistic models of how single cells respond to different perturbagens can help integrate disparate big data sets or predict response to varied drug combinations. However, the construction and simulation of such models have proved challenging. Our lab previously constructed one of the largest mechanistic models for single mammalian cell regulation of proliferation and death (774 species, 141 genes, 8 ligands, 2400 reactions). However, this, as many other large-scale models, was written using licensed software (MATLAB) with intricate programming structure, impeding alteration, expansion, and sharing. Here, we generated a new foundation for this model, which includes a python-based creation and simulation pipeline converting a few structured text files into an SBML-compatible format. This new open-source model (named SPARCED) is high-performance- and cloud-computing compatible and enables the study of virtual cell population responses at the single-cell level. We applied this new model to a subset of the LINCS MCF10A Data Cube, which observed that IFNg acts as an anti-proliferative factor, but the reasons why were unknown. After expanding the SPARCED model with an IFNg signaling module (to 950 species, 150 genes, 9 ligands, 2500 reactions), we ran stochastic single-cell simulations for two different putative crosstalk mechanisms and looked at the number of cycling cells in each case. Our model-based analysis suggested, and experiments support that these observations are better explained by IFNg-induced SOCS1 expression sequestering activated EGF receptors, thereby downregulating AKT activity, as opposed to direct IFNg-induced upregulation of p21 expression. This work forms a foundation for increased mechanistic model-based data integration on a single-cell level, an important building block for clinically predictive mechanistic models.

22. *Single-cell analysis of drug-induced mesenchymal transition in patient-derived GBM stem-like cells*

Presenter: Park, James
Institute for Systems Biology

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Adrian Lopez Garcia de Lomana (University of Iceland, IS)
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Glioblastoma stem-like cells (GSCs), a rare subpopulation of tumor cells that drive tumorigenesis and recurrence, exhibit a variety of phenotypes including molecular signatures corresponding to the classical, proneural, and mesenchymal GBM subtypes. In addition, GSCs are plastic and have been shown to shift from a classical or proneural subtype into the more aggressive, drug-resistant mesenchymal subtype, a change that correlates with treatment-induced phenotypic changes in recurrent GBM tumors. Such heterogeneity and plasticity complicate our ability to treat tumors effectively. Understanding the regulatory mechanisms that drive mesenchymal transitions in GSCs would improve our ability to minimize or prevent this transition from occurring, potentially improving patient care and clinical outcomes. Here, we show that in vitro treatment of a patient-derived (PD) GSC population susceptible to pitavastatin, a statin associated with anti-proliferative effects on glioma cells, can induce a mesenchymal transition and corresponding increase in resistance in surviving PD-GSCs. Conversely, identical treatment of another PD-GSC population, derived from another patient, resulted in minimal changes in the proportion of molecular subtypes or overall drug resistance in that GSC population. Using a combination of time-course, single-cell RNA-seq analysis and network inference, we developed regulatory network models and identified a coordinated sequence of regulatory events driving the drug-induced mesenchymal transition. Comparison of the regulatory network models underlying the two PD-GSC populations revealed distinct network structures and treatment response dynamics, both of which differ from previously reported regulatory mechanisms driving mesenchymal transitions in GBM. Experimental results indicated distinct TFs were associated with the response and transition in the respective PD-GSC populations. From these regulatory network models, we also identified targets for secondary drugs, which when used in combination with pitavastatin resulted in greater elimination of PD-GSCs than pitavastatin-treatment alone. In a broader context, these results highlight the context- and patient-specific trajectories along which mesenchymal transitions can occur. These results further emphasize the need to understand the non-genetic mechanisms associated with cell state transitions and may open an avenue toward the identification of new targets and therapeutic strategies aimed at preventing the unintended consequence of treatment-induced mesenchymal transitions in GBM.

23. *Investigating Dynamics of Tumor-Associated Macrophages in Triple-Negative Breast Cancer During Immunotherapy Using a Quantitative Systems Pharmacology Model*

Presenter: Wang, Hanwen
Johns Hopkins University

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Introduction: Triple-negative breast cancer (TNBC) is an aggressive subtype of breast cancer that lacks expression of estrogen receptor, progesterone receptor, and human epidermal growth factor receptor-2. Once metastatic, TNBC is incurable with a substantially lower overall survival rate compared to other types of breast cancer. Despite recent advances in targeted therapies, anti-body drug conjugates, and immunotherapy, novel therapies to overcome resistance and improve patient outcomes are needed. Therefore, new therapeutic targets are under investigation in preclinical and clinical studies, such as CD47 which is expressed by TNBC cells to inhibit phagocytosis by tumor-associated macrophages (TAMs). In parallel with clinical efforts, quantitative systems pharmacology (QSP) models are developed to make efficacy prediction and assist with clinical trial design for treatments of interest [1].

Methods: We expanded our previously published QSP platform (Wang et al., J Immunother Cancer, 2021) to include dynamics of TAMs. The model comprises of four compartments: central, peripheral, tumor, and tumor-draining lymph nodes. It is modularized to describe the dynamics of major cellular and molecular species involved in cancer-immune cell interactions, including cancer cell, antigen-presenting cell, CD8+ effector T cell, CD4+ helper T cell, regulatory T cell, myeloid-derived suppressor cells, and TAMs. Incorporated mechanisms were supported by experimental implications, and parameters were estimated based on preclinical studies of TNBC. We generated a virtual patient population by sampling values of a subset of model parameters within physiologically reasonable ranges and simulated the tumor response to nab-paclitaxel and atezolizumab, using the same dose regimen of the IMpassion130 trial.

Results: We first compared the dynamics of TAMs including relevant checkpoint interactions with preclinical observations. Particularly, with calibrated parameters, the model predicted that phagocytosis of cancer cells by TAMs can be enhanced by about eight- and two-fold by blocking CD47 and PD-1, respectively, which is consistent with experimental findings in vitro. Further, we generated around 1,000 virtual patients with T cell densities and cytokine concentrations that fall within the clinically measured ranges in TNBC tumors and conducted in silico clinical trials of atezolizumab and nab-paclitaxel. The predicted response rate and duration of response were consistent with our previously published model as well as results from the IMpassion130 trial [2]. While the model suggested that TAMs-related molecular species do not serve as predictive biomarkers for this combination regimen, it showed that checkpoint interactions such as CD47-SIRPa can suppress antitumoral response during anti-PD-L1 treatment and are thus potential therapeutic targets.

Conclusion: We added a new mechanistic module into our previously published QSP platform to investigate dynamics of TAMs in TNBC during immunotherapy. Through proper recalibration, we demonstrated that the expanded model retains its predictive power while providing a computational framework to predict clinical response to macrophage-targeted agents.

References: **[1]** Bradshaw EL, et al. Applications of Quantitative Systems Pharmacology in Model-Informed Drug Discovery: Perspective on Impact and Opportunities. *CPT Pharmacometrics Syst Pharmacol.* 2019;8(11):777-791. **[2]** Emens LA, et al. Atezolizumab and nab-Paclitaxel in Advanced Triple-Negative Breast Cancer: Biomarker Evaluation of the IMpassion130 Study. *J Natl Cancer Inst.* 2021;113(8):1005-1016.

24. Temporal and spatial topography of proliferation in cancer

Presenter: Kabraji, Sheheryar

Dana Farber Cancer Institute

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Background: Tumors are complex ecosystems where exogenous and endogenous cues are integrated to either stimulate or inhibit cancer cell proliferation. However, the nature of these complex cell cycle states, their spatial organization, response to perturbation, and implications for clinical outcomes, are poorly characterized in tumor tissues.

Methods: We used multiplexed tissue imaging to develop a robust classifier of proliferation, the multivariate proliferation index (MPI), and applied this to map proliferative and non-proliferative cells in 650 unique tumors across five cancer types. Next, we created a framework for studying cell cycle dynamics using images of fixed tissues. This framework (cell cycle difference combined with classical multidimensional scaling, ccD-CMD) is based on time inference, a computational method for modelling dynamic processes in the absence of temporal data. Finally, we used single cell RNA sequencing to probe the gene expression programs underlying cell cycle states in residual disease.

Results: The MPI outperforms single markers, like Ki67, when classifying proliferative index across diverse tumor types and reveals the proliferative architecture of tumors in situ. We find that proliferative and non-proliferative cancer cells are organized across microscopic (cell-to-cell) and macroscopic (tissue-level) scales. Both domains are reshaped by therapy, and local clusters of proliferative and non-proliferative tumor cells preferentially neighbor distinct tumor-infiltrating immune cells. We found that in primary tumors, subsets of non-proliferating cancer cells (MPI 0) expressed non-overlapping markers of quiescent cancer cells, cancer stem cells, and dormant cancer cells and were spatially segregated within the same tumor. In high-dimensional marker space, populations of proliferative cancer cells express canonical patterns of cell cycle protein markers, a property we refer to as "cell cycle coherence". Untreated tumors exist in a continuum of coherence states, ranging from optimal coherence, akin to freely cycling cells in culture, to reduced coherence characterized by either cell cycle polarization or non-canonical marker expression. Coherence can be stereotypically altered by induction and abrogation of mitogen signaling in a HER2-driven model of breast cancer. Cell cycle coherence is modulated by neoadjuvant therapy in patients with localized breast cancer. Microscopic residual tumor cells after HER2 inhibition from patients and tumor models have similarly skewed cell cycle dynamics. Single cell RNA sequencing of residual tumor cells with skewed cell cycle dynamics suggests that upregulation of the nucleolar stress program is necessary to survive HER inhibition.

Conclusions: The MPI robustly defines proliferating and non-proliferating cells in breast cancer tissues, with immediate implications for clinical practice and research. The coherence metrics capture the diversity of post-treatment cell cycle states directly in clinical samples from patients with breast cancer, a fundamental step in advancing precision medicine. More broadly, replacing binary metrics with multivariate traits provides a quantitative framework to study temporal processes from fixed static images and to investigate the rich spatial biology of human cancers.

25. Identifying drivers of aggressiveness in prostate cancer molecular subtypes

Presenter: Orman, Michael
University of Colorado Anschutz Medical Campus

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The lethality of prostate cancer (PCa) is driven by its transition from localized to metastatic disease. In recent years, several tumor profiling studies in PCa patients have revealed the molecular characteristics of both localized and metastatic PCa tumors. These studies have provided an abundance of molecular and clinical information, however, an understanding of the molecular determinants driving aggressiveness in PCa remains unclear. To address this gap, we have performed a meta-analysis that integrates genetic, transcriptomic, and clinicopathologic data across four independent PCa cohorts. Our approach begins by determining a set of common, clinically-significant alterations observed in primary PCa. This analysis revealed MAP3K7 and USP10 loss-of-function alterations as frequently occurring alterations that are associated with progression-free survival. Next, our approach compares primary and metastatic tumors harboring either USP10 or MAP3K7 alteration. This analysis identified distinct sets of genes associated with aggressiveness in patients harboring either USP10 or MAP3K7 loss. Further inspection of these genes confirmed that some have been previously linked to cancer while other genes remain unstudied. Results from this work may guide future studies of the molecular pathways regulating aggressiveness in USP10-deleted or MAP3K7-deleted PCa. Additionally, the analysis pipeline generated in this work is flexible to accommodate user-defined molecular subtypes. Thus, this work also yields a generalizable tool for identifying novel regulators of aggressiveness within molecularly-defined PCa subtypes.

26. *Exploring the metabolic landscape of pancreatic ductal adenocarcinoma cells*

Presenter: Saha, Rajib
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Andrea Goertzen (University of Nebraska-Lincoln, Lincoln, NE)
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Rajib Saha (University of Nebraska-Lincoln, Lincoln, NE)

Pancreatic ductal adenocarcinoma (PDAC) is a major research focus because of its poor therapy response and dismal prognosis. PDAC cells adapt their metabolism to the surrounding environment, often relying on diverse nutrient sources. Because traditional experimental techniques appear exhaustive to find a viable therapeutic strategy, a highly curated and omics-informed PDAC genome-scale metabolic model was reconstructed using patient-specific transcriptomics data. From the model-predictions, several new metabolic functions were explored as potential therapeutic targets in addition to the known metabolic hallmarks of PDAC. Significant downregulation in the peroxisomal beta oxidation pathway, flux modulation in the carnitine shuttle system, and upregulation in the reactive oxygen species detoxification pathway reactions were observed. These unique metabolic traits of PDAC were correlated with potential drug combinations targeting genes with poor prognosis in PDAC. Overall, this study provides a better understanding of the metabolic vulnerabilities in PDAC and will lead to novel effective therapeutic strategies.

27. Model-Based Prediction of an Effective Adhesion Parameter Guiding Multi-Type Cell Segregation

Presenter: Rossbach, Philipp

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The process of cell-sorting is essential for development and maintenance of tissues and tumor progression. In particular, deficiencies in segregation underlie cancer invasion and metastatic spreading of tumor cells. With the Differential Adhesion Hypothesis, Steinberg proposed that cell-sorting is determined by quantitative differences in cell-type-specific intercellular adhesion strengths. Those adhesive bonds can alter the intra-cellular structure and directly affect cell mobility. An implementation of the Differential Adhesion Hypothesis is the Differential Migration Model (DMM) by Voss-Böhme and Deutsch. There, an effective adhesion parameter (EAP) was derived analytically for systems with two cell types, which predicts the asymptotic sorting pattern. However, the existence and form of such a parameter for more than two cell types is unclear. Here, we generalize analytically the concept of an effective adhesion parameter to three and more cell types and demonstrate its existence numerically for three cell types based on in silico time-series data that is produced by a cellular-automaton implementation of the Differential Migration Model. Additionally, we classify the segregation behavior using statistical learning methods and show that the estimated effective adhesion parameter for three cell types matches our analytical prediction. Finally, we demonstrate that the effective adhesion parameter can resolve a recent dispute about the impact of interfacial adhesion, cortical tension and heterotypic repulsion on cell segregation.

28. Tissue-engineered bone metastasis model to quantitatively capture dynamic tumor cell heterogeneity

Presenter: Ryan, Patrick
University of Massachusetts Amherst

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Circulating tumor cells frequently land on the bone where they undergo apoptosis or lay dormant for varying periods. A subset of disseminated tumor cells (DTCs) awaken in aging and develop lethal metastasis but the underlying mechanism remains largely uncertain mainly due to the lack of relevant models. Bone remodeling becomes dysregulated in aging leading to increased osteoclastogenesis and decreased bone mass and it has been well documented that DTC growth is functionally linked to bone remodeling as bone metastasis almost always accompanies osteolytic or osteoblastic lesions. Studying the systemic interactions between cancer and bone remodeling relies on mouse models or in vitro bone chip or tissue culture plastic (TCP) assays. Mouse models are limited to study bone cancer metastasis due to inherent genetic differences between humans and mice and a lack of anatomical inaccessibility of the bone. Current in vitro assays are poor at recapitulating the ECM structure of bone, fabricated from opaque materials and not amenable to imaging studies, and expensive and burdensome to prepare. Development of new experimental bone metastasis models and algorithms for longitudinal quantitative monitoring of individual DTC fate is imperative to better understand bone metastasis.

We have developed a tissue-engineered bone model to study DTC tumor response in the bone microenvironment using a novel biomaterial, osteoid-inspired demineralized bone paper (DBP), a thin slice of demineralized bovine compact bone. Osteoblasts cultured on DBP rapidly deposit minerals and form mineralized bone tissue analogs. By layering multiple osteoblast-DBPs, we have established 3D bone models with controlled cellularity. A thin (20um) DBP slice facilitates fluorescent microscopic imaging of cells even after mineralization allowing for high content imaging based studies.

First, we simulated bone metastasis by inoculating 100-300 fluorescently-labeled tumor cells in our DBP model. Subsequent fluorescent monitoring of GFP-tumor cells allows for time-course individual tumor cell fate monitoring. Our model recapitulates the heterogeneous response of DTCs either becoming dormant, apoptotic, or proliferative in a quantitative manner. These results show significant differences from control experiments on TCP. Additionally, DTC morphology and behavior is dramatically altered on DBP vs TCP models.

Next, we emulated the aging effect of DTC growth via repeating the experiments with varying extents of osteogenic cellularity. By incorporating 3D layered bone models into our DBP assay, we showed that increased bone mass reduced DTC growth and increased DTC dormancy, indicative that our DBP models recapitulate the response of DTCs in an aged/young bone microenvironment.

Lastly, by co-culturing osteoblasts with osteoclast progenitor cells under chemical stimulation to induce osteoclastogenesis we have recapitulated the bone remodeling cycle and its subsequent effect on DTC activity.

We envision that the established bone metastasis models represent a unique platform to reproduce DTC fate with high fidelity, provide clinically relevant results, and allow for high throughput, high content, and quantitative imaging based experiments.

29. *Unified Small Cell Lung Cancer Growth Mechanisms from Multimodel Inference and Dataset Integration*

Presenter: Beik, Samantha
Vanderbilt University

Samantha Beik (Vanderbilt University, Nashville, TN)
Leonard Harris (University of Arkansas, AR)
Vito Quaranta (Vanderbilt University, Nashville, TN)
Carlos Lopez (Altos Laboratories, Redwood City, CA)

Biological studies often entail measuring amounts of biological entities (proteins, cells, genetic material) and connecting their presence with changes in a biological system, such as a cell or tumor. We can use mathematical equations, brought together within a mathematical model, to represent changes in amounts of these biological entities over time or after a stimulus. However, building a set of equations to represent a biological process is challenging, requiring choosing which biological details to include in a model and which may overly complicate it. We aim to understand tumor growth in small cell lung cancer (SCLC), to determine which behaviors of tumor cells lead to treatment resistance in this disease. There are many possible equations to include in an SCLC model, but it was unclear which ones would best represent SCLC biological processes. Thus, we generated thousands of models with different combinations of equations. We wondered which equations (thus which biological processes) were most important for the model to act similarly to real-life tumors. We compared each possible model to different tumor datasets and found that the important processes were consistent: those representing the ability of cells to change identities to better support the tumor. Further, we saw which equations in the models did not affect how well those models matched the data, indicating that different experiments are needed to determine their importance. We predict that cells in the SCLC tumor changing identities to support their population are a reason that SCLC recurs post-treatment in almost every patient.

30. Developmental Basis of SHH Medulloblastoma Heterogeneity

Presenter: Pister, Veronika
MIT

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Medulloblastoma (MB) is one of the most common malignant pediatric brain tumors. The sonic hedgehog (SHH) subtype accounts for 30% of MB cases and likely arises from mutations in granule cell precursors (GCPs), neuronal progenitors of the cerebellar cortex that differentiate into granule neurons. SHH MB is extremely heterogeneous, but it is unknown whether this heterogeneity relates the tumors' developmental origins. To investigate this question, we performed single-nucleus RNA-Sequencing on seven highly differentiated tumors with extensively nodular histology and observed malignant cells resembling each stage of granule neuron development. Using novel computational approaches, we connected these results to published datasets and found that established molecular subtypes of SHH MB are enriched for specific developmental cell types. Additionally, some genomic copy number variations are associated with specific developmental stages, and we observed distinct metabolic and histological profiles for tumors containing cells resembling late-stage granule neurons.

31. *Multiscale modeling of cancer metabolism: from single-cell metabolic flux to hundreds of cancer organoids*

Presenter: Macklin, Paul
Indiana University

Kurtoglu Furkan (Indiana University, Bloomington, IN)
Niki Tavakoli (University of Southern California, Los Angeles, CA)
Emma Fong (Ellison Institute for Transformative Medicine, Los Angeles, CA)
Kali Konstantinopoulos (Indiana University, Bloomington, IN)
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Nicholas A Graham (University of Southern California, Los Angeles, CA)
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Stacey D Finley (University of Southern California, Los Angeles, CA)
Paul Macklin (Indiana University, Bloomington, IN)

Altered metabolism is a key driver in cancer cell adaptation to microenvironmental limits and therapeutic resistance. To experimentally study this system, we developed an in vitro platform that grows hundreds of cancer organoids as they metabolically interact with each other and co-cultured cells such as cancer associated fibroblasts, along with detailed measurements of metabolite consumption. This allowed us to build and validate detailed mathematical models of single-cell metabolism using flux balance analysis (FBA) techniques. We next sought to integrate the validated metabolism model into an agent-based model of the entire 1500 mm^3 well of cancer organoids. However, this is computationally challenging: (1) FBA techniques require solving a constrained optimization problem for every cell at every time step, and (2) we must simulate diffusion of exchanged metabolites in a large well volume.

To solve these challenges, we massively explored the FBA model across broad ranges of inputs to train a deep neural network (DNN) surrogate model that rapidly and accurately approximates the FBA model, and imported the DNN into each individual cell agent. In the integrated model, cell agents consume metabolites (based on 3D diffusion solutions), evaluate the DNN metabolic model, compute biomass creation, and use this to drive cell growth and division. To simulate the large computational domain, we approximated biotransport in the fluid-filled top of the well as 1D diffusion, simulated full 3D diffusion in the organoid-filled portion of the well, and developed a mass-conserving exchange between these two numerical solutions. Taken together, these techniques allow us to dynamically simulate behavior across scales: from metabolic fluxes in individual cells, to exchange of metabolites between individual cells and organoids, to the emergent growth of an entire well of hundreds of organoids. After presenting initial results, we will highlight ongoing high-throughput multiscale investigations of virtual knockouts.

32. *Leveraging intratumor heterogeneity to uncover mechanisms of resistance in chromosome 8 amplified malignant peripheral nerve sheath tumors via spatial proteomics*

Presenter: Gosline, Sara
Pacific Northwest National Laboratory

James Pino (Pacific Northwest National Laboratory, Seattle, WA)
XIaochun Zhang (Washington University of St. Louis, St. Louis, MO)
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Sara JC Gosline (Pacific Northwest National Laboratory, Seattle, WA)

Malignant Peripheral Nerve Sheath Tumors (MPNST) are a rare sarcoma for which there are limited clinical options and a five-year survival rate that hovers at around 50%. In these tumors, chromosome 8 (chr8) amplification is seen in roughly 60% of patients and correlates with poor prognosis. Our recent work (Dehner et al.), we used single cell RNA-seq to measure the effects of chr8 amplification, which occurs at various degrees throughout the bulk tumor. However, we were unable to determine which genes on chromosome 8 lead to poor phenotype nor do we understand the mechanism by which they do so.

In this work, we seek to leverage the intratumor heterogeneity of MPNST chr8 amplification to identify the protein signaling networks that give rise to poor prognosis in chr8-amplified patients. We describe an approach that uses DNA fluorescence in situ hybridization (FISH) to quantify the chr8 amplification within individual tumors. We then overlay this information with spatial proteomics measurements via our NanoPOTS framework, which can capture ~6000 proteins per 100uM region. Together these two technologies enable comparisons of protein activity across regions with variable chr8 amplification levels.

We evaluated this approach on preliminary samples that exhibit a range of chr8 amplification from 32.5% to 44%. We then employed a computational pipeline that compared the tumor samples with low (<40% chr8 amplification) and high (>40% chr8 amplification) to identify differential expressed proteins and phosphosites. Given the limited differences between the regions, we identified 20 differentially expressed proteins and 81 differentially expressed phosphosites. Using these molecules, we constructed a mechanistic signaling network that linked these proteins and phosphosites to genes that reside on chromosome 8. This network identified upstream regulators responsible for the differences in signaling and are currently working to validate these in our MT PDX models, including NFKB1, a transcription regulator known to be involved with many gene expression events, PPBP, a known potent chemoattractant and activator of neutrophils, MMP9 (Matrix Metallopeptidase 9), which is involved with the breakdown of extracellular matrix. These proteins could contribute or be responsible for structural and signaling changes needed for cancer progression.

Going forward we plan to expand our approach to additional tumors to fully leverage the heterogeneity of MPNST to identify specific pathways activated in chr8 amplified tumors that can be targeted by existing drugs.

33. Adipocyte-origin exosomes drive EMT and metastasis in TNBC models, but only in insulin resistant or diabetic contexts

Presenter: Qiu, Yuhan
Boston University

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Andrew Chen (Boston University, Boston, MA)
Conor Ross (Boston University, Boston, MA)
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Carla S. Mazzeo (Boston University, Boston, MA)
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Naomi Y. Ko (Boston University, Boston, MA)
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Objective: Adipose tissue in Type 2 Diabetes (T2D) is inflamed. Patients with triple negative breast cancer (TNBC) and comorbid T2D have higher risks of metastasis and shorter survival. However, mechanisms that couple T2D to TNBC outcomes are unknown. Here we hypothesize that exosomes, small vesicles secreted by tumor microenvironment (TME) breast adipocytes, drive epithelial-to-mesenchymal transition (EMT), metastasis in TNBC.

Methods: Exosomes from 3T3-L1 mature adipocytes, either insulin-sensitive (IS) or insulin-resistant (IR), were characterized and quantified by NanoSight. Exosomal miRNAs were profiled using miRNA array. Murine 4T1 cells, a TNBC model, were treated with exosomes in vitro (3dy) and injected into mouse mammary fatpads. Histology and immunohistochemistry detected TME differences (angiogenesis; adipocyte number, size). Distant metastases were visualized, quantified, isolated by clonogenic assay, and analyzed by RNA seq. Differentially expressed genes were identified and pathway enrichment analysis was conducted.

Results: In primary tumors, EMT gene transcription and angiogenesis biomarker CD31 were elevated in IR group vs. control and IS groups. IR exosome-driven brain metastases showed more mesenchymal morphology. Network analysis of gene expression revealed distinct molecular signatures of proliferation, metabolism, EMT, adhesion and motility. Moreover, differential gene expression of cancer cells in brain vs. spleen revealed new targets that prevent brain metastasis. miRNA array identified key regulators like miR-let7-b differentially expressed between IS vs IR exosomes.

Conclusions: IR adipocyte exosomes modify TME, increase EMT and promote metastasis to distant organs, likely through miRNA pathways. We suggest metabolic diseases (e.g., T2D) reshape TME, promoting metastasis and decreasing survival. Therefore, TNBC patients with T2D should be closely monitored for metastasis, with metabolic medications considered.

34. *Uncovering intra-tumoral heterogeneity and mechanism of response to treatment using single-cell biclustering*

Presenter: Singh, Amartya
Rutgers Cancer Institute of New Jersey

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Jui Wan Loh (Rutgers University, New Brunswick, NJ)
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Single-cell RNA sequencing studies can uncover cellular heterogeneity and map dynamic changes during differentiation, treatment, and evolution. Yet, distinguishing between technical and biological sources of variation poses a significant challenge to appropriate and effective analysis and interpretation. Normalization of the counts and selection of the most variable and biologically informative genes are often the first steps in the analysis. Clustering is then performed to find groups of cells with similar transcriptional profiles associated with distinct cell types or states. However, regressing out presumed sources of variation and identifying gene expression signatures based on a lower dimensional representation inherently limits the examination of the underlying heterogeneity, as cells within a cluster may still have subtle but crucial differences that are not captured by traditional exclusive and exhaustive clustering methods.

We developed the Tunable Biclustering Algorithm (TuBA) for unsupervised gene expression analyses and identification of non-exhaustive and non-exclusive subsets of genes and samples. TuBA relies on the associations between the sample-sets in the top (for high expression) or bottom (for low expression) expression percentiles to infer co-expression signatures without explicitly modeling distributions. Pairs of genes with significant matches in their top or bottom sample-sets are represented graphically as pairs of nodes connected with edges that correspond to the shared samples. TuBA uses this graphical representation to identify biclusters in an iterative way.

To apply TuBA to single-cell unique molecular identifier (UMI) counts, we first propose that feature selection can be performed without explicitly accounting for systematic biological biases (such as cell size) and technical biases (such as differences in sampling depths), since these biases have a consistent impact on the variance of individual genes. We show that the application of TuBA to single-cell UMI data after the proposed feature selection process and using appropriately normalized counts uncovers co-expression signatures indicative of inter- and intra-tumor heterogeneity.

To demonstrate the utility and performance of this approach, we analyzed longitudinal samples collected from three chronic lymphocytic leukemia (CLL) patients before treatment and during ibrutinib monotherapy at weeks 2 and 24. These patients respond to treatment and lacked resistance mutations; however, they had persistent minimal residual disease (MRD) during therapy. Little is known about the transcriptomic architecture of MRD population under ibrutinib which inactivates Bruton's tyrosine kinase in the B-cell receptor (BCR) pathway. Accordingly, we observed down-modulation of genes involved in the BCR and NFκB signaling pathways as well as genes associated with proliferation and metabolism after treatment. On the other hand, we detected an up-regulated bicluster in post-treatment cells enriched with the transcriptional signature of activator protein 1 (AP-1) and mitogen-activated protein kinase (MAPK) signaling pathways. These results corroborate with the extended analysis of serial proteomic and bulk RNA sequencing data from these and other ibrutinib-treated CLL patients and suggest the relevance of up-regulated pathways in adaptation and acquisition of by-pass oncogenic signals in MRD.

35. *Modeling the heterogeneity and therapeutic response of Triple-negative breast cancer*

Presenter: Doha, Zinab

Oregon Health & Science University

Zinab Doha (Oregon Health & Science University, Portland, OR)

Triple Negative Breast Cancer (TNBC) is a heterogeneous disease that is characterized by its distinct molecular profile, aggressive nature and lack of targeted therapies. Reasons for this aggressive phenotype are currently the focus of intense research, but progress has been slow due to the lack of proper TNBC model systems. We have generated a novel genetically engineered mouse model by combining MYC upregulation and PTEN loss (Myc;Ptenfl). We demonstrate that this new genetic model develops spontaneous mammary tumors that closely resemble the heterogenous microenvironment of human TNBC in several key aspects as evidenced by histology, immunohistology, multiplex imaging and single-cell RNA sequencing (scRNA-seq) analysis. This MycPtenfl model provide a unique tool for assessing the spectrum of patient triple-negative tumor behavior and drug response. scRNA-seq analysis reveals Myc;Ptenfl with MYC high tumors upregulate oxidative phosphorylation and homologous recombination (HR) DNA repair pathways, causing resistance to DNA damaging agents such as PARP inhibitors (PARPi). We found by multiplex imaging and scRNA-seq analysis that inhibition of MYC Serine 62 phosphorylation using small-molecule activators of PP2A in combination with PARPi, induced downregulation of not only MYC but, also HR genes with increased DNA damage leading to subsequent synergistic lethality in Myc;Ptenfl TNBC tumors. Notably, combination therapy synergy led to an increase in anti-tumor lymphoid to immunosuppressive myeloid cell ratios, which further induced synergistic growth inhibition, providing an immune escape mechanism induced by MYC and DNA repair. Our results show how dampening MYC oncogene addiction can leverage cancer cell sensitivity to PARPi, and provide a basis for expanding the use of PARPi beyond BRCA-mutant breast cancers.

36. Investigating upstream mechanisms of NPEPPS-mediated drug resistance in muscle-invasive bladder cancer

Presenter: Feldman, Lily Elizabeth

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The standard of care for eligible patients with muscle-invasive bladder cancer (MIBC) is cisplatin-based neoadjuvant chemotherapy followed by radical cystectomy. However, platinum-based treatments leave up to 70% of MIBC patients with residual disease, and the 5-year survival rate for these individuals is less than 30%. Understanding cisplatin therapy resistance mechanisms will improve MIBC treatment by developing chemotherapy response biomarkers and new MIBC precision medicine strategies. We previously identified NPEPPS, an M1 aminopeptidase, as a novel mediator of platinum drug response through its regulation of volume regulating anion channels (VRACs) which control platinum drug import. We have shown that NPEPPS is upregulated across 5 cisplatin resistant human MIBC cell lines and that increasing NPEPPS expression alone increases cell viability under cisplatin treatment. We have also shown that loss of NPEPPS expression from resistant cell lines is sufficient to restore normal levels of platinum uptake and improve sensitivity. These findings point to NPEPPS as a novel therapeutic target to improve platinum-based drug response rates in patients with MIBC, but the mechanisms driving increased NPEPPS expression in response to platinum-based drugs remain unknown. We recently used an integrative bioinformatics approach to generate and prioritize a list of transcription factors (TFs) which may be cisplatin-responsive to induce differential NPEPPS mRNA expression. The next steps in this investigation will be to use siRNA to suppress each of the top ten prioritized TFs, then treat with cisplatin and measure whether the normal induction of NPEPPS is blocked in the context of TF KD. These results will provide a novel characterization of cisplatin-induced transcription factor activity in MIBC cells and improve our understanding of the upstream regulatory pathways controlling NPEPPS expression in the context of cisplatin treatment.

37. Computational methods for identifying treatment-related genomic alterations

Presenter: Tran, Thinh N.

Gerstner Sloan Kettering Graduate School, Memorial Sloan Kettering Cancer Center, New York, NY

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Nikolaus Schultz (Memorial Sloan Kettering Cancer Center, New York, NY)

Cancer's ability to continuously evolve in response to the selective pressure of treatment continues to challenge the efficacy of targeted therapeutic regimens. Multiple secondary alterations are known to emerge after treatment with targeted and endocrine therapies, which confer resistance and result in disease progression. These treatment-related genomic changes have been characterized in multiple cancer types for different treatments, mostly in studies focusing on one cancer type and at the gene level. As pre- and post-treatment clinical sequencing becomes routine, there emerges the need for a computational pipeline to systematically compare untreated to treated samples and identify important treatment-related alterations, which could then inform future therapy choices. To identify alterations enriched in cohorts of post-treatment samples and minimize confounding effects from comparing samples from different patients, we adopted a difference-in-differences (DiD) model, which creates a quasi-experimental setup to compare changes in alteration frequencies of cancer genes over time. Specifically, by comparing treated samples to control groups of untreated primary and metastasis samples, DiD can reduce the confounding effects of time-related factors, e.g. genetic drift, on changes in alteration frequencies and thus attribute changes we see in post-treatment samples to a specific treatment of interest. We applied our model on genomic and treatment data for patients in the MSK-IMPACT clinical sequencing cohort, incorporating untreated samples from TCGA datasets of corresponding cancer types as treatment-naive controls. The model accurately identifies known resistance mutations in lung, breast and prostate cancers, as well as enrichment of alterations in multiple genes within the PI3K/RTK/RAS, TP53 and MYC pathways in lung and breast samples after treatment with EGFR tyrosine kinase inhibitors and hormone therapy, respectively.

38. A Life History Framework of Therapeutic Resistance

Presenter: Richker, Harley
Arizona State University, Arizona Cancer Evolution Center

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Evolutionary life history theory explains species-level trade-offs between survival and reproduction. Recently, the life history framework has been expanded to understand similar trade-offs at the cellular level in cancer cell phenotypes. Heterogeneity in mutational profiles across tumor cells allows for variation in cellular traits such as replicative rate and resistance to apoptosis, both of which are prognostically significant. Utilizing a pathway analysis on NanoString data from a cohort of murine adaptive therapy trials we sought to investigate how the modality of treatment shifted the enrichment of genes associated with cell survival and reproduction. The tempo, dose, and duration of cancer therapy should select for specific cancer cell life history traits that are dependent on the ecological shifts presented by these various regimes. We seek to determine how variation in the timing and dose of chemotherapy agents can serve as a selection pressure towards one end of the life history spectrum. Our aim is that by understanding cancer cell life history through studying expression profiles, we can inform the evolution of future adaptive therapy protocols.

39. Identifying and characterizing aggressive prostate cancer subtypes

Presenter: Yette, Gabriel

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Identifying genetic perturbations that promote aggressive prostate cancer (PCa) is the first step to improving patient outcomes. This can help discern indolent from aggressive PCa subtypes and predict what subtypes will be susceptible or resistant to treatments. Because PCa cells largely remain dependent on androgen receptor (AR) signaling, androgen deprivation therapy and anti-androgens are commonly used treatments. However, cells develop resistance to these treatments. One aggressive PCa subtype of focus involves the co-loss of genes MAP3K7 and CHD1. Cells modeling this subtype proliferate more in the presence and absence of androgens. In addition, these cells exhibit increased AR signaling and resistance to the anti-androgen, enzalutamide. One mechanism for enzalutamide resistance is through expression of constitutively active AR-V7, which arises from alternative splicing of AR RNA and lacks the enzalutamide target region. We found that MAP3K7/CHD1 knockdown cells indeed had increased AR-V7 expression, thereby promoting increased AR signaling, enzalutamide resistance, and PCa cell survival. Interestingly, we found patients with low expression of MAP3K7 had increased expression of splicing factors compared to patients with high MAP3K7 expression. We hypothesized that this increase in splicing factors could contribute to enzalutamide resistance by generating more AR-V7 transcripts. Transient knockdown of U2AF2, previously linked to AR-V7 splicing, resulted in re-sensitizing MAP3K7/CHD1 knockdown cells to enzalutamide. Current efforts are aimed at identifying downstream targets of AR-V7 that contribute to aggressiveness and conducting a CRISPR/Cas12a dull-knockout screen to identify PCa subtypes associated with commonly lost regions of chromosomes 8p and 16q.

40. A multiscale agent-based in silico model of metastatic cancer migration and invasion through a remodeling extracellular matrix

Presenter: Benson, Temitope

Prof. Ashlee N. Ford Versypt

Temitope Benson (Department of Chemical and Biological Engineering,
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University at Buffalo, The State University of New York)

Ashlee N. Ford Versypt (Department of Chemical and Biological Engineering,
Core Faculty, Institute for Computational and Data Sciences,
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The spread of cancer cells from a localized tumor mass in one part of the body to another is known as metastasis. It plays a crucial role in cancer-related death in cancer patients and reducing the efficacy of cancer treatment. Cancer cells in a tumor mass interact with one another as well as their local tumor microenvironment, particularly the extracellular matrix (ECM), during metastasis. The ECM undergoes structural remodeling of biochemical, physical, and mechanical characteristics because of this interaction. Cancer cells exhibit different mode of migration and invasion properties which includes single and collective migration modes.

Single and collective cancer cell migration from the tumor is influenced by this structural remodeling. The processes and techniques that produce these cancer cell migratory characteristics are still unknown. Our group used the free open-source software CompuCell3D to create a computer model that mimicked in vivo cancer cell movement across the ECM during structural remodeling. Here, we discuss how we used in vitro migration tests for varied ECM collagen fiber concentrations and pore diameters to evaluate phenotypic changes from single cell to collective cell migration to validate this model. We also investigate the impact of cell adhesion. Chemotaxis-induced cancer cell motility is also investigated and quantified. The cancer cells are represented as discrete agents in our model, and the ECM components, including collagen fibers and remodeling enzyme(s), are modeled using a system of partial differential equations. The goal is to be able to validate the in vitro model and then go on to in vivo investigations to provide cancer metastasis prediction capacity.

41. *Reconstructing the oxygenation landscape of bladder tumors in mice.*

Presenter: Ojwang, Maureiq
Moffitt Cancer Center

Maureiq Ojwan' (Moffitt Cancer Center)
Katarzyna Rejniak (Moffitt Cancer Center)
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The tortuous tumor vasculature and irregular cellular architecture can cause heterogeneities in tissue oxygenation resulting in multiple normoxic and hypoxic regions. Hypoxia induces more aggressive tumor cell phenotypes and suppresses functionality of immune cells; thus it is important to investigate the cellular composition of these hypoxic regions. In collaboration with immunology lab, we analyzed twelve histology samples of mouse bladder tumor treated either with T cell adoptive therapy, gemcitabine chemotherapy, or combination of both, in addition to an untreated control.

For mathematical simulations, we developed an in-silico hybrid agent-based model with Michaelis-Menten kinetics for oxygen cellular uptake and a constant influx of oxygen from the vasculature. The model uses histology images digitized with Aperio software to segment the individual tumor, immune, and stromal cells, as well as blood vessels. These form the base for all subsequent simulations. First, the Clark-Evans test and Ripley's K analyses were used to determine the spatial patterns of clumped vs. dispersed cells and vessels. Next, the equilibrium tissue oxygenation was simulated for each histology to determine the distributions of oxygenated vs. hypoxic tumor and immune cells. In particular, the distributions of infiltrating immune cells: CD8+, CD4+, and myeloid-derived suppressor cells (MDSCs) in untreated vs. treated tumors were examined for both tumor and nontumor regions. In general, there were more hypoxic cells in the tumor regions than in nontumor regions across different treatments and across tissues sizes. All tissues had fewer hypoxic cells in the nontumor regions as excepted for normal vasculature, except the large tissues with the combined treatments. The small tissues with the combined treatments had well-oxygenated CD8+ cells; the others had a similar trend of hypoxic cells. Also, the small tissues treated with adoptive T cell therapy had well-oxygenated MDSCs compared to gemcitabine and untreated tumors.

These cell-scale simulations based on histology images are the first step in correlating the oxygen distribution patterns of bladder tumor-T cell infiltration potential. In the future, this approach will be combined with acidity and glucose maps to identify tumor niches of specific phenotypes from metabolic landscapes.

42. Cancer-associated fibroblasts promote epithelial-mesenchymal transition in pancreatic ductal adenocarcinoma

Presenter: Kowalewski, Karl
University of Virginia

Karl Kowalewski (University of Virginia, Charlottesville, VA)
Matthew Lazzara (University of Virginia, Charlottesville, VA)

The pancreatic ductal adenocarcinoma (PDAC) microenvironment is richly populated by cancer-associated fibroblasts (CAFs), which actively participate in tumor progression. There are at least two CAF subtypes: myofibroblastic CAFs (myCAFs), which remodel the stroma, and inflammatory CAFs (iCAFs), which secrete cytokines and growth factors. We hypothesize that iCAFs promote epithelial-mesenchymal transition (EMT), a cell process that occurs early in PDAC metastasis and promotes chemoresistance. To investigate this possibility, we worked with cell culture models of PDAC and analyzed publicly-available patient datasets. CAF-conditioned medium generally induced EMT in PDAC cell lines, and this effect was enhanced when CAFs were treated with ILα, which promotes the iCAF phenotype. Moreover, iCAF-conditioned medium promoted MAPK signaling in neoplastic PDAC cells that was required for EMT. Analysis of publicly available single-cell RNA sequencing datasets nominated CAF-tumor cell paracrine interactions to investigate and further suggested that hypoxia, a ubiquitous feature of PDAC tumors, promotes the iCAF phenotype. Cell culture studies confirmed that hypoxia promotes an iCAF phenotype, raising the intriguing possibility that hypoxia could cooperate with CAFs to promote EMT in PDAC.

43. Analysis of Single-cell Transcriptomics to Investigate the Role of the Microenvironment on Small Cell Lung Cancer Phenotypic Transition and Subtype Composition using 3D organoids

Presenter: Omokehinde, Tolu

Vanderbilt University

Tolu Omokehinde, Ph.D, Amanda Linkous, Ph.D

Small Cell Lung Cancer (SCLC) is a highly aggressive, neuroendocrine tumor. Traditional reductionist approaches have proven ineffective to ameliorate the dismal outcomes for SCLC – survival at 5 years remains less than 5%. Accumulating evidence indicates that, contrary to previous textbook knowledge, virtually every SCLC tumor is comprised of multiple subtypes. A major obstacle to improving treatment is that SCLC tumor cells disseminate early, with a strong propensity for metastasizing to the brain. In addition to the brain, clinical evidence has demonstrated SCLC tumors can metastasize to the liver, bone, and various other secondary sites. Previous work in our lab utilized innovative and mathematical modeling tools, to identify 5 SCLC subtypes while demonstrating that certain SCLC subtypes have higher plasticity suggesting they may transition between other phenotypes. This represents a significant advancement in the field of SCLC tumor subtype and phenotypic identification. It has been suggested that SCLC can phenotypically transition and may be dependent on environmental cues, potentially leading to drug tolerance. To address the role of microenvironmental changes in SCLC disease progression, we have developed 3D human embryonic stem cell-derived lung and brain organoids. Here, we demonstrate the advantages of lung organoids as a useful model to recapitulate primary disease, cerebral organoids as a platform for SCLC brain metastasis, and assessment of subtype interactions when coupled, to longitudinal monitoring by high-content imaging or high-throughput omics data generation. These models will allow us to study how the lung and brain microenvironments may affect subtype and phenotypic transition within SCLC.

44. Mapping intercellular communication networks with scRNAseq in a mouse model of HER2+ breast cancer reveals unique signaling networks associated with tumor metastases

Presenter: Acharya, Chaitanya R.
Duke University

Chaitanya Acharya (Duke University)
Josh Ginzel (Duke University)
Kim Lyerly (Duke University)
Joshua Snyder (Duke University)

Background and Hypothesis: HER2+ breast cancers constitute 20% of all primary breast tumors, and are mostly treated with a combination of chemotherapy and HER2-targeted monoclonal antibody trastuzumab despite significant rates of primary or secondary therapeutic resistance. HER2 is most commonly observed as wild type, and two other isoforms, namely d16 and p95. We previously described how d16 and p95 tumors have distinct developmental trajectories. We hypothesize that distinct cellular mechanisms drive intra-tumoral heterogeneity between d16 and p95 tumors.

Methods and Results: To test our hypothesis, we analyzed single-cell transcriptomes of ~50K cells from 15 tumors obtained from Cancer rainbow mice. While we have previously shown that d16 HER2 tumors are more proliferative and less invasive than p95 HER2 tumors (which are slightly more immunogenic), we theorized that mapping cell-cell interaction networks will shed more light on the cellular heterogeneity and invasive potential of p95 HER2 tumors. Using receptor ligand interaction network of single cells, we could infer cellular cross-talk between various cell types (epithelial, stromal fibroblast and immune cell compartments) in d16 and p95 HER2 tumors. Our analysis revealed 9,031 ligand-receptor interactions in p95 tumors compared to 5,126 in d16 tumors, with maximum interactions happening between stromal fibroblasts and epithelial cells. Network centrality analysis also revealed an uptick in cellular communication between immune cells and epithelial cells within p95 tumors especially, epithelial cells communicating with regulatory T cells (Tregs). In a comparative analysis of p95 and d16 signaling networks, several pathways such as WNT signaling is upregulated in p95 HER2 tumors. Further analysis of inferred WNT signaling pathway revealed relative contribution of individual ligand-receptor pairs such as Wnt7b-(Fzd7+Lrp5), Wnt4-(Fzd7+Lrp6) and Wnt10a-(Fzd7+Lrp6). We then employed a pattern recognition method based on non-negative matrix factorization to identify global communication patterns in p95 and d16 HER2 tumors. We identified four specific communication patterns that connect cell groups with signaling pathways. The first pattern is dominated by tumor epithelial cells, which are associated with pathways such as WNT and CEACAM. The second pattern is dominated by myeloid-derived cells such as macrophages and neutrophils. These patterns are associated with pathways such as VCAM. The third pattern is dominated by lymphoid cells such as Tregs and CD8 T cells, and the pathways associated with this pattern include IL2, CD137, PDL1 and PDL2. The final pattern is dominated by stromal fibroblasts and is associated with PTPRM. Even though, both p95 and d16 tumors exhibited the same global communication patterns, the pathways associated with each pattern were different. Additionally, we plan to explore transcription factor communication network using single-cell regulatory network and inference techniques adopted by SCENIC activity approach. This investigation, hopefully, will elucidate transcription factor regulons that drive the main differences between p95 and d16 tumors.

Conclusions: Building on our previous work which described distinct cancer trajectories of p95 and d16 tumors, we constructed cellular signaling networks that attempt to distinguish p95 from d16 tumors. We identified distinct signaling pathways that potentially describe p95 tumor invasiveness. Similar differences are being currently explored in human HER2+ breast tumors.

45. Systems-Mechanobiology of Cancer

Presenter: Spill, Fabian
University of Birmingham, UK

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Jorge Escribano (Zaragoza, Spain),
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Roger Kamm (MIT, MA),
Jose Manuel Garcia-Aznar (Zaragoza, Spain),
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Experimental biologists study diseases mostly through their abnormal molecular or cellular features. For example, they investigate genetic abnormalities in cancer, hormonal imbalances in diabetes, or an aberrant immune system in vascular diseases. However, many diseases, including cancer, are also characterised by a mechanical component which is critical to their deadliness. Most notably, cancer typically kills through metastasis, where the cancer cells acquire the capability to remodel their adhesions and to migrate. Solid tumours are also characterised by physical changes in the extracellular matrix – the material surrounding the cells. While such physical changes are long known, only relatively recent research revealed that cells can sense altered physical properties and transduce them into chemical information. An example is the YAP/TAZ signalling pathway that can activate in response to altered matrix mechanics and that can drive tumour phenotypes such as the rate of cell proliferation.

Systems-biology models aim to study diseases holistically. In this talk, I will argue that physical signatures are a critical part of many diseases and therefore, need to be incorporated into systems-biology. Crucially, physical disease signatures bi-directionally interact with molecular and cellular signatures, presenting a major challenge to developing such models. I will discuss the example of how blood vessel cells interact mechano-chemically with each other to regulate the passage of cancer cells during metastasis. I will show a series of computational models that have led to insights into the intricate interplay of mechanical and molecular factors in regulating endothelial and cancer cell biology.

46. Boolean model of the Epithelial Mesenchymal Transition linked to mechanosensing, contact inhibition and growth signaling charts the context-dependence of biomechanically triggered EMT and MET

Presenter: Regan, Erzsébet
The College of Wooster

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Emmalee Sullivan (Ohio University, Cleveland, OH)
Marlayna Harris (Stanford University School of Medicine, Stanford, CA)
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During the emergence of carcinomas, loss of contact inhibition leads to proliferation within intact monolayers, as well as a weakening of cell-cell junctions critical for invasion into the surrounding tissue and migration leading to metastasis [R]. This transformation from epithelial to invasive and migratory mesenchymal phenotype is called the Epithelial to Mesenchymal Transition (EMT). The reverse mesenchymal-epithelial transition (MET) often aids the settling of migratory cells into their distant metastases. As metastatic cancers account for 90% of cancer deaths worldwide, the search for effective therapeutic approaches led to substantial development of predictive models dealing with the complex interaction between contact-mediated signaling, engagement of the EMT transcriptional program, and its reversal during MET. While current models focus on biochemical triggers of EMT such as TGF-Î2, the importance of the biophysical microenvironment in controlling EMT is demonstrated by experiments that include full EMT in the absence of any biochemical transforming signal on stiff nano-patterned ECMs, mechanical stress-induced or cell-shape dependent EMT transcription factor induction, and stiffness-dependent apoptosis vs EMT in response to TGF-Î2. These effects are outside of the scope of published models to date. To address this, we built a large modular Boolean network model of EMT triggered by biomechanical and growth signaling crosstalk, linked to a published network of epithelial contact inhibition, proliferation, and apoptosis. Our model reproduces the ability of the core EMT transcriptional network to maintain distinct epithelial, hybrid E/M and mesenchymal states, as well as EMT driven by mitogens such as EGF on stiff ECM. We also reproduce the observed lack of stepwise MET that does not pass through the hybrid E/M state, as well as the need for strong autocrine TGFβ signaling to maintain a mesenchymal state in the absence of mitogens, on softer matrices, or at high cell density. Finally, our model captures the inhibitory effects of TGFβ on proliferation and anoikis resistance in mesenchymal cells, as well as its ability to trigger apoptosis on soft ECM vs. EMT on stiff matrices. In addition, we offer experimentally testable predictions related to the effect of neighbors on partial vs. full EMT, the tug of war between mitosis and the maintenance of migratory hybrid E/M states, as well as cell cycle defects in dynamic, heterogeneous populations of epithelial, hybrid E/M and mesenchymal cells. Our model serves as a stepping-stone towards modeling the effects of the biomechanical environment on cancer cell stemness linked to the hybrid E/M state, as well as the mutually inhibitory crosstalk between EMT and senescence. As these two phenomena coexist in damaged or aging epithelia as well as tumors, testing and then expanding our modular model is a key step towards building detailed models both tumor progression and wound healing in the context of an aging tissue.

47. Ascertainment of non-coding genes as key molecular players in cervical squamous cell carcinoma through the systems biology approach

Presenter: Thippana, Mallikarjuna
University of Hyderabad

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Manimaran P (University of Hyderabad, Hyderabad, India)
Vaibhav Vindal (University of Hyderabad, Hyderabad, India)

Background: Cervical cancer (CC) is the primary cause of mortality among women in developing countries. Preventing cervical cancer is partially possible by early vaccination against the human papillomavirus, the most common cause of the disease. Nevertheless, it is imperative to understand the genetics of the disease progression to develop new therapeutic strategies. With the advent of NGS technologies, a vast amount of biological data for cancers can be used to extract meaningful insights. The Cancer Genome Atlas project provides multi-omics profiling datasets for each type of cancer under the study. We performed integrative network analysis to explore competing endogenous RNAs (ceRNAs) as potential candidate drivers for cervical squamous cell carcinoma.

Objective: The present study aims to identify potential genes and pathways associated with cervical carcinoma progression through integrative networks and systems biology approach.

Methods: Differential gene expression patterns (i.e., protein-coding and non-coding genes) were filtered by differential expression analysis on transcriptomic profiling data of cervical cancer and normal cervix tissue. Dysregulated PPI network analysis on differentially expressed protein-coding genes results in key hub genes regulating the network. The key hub genes were validated by the SVM model. However, non-coding genes play a significant role in regulating protein-coding genes resulting in the progression of the disease and hence the interactions between the key genes and their target miRNA and lncRNA were studied with the help of integrative network analysis. Further, functional enrichment and survival analysis were performed to understand their role in disease progression.

Results: In this study, we analyzed cancer-specific expression networks, i.e., differentially expressed lncRNA and their target microRNA network & differentially expressed microRNA and their target hub mRNA networks which were identified via differential expression and gene co-expression network analysis on transcriptomic data of cervical cancer. Subsequent regulatory network analysis on each network separately identifies key genes in the network that have a crucial role in regulating the network, i.e., regulation of disease progression. It is found that microRNA (miR-184) is present in both target networks and involved in several types of cancer by playing a role in regulating gene expression. Network analysis of DElncRNA – target DEmiRNA network shows that KCNQ1OT1, as a hub long non-coding RNA plays an important regulatory role in transcription of target genes via epigenetic modifications. Also, DEmiRNA – hub DEmRNA regulatory network analysis results in hsa-miR-637 as hub micro-RNA has a role in posttranscriptional regulation of target gene expression. Further, the competing endogenous RNAs (ceRNAs) network clearly shows the relationship between lncRNAs and miRNAs. These genes are mostly enriched in biological processes of cell division, mitotic nuclear division, cell cycle checkpoint and cell proliferation in gene ontology analysis. The KEGG pathway enrichment analysis of the proteins lists them as mainly associated with the cell cycle. We also performed a survival analysis to check the potential candidate's prognosis for their overall survival in cervical cancer patients.

Conclusions: Our findings provide key insights into the regulatory role of lncRNA and miRNA via cancer-specific expression networks in the pathogenesis of cervical cancer. Further, they can be validated for the development of diagnostic markers and therapeutic studies.

Keywords: Cervical cancer, Non-coding genes, ceRNA, Candidate drivers, Regulatory networks and Support vector machine learning algorithm

48. Potential role of micro RNAs in pancreatic cancer manifestation

Presenter: Kundu, Atreyee
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Cancer cells are different from normal cell in regard to phenotypic and functional expression. Cancer is the outcome of aberrant gene expression affecting various cellular signaling pathways. Micro RNAs (MIRs) are small, non-coding RNAs regulating the expression of various protein coding genes post-transcriptional and are known to play critical roles in the complicated cellular pathways leading to cell growth, proliferation, development and apoptosis. MiRs are involved in various cancer related pathways and function both as tumor suppressor and cancer causing genes. There is need of significant biomarkers and better prognostication of response to particular treatment and liquid biopsy could be useful to appraise such potential biomarkers. This review has focused on the involvement of anomalous expression of miRs in human pancreatic cancer and the investigation of miR-based biomarkers for disease diagnosis and better therapeutic selection.

49. AKT inhibition reduces cell motility in CD90+ Hepatocellular Carcinoma

Presenter: Lifferth, Jonathan
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Background: Hepatocellular Carcinoma (HCC) is typically characterized by elevated expression of EGFR and EphA2. Additionally, HCC cell lines can be classified into three subtypes based on the presence of cancer stem cell markers: EpCAM+ (epithelial, tumorigenic), CD90+ (mesenchymal, metastatic), and EpCAM-/CD90- (neutral) [1, 2].

Abstract: Using PySB [3] and PyDREAM [4], we constructed a mathematical model of EGFR and EphA2 signaling pathways in HCC. Model parameters were based on time-series reverse phase protein assay (RPPA) data measuring concentrations of molecules in the EGFR/EphA2 signaling pathways including phosphorylated and unphosphorylated forms of EGFR, EphA2, AKT, and others. This model showed asymmetrical signal dependence on AKT in CD90+ cell lines. This indicates that metastasis of HCC cell lines (measured as cell speed) is more dependent on AKT activity in CD90+ cells than in EpCAM+ cells. To evaluate the effect of an AKT inhibitor (MK2206) on cell speeds between the two subtypes, bright field microscopy images were captured of two HCC cell lines (1 EpCAM+ and 1 CD90+) at 3 concentrations (0 uM, 1 uM, and 2.5 uM). Additionally, to compare cell speeds without treatment, bright field microscopy images were captured for six HCC cell lines (4 EpCAM+ lines and 2 CD90+ lines) over 48.5 hours at 30 minute intervals. Images were segmented using iLastik [5]. Cell coordinate identification and cell tracking were performed with TrackMate, an ImageJ plugin [6].

Kernel density estimates (KDE) of the distribution of mean cell speeds for all cell lines found that certain cell lines experience significant variability between experiments. This variability was quantified by calculating and comparing Kolmogorov-Smirnov values for mean cell speed distributions between each experiment for each cell line. KDEs were also used to compare the JHH6 (CD90+) and JHH7 (EpCAM+) cell lines under varying concentrations of MK2206. These KDEs demonstrate a shift in cell speed distribution towards lower speeds in the JHH6 cell line with increasing concentration of MK2206. No clear trend is observed for JHH7. Linear regression between cell mean speeds identified a reduction of cell speed in the JHH6 cell line ($P=0.01$) not in the JHH7 cell line ($P=0.16$). Mean cell speed in JHH6 cells decreased by 10.3% at 1-¼M and 17.7% at 2.5-¼M.

Conclusion: Here we demonstrate that AKT inhibition with MK2206 reduces cell motility in CD90+ HCC cells and not in EpCAM+ HCC cells. This finding reveals that detection of CD90 or EpCAM in a specific HCC tumor may be useful in improving efficacy of targeted treatment of HCC. The use of MK2206 or other AKT inhibitors merits further investigation for treating CD90+ HCC. Additionally, researchers must consider how experimental sensitivity of certain cell lines (JHH4 and Huh7) may result in significant variation between cell motility behavior in HCC.

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50. AP1 protein interactions affect the dynamics of cell state transitions in Melanoma

Presenter: Degefu, Yonatan
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Natacha Commandante-Lou (University of Michigan)
Doug Baumann (University of Virginia)
Mohammad Fallahi-Sichani (University of Virginia)

Cellular plasticity plays an important role in driving non-genetic heterogeneous tumor cell states that are resistant to therapies. Regulatory transcription factor networks such as AP1 proteins have been shown to be key players in cellular plasticity. AP1 proteins also serve as pioneer factors that establish chromatin states and predispose cells to diverse transcriptional programs and thereby guide cells towards epigenetic reprogramming. They consist of three subfamilies, including FOS, JUN and ATF proteins that form dimers to induce specific changes in gene expression. In melanoma tumors, for example, previous studies have shown that AP1 proteins are involved in resistance to MAPK inhibitors, and therapy-induced dedifferentiation. However, the exact mechanisms of how melanoma cells acquire such plasticity, and the diversity of responses that result from perturbations in the MAPK signaling pathway under the control of AP1 proteins are not well understood. Filling this gap of knowledge would require a systems-based approach to understand the interdependencies among different AP1 proteins and their effect on melanoma cell state heterogeneity at the single-cell level. To investigate the variation of AP1 proteins with baseline heterogeneity in differentiation states, our lab recently used highly multiplexed iterative imaging to measure the single-cell levels of 17 different AP1 proteins (including six phosphorylation states), and 4 differentiation state markers across 19 BRAF-mutant melanoma cell lines. Using a random forest classifier, differentiation state of a melanoma cell was predictable based on AP-1 protein levels with an accuracy of 74%. SHAP analysis identified p-cFOS, FRA2, ATF4, cFOS, p-FRA1, and cJUN as the most important AP1 proteins that predicted single cell differentiation state. The model-predicted results were validated using combinations of AP-1 siRNA knockdown experiments in heterogeneous melanoma cell populations. In our current studies, we are working to develop mechanistic models of cell state transitions based on dynamic interactions between the identified key AP-1 proteins. These models will reveal how regulated interactions between a few key AP-1 players can lead to heterogeneous cell states and their dynamic transitions across genetically diverse melanoma cell populations.

51. *epitoPeR enables scale up of high-content CRISPR screens*

Presenter: Choudhary, Krishna
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Ruzbeh Mosadeghi (University of California, San Francisco, CA)
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Motivation: Pooled CRISPR screens are widely applied as a productive tool for biological discovery in many applications, including cancer target discovery and drug development. CRISPR screens are typically based on a monocular phenotype, such as cell death. While this has helped illuminate basic mechanisms in gene essentiality and resistance to cell death, it has generally failed to yield gene function information for the vast majority of the genome. Pooled screening lacks the richness that is attainable by image-based phenotypic screens.

Image-based screens yield more granular knowledge about cancer cell states, e.g. cell morphology, subcellular protein localization, etc. However, the challenges of executing image-based CRISPR screens and performing large-scale image analysis have restricted the scalability and accessibility of high-content image-based CRISPR screens.

Results: We have developed an optical barcoding technology that uses epitope combinations attached to a fluorescent protein to barcode CRISPR guide identities in conjunction with cyclic immunofluorescence microscopy to lower the cost of high-content CRISPR screens. By attaching subcellular localization signals to barcode-carrying proteins, we can perform combinatorial screens and identify genetic interactions. To support analysis of the resulting large-scale imaging data, we have developed a computational pipeline, named epitoPeR, which provides a high yield of single cells with a low rate of errors in decoding CRISPR guides, and enables high-performance analysis of high-content images. Our pipeline enables automated workflows on high-performance computing platforms and provides methods for image pre-processing, including stitching and registering images from cyclic microscopy, barcode analysis, image segmentation, and quality control. We have benchmarked our computational methods by comparing with existing methods for similar image analysis tasks, as well as testing with simulated data. We provide several strategies to lower the burden of optimizing epitope stains and data collection strategies that overall help screen more genes and gene combinations than possible currently for a comparable cost and time investment. The combined advances in our optical barcoding technology and computational methods will accelerate the discovery of novel cancer targets and drug development.

52. Drug Mechanism Enrichment Analysis: interpreting drug rank lists using common mechanisms of action

Presenter: Garana, Belinda B.
University of Southern California, Los Angeles, CA

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James H. Joly (Nautilus Biotechnology, San Carlos, CA)
Nicholas A. Graham (University of Southern California, Los Angeles, CA)

Although targeted therapies have the potential for efficacy on tumor tissues with minimal effect on normal tissues, only fifteen percent of cancer patients are eligible for targeted therapies based on the presence of genetic aberrations. Additionally, of the patients eligible for targeted therapeutics, only half respond to treatment. Thus, improved methods to match tumors with effective therapeutics are desperately needed to realize the promise of precision oncology. Many computational approaches have been developed to rank drugs based on molecular signatures. One shortcoming of these approaches is that they output a long, rank-ordered list of drugs from which it is difficult to extracting biological or mechanistic insight. Thus, we developed Drug Mechanism Enrichment Analysis (DMEA), an approach to identify enriched drug mechanisms of action (MOAs) in rank-ordered drug lists. The method is based on gene set enrichment analysis (GSEA) and derives its power by integrating prior knowledge about the MOA and/or molecular targets of individual drugs.

To test DMEA, we first demonstrated that our approach can successfully identify enriched drug MOAs in simulated data. Then, we validated DMEA using several types of rank-ordered drug lists including drug perturbation connectivity scores, genetic loss of function and targeted inhibitor connectivity scores, and weighted gene voting molecular classification scores of intrinsic and acquired drug resistance. For all these test cases, DMEA successfully detected the expected MOA and highlighted opposing MOAs. Finally, we illustrated that DMEA can be used to discover senescence-inducing and senolytic drug MOAs for a primary human mammary epithelial cell model of senescence.

Taken together, DMEA is a bioinformatic tool that identifies enriched drug MOAs to facilitate biological understanding of rank-ordered drug lists. As demonstrated, DMEA enables researchers to better understand and identify therapeutic MOA for disease. DMEA is publicly available to use as a web application (https://dmea.app/) or download as an R package. For more information on how to use DMEA, please visit our website (https://belindabgarana.github.io/DMEA).

53. *Dimension reduction in simulation models for the analysis of 3D spheroids for the Optimization of radiotherapy in tumor treatment*

Presenter: Franke, Florian
HTW Dresden

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Hans-Joachim Böhme (HTW Dresden, Dresden, Germany)
Anja Voss-Böhme (HTW Dresden, Dresden, Germany)
Steffen Lange (HTW Dresden, Dresden, Germany)

Understanding the complicated dynamics of tumors in order to develop more efficient therapeutic strategies for tumor suppression is one of the most challenging problems in biomedicine. While three-dimensional tumor spheroids are increasingly recognized as a preferred, in-vitro model system to assess a so-called curative effect of combinatorial radio(chemo)therapy, these experiments remain laborious and make observation of long-term therapeutic response challenging. In principle, mathematical models for the 3D tumor dynamics in spheroids have the potential to allow estimates of experimentally inaccessible quantities and predict therapeutic response. In particular, cellular automata allow to effectively represent the spatial composition of a 3D structure at the cellular level, incorporating essential but often neglected effects like an oxygen gradient across the avascular spheroid. However, the reliable, data-driven calibration of the necessary model parameters in the case of three dimensions is computationally expensive and thus very laborious and often not feasible. We exploit the radially symmetric structure of the tumor, evident from the experimental data, to first develop a simplified 1D differential equation model for the spheroid dynamics in radial direction. This dimensionally reduced model can then be analyzed theoretically as well as simulated efficiently making calibration of model parameters feasible. We derive internal relationships between the parameters of the reduced 1D model and the 3D cellular automaton in order to transfer the calibration to the 3D model. In this sense, the 1D continuum model acts as catalyst for the calibration of the 3D cell-based model.

54. *Toward Comprehensive, Single-molecule Proteomics: Protein Identification by Short-epitope Mapping*

Presenter: Joly, James
Nautilus Biotechnology

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The impact of systems biology research has been hampered by incomplete access to the proteome. A weak correlation between RNA and protein expression has been repeatedly demonstrated across a wide range of methods and contexts, revealing a need for new proteomics approaches. Protein Identification by Short-epitope Mapping (PrISM) is a novel proteomics method designed to enable comprehensive proteome quantification at single-molecule sensitivity. In this work, we present computational modeling demonstrating the feasibility of this method and experimental application of the approach leveraging intact, single-molecule protein measurements to identify a small number of model proteins. The implementation of PrISM as presented works by acquiring iterative, non-traditional affinity reagent binding measurements on single, intact protein molecules. Counterintuitively, using affinity reagents with poor specificity (i.e. cross-reacting to many proteins) enables identification of tens of thousands of proteins with only hundreds of affinity reagents. To experimentally demonstrate PrISM, we use a small number of affinity reagents to quantify a larger number of proteins. In simulations, PrISM can identify more than 98% of proteins in human, mouse, yeast, and E. coli using 300 affinity reagents targeting short trimer epitopes. PrISM is robust to experimental confounders including non-specific binding and noisy, incomplete, or inaccurate estimation of affinity reagent to protein binding characteristics. Simulations of the approach with a chip containing 10 billion protein molecules measured in parallel show a dynamic range of detection of up to 9.5 and 11.5 orders of magnitude for HeLa cells and plasma, respectively. Using such a chip, we believe that the PrISM method is capable of identifying and quantifying over 95% of the human proteome in a single experiment, potentially revolutionizing biomarker discovery and cancer research.

55. *Ultrasensitive response explains the benefit of combination chemotherapy despite antagonism*

Presenter: Patterson, Sarah
UNC Chapel Hill - Adam Palmer Lab

Sarah Patterson (University of North Carolina at Chapel Hill, Chapel Hill, NC)
Dr. Adam Palmer (University of North Carolina at Chapel Hill, Chapel Hill, NC)

For over 50 years aggressive lymphomas have been treated with combination chemotherapy, most often as several cycles of concurrent drug administration. Concurrent administration is in theory optimal when combination therapies have synergistic (more than additive) drug interactions. We investigated pharmacodynamic interactions in the 4-drug "CHOP" regimen for Peripheral T-Cell Lymphomas (PTCL) by isobologram analysis in 7 PTCL cultures. We found that CHOP consistently exhibits antagonism between some of its drugs, but never synergies, which is unexpected given that it cures approximately half of patients with PTCL. We next used month-long in vitro models of treatment cycles to test whether staggered treatment schedules could enhance tumor cell kill by avoiding antagonism, relative to concurrent treatment using the same doses. Surprisingly, we observed that tumor cell kill is maximized by concurrent drug administration despite antagonistic drug-drug interactions. We propose that an ultrasensitive dose response, as described in radiology by the linear-quadratic (LQ) model, can reconcile these seemingly contradictory observations. The LQ model describes the relationship between cell survival and dose, and in radiology has identified scenarios favoring hypofractionated radiation – the administration of fewer, larger doses rather than multiple smaller doses. Specifically, a large quadratic component in a dose-response describes cells requiring an accumulation of DNA damage in order to die, which we also observed for the DNA-damaging chemotherapies in the CHOP regimen. By adapting the LQ model to combination chemotherapy, we find that even when chemotherapies have antagonistic interactions, tumor cell kill is maximized by concurrent administration of multiple drugs, explaining the clinical efficacy of CHOP. Thus, our study identifies a new mechanism by which combination therapy can be clinically advantageous that does not depend on drug-drug interactions.

56. *Overcoming the Challenges to Cancer Patient Digital Twins with Simulations of Tumor Evolution and Protein Structure Dynamics*

Presenter: McCoy, Matthew
Georgetown University Medical Center

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Cancer Patient Digital Twins (CPDT) are patient parameterized simulations of cancer growth and progression. These computational models are constructed in a way that allow for the comparison of different therapeutic strategies, and interpreted in a way that can inform the selection of an optimal intervention for a given real world patient. Using a model of subclonal tumor evolution, we compared two strategies for selecting the dosing sequence of two non-cross resistant drugs over a five-year course of treatment. Sampling across a clinically realistic range of the patient-definable parameter space resulted in tens of millions of CPDTs, each a unique combination of parameters with a predicted survival on each of the therapy selection strategies. We found that a strategy that considered tumor evolution and minor subclones, termed Dynamic Precision Medicine (DPM), improved outcomes in a significant portion of the CPDT population when compared to a standard precision medicine (PM) strategy that only considers the static properties of the predominant subclone at given timepoints, on average doubling survival over a comprehensive range of clinically relevant initial conditions (Beckman et al., PNAS 2012). This was true even when the DPM was used only during the first of two 45-day treatment windows. Surprisingly, the results showed that if DPM suggested a different dosing sequence than suggested by PM for even one of these first two interventions, the patient outcomes were significantly improved (McCoy et al., medRxiv 2020). This subpopulation of CPDTs stands to greatly benefit by selecting the optimal treatment strategy, however mapping the patient data in the subgroup that benefitted to corresponding baseline evolutionary model parameters that control subclonal growth and drug resistance and evolutionary phenotypic transition rates remains a significant challenge. This is due to the complex interaction between parameters. We are developing clustering and visualization tools to address this challenge.

Current technology is also limited when it comes to generating data that can used to define model parameters that capture complex phenomenon like the evolutionary rate of developing phenotypic drug resistance. For example, DNA sequencing data reveals many mutations of unknown clinical and biological significance, reducing its information content. One approach we are developing utilizes simulations of protein dynamics to predict changes to these subclonal properties that result from somatic mutations. Preliminary results suggest that simulating the conformational flexibility of variant protein structures can be linked to the phenotypic and functional changes using artificial intelligence (McCoy et al., Biophysical Journal 2021), and is even predictive of drug sensitivities that result from missense mutations in the drug target (Hamre et al., Comput Biol Med. 2021). Future work aims to utilize this approach to link mutations detected in a patient's tumor genome to subclonal parameters in an evolutionary model.

While the required resolution and fidelity of the model will depend on the specific cancer type, significant and generalized challenges still exist to parameterizing the high dimensional parameter space and to interpreting the simulation results in a clinically meaningful way.

57. Computational analysis of 4-1BB-induced NFκB signaling suggests improvements to CAR cell design

Presenter: Tserunyan, Vardges
University of Southern California

Vardges Tserunyan (University of Southern California, Los Angeles, CA),
Stacey Finley (University of Southern California, Los Angeles, CA)

Chimeric antigen receptor (CAR)-expressing cells are a powerful modality of adoptive cell therapy against cancer. The potency of signaling events initiated upon target encounter depends on the costimulatory domain within the structure of the CAR. One such costimulatory domain is 4-1BB, which affects cellular response via the NFκB pathway. However, the quantitative aspects of 4-1BB-induced NFκB signaling are not fully understood. To elucidate this problem, we developed an ordinary differential equation-based mathematical model representing canonical NFκB signaling activated by CD19scFv-4-1BB. After a global sensitivity analysis on model parameters, we ran Monte Carlo simulations of cell population-wide variability in NFκB signaling. Then, we quantified the mutual information between the extracellular signal and different levels of the NFκB signal transduction pathway. We found that in response to a wide range of antigen concentrations, the magnitude of the transient peak in NFκB nuclear concentration varies significantly, while its timing is relatively consistent. Global sensitivity analysis showed that the model is robust to variations in parameters, and thus, its quantitative predictions would remain applicable to a broad range of parameter values. Next, we found that overexpressing NEMO and disabling IKK2 deactivation can increase the mutual information between antigen levels and NFκB activation.

Our modeling predictions provide actionable insights to guide CAR development. Particularly, we propose specific manipulations to the NFκB signal transduction pathway that can fine-tune the response of CD19scFv-4-1BB cells to the antigen concentrations they are likely to encounter.

58. Combination therapy including inavolisib suppresses transcriptional cell cycle signatures in ESR1-mutant ER-positive breast cancer cells

Presenter: Marohl, Taylor

Department of Biomedical Engineering, University of Virginia

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Jenille Tan (Genentech Inc., South San Francisco, CA)
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Targeted therapies have improved progression-free survival in hormone-receptor positive (HR+) breast cancers, however, achieving durable responses remains a challenge. Mutations in ESR1 and PIK3CA are common in advanced settings and can potentially drive resistance to aromatase inhibitors. CDK4/6 inhibitors have been successfully combined with endocrine therapies to increase efficacy, yet eventually disease progression still occurs in many cases. PI3Ka-specific inhibitors have shown promise in treating PIK3CA mutant breast cancer tumors. As the number of therapeutic options rises, it becomes increasingly complex yet important to explore the molecular underpinnings of how these agents combine with one another and how their effects are influenced by the mutational landscape. Here we aim at understanding how estrogen receptor antagonists, CDK4/6 inhibitors, and PI3K inhibitors affect cell transcriptional profiles as single agents or in combination. We also aim at studying the impact of ESR1 mutation on cell transcriptional response to these treatments.

We profiled ER antagonist and selective degrader giredestrant (GDC-9545), CDK4/6 inhibitor palbociclib, and PIK3a-specific inhibitor inavolisib (GDC-0077) as single agents and in combination in PIK3CA-mutant MCF7 cells expressing ESR1 wild-type (WT) or ESR1-Y537S (MUT). Cells were harvested at 24 and 96 hours for transcriptional profiling by both bulk and multiplexed single-cell RNA-seq. GSEA was used to identify transcriptional profiles.

WT cells treated with any regimen that included giredestrant and harvested at 96 hours were quite transcriptionally homogenous. By contrast, MUT cells harvested at 96 hours separated into multiple clear sub-groups, which were distinguished primarily by presence or absence of inavolisib treatment. MUT cells with treatments including inavolisib have significantly lower scores of cell cycle signatures compared to cells treated without inavolisib. Among cells treated without inavolisib (single-agent giredestrant or giredestrant/palbociclib combination), a third subset of cells, primarily consisting of those treated with single-agent giredestrant, had particularly high scores of cell cycle signatures. The transcriptional profiles of this small set of cells were most like those of cells treated without giredestrant at all (DMSO, palbociclib, or inavolisib). These results indicate that in ESR1-MUT cells specifically, ER antagonism by giredestrant as a component of combination treatment may be more effective at suppressing proliferative transcriptional signatures than as single-agent. Furthermore, the lack of differential transcriptional response to inavolisib in WT cells, but strong differential response in MUT cells, suggests that PI3K inhibition may play a particularly important role in tumors harboring mutant ESR1 when combined with ER antagonists.

59. *Modeling age-specific incidence of colon cancer via niche competition*

Presenter: Lange, Steffen
HTW Dresden

Steffen Lange (Hochschule fuer Technik und Wirtschaft, Dresden, Germany)
Richard Mogwitz (Hochschule fuer Technik und Wirtschaft, Dresden, Germany)
Denis Hünniger (Hochschule fuer Technik und Wirtschaft, Dresden, Germany)
Anja Voss-Böhme (Hochschule fuer Technik und Wirtschaft, Dresden, Germany)

Cancer development is a multistep process often starting with a single cell in which a number of epigenetic and genetic alterations have accumulated thus transforming it into a tumor cell. The progeny of such a single benign tumor cell expands in the tissue and can at some point progress to malignant tumor cells until a detectable tumor is formed. The dynamics from the early phase of a single cell to a detectable tumor with billions of tumor cells are complex and still not fully resolved, not even for the well-known prototype of multistage carcinogenesis, the adenoma-adenocarcinoma sequence of colorectal cancer. Mathematical models of such carcinogenesis are frequently tested and calibrated based on reported age-specific incidence rates of cancer, but they usually require calibration of four or more parameters due to the wide range of processes these models aim to reflect. We present a cell-based model, which focuses on the competition between wild-type and tumor cells in colonic crypts, with which we are able reproduce epidemiological incidence rates of colon cancer. Additionally, the fraction of cancerous tumors with precancerous lesions predicted by the model agree with clinical estimates. Furthermore, the model is successfully applied to rectal and gastric cancer, whose tumor-originating cells are proposed to be also compartmentalized into niches. The correspondence between model and reported data suggests that the fate of tumor development is majorly determined by the early phase of tumor growth and progression long before a tumor becomes detectable. Due to the focus on the early phase of tumor development, the model has only a single fit parameter, the time scale set by an effective replacement rate of stem cells in the crypt. We find this effective rate to be considerable smaller than the actual replacement rate, which implies that the time scale is limited by the processes succeeding clonal conversion of crypts.

60. Accelerating Cell Based Cancer Simulations Using Deep Neural Networks

Presenter: Sundus, Aneequa
Indiana University Bloomington

Furkan Kurtoglu (Indiana University Bloomington)
Paul Macklin (Indiana University Bloomington)

Cancer modeling in systems biology ranges from use of ODEs to agent-based modeling. Agent-based simulation software is widely used for creating cell-based simulations. These models can be intuitive to understand due to rule-based nature of assigning biological behavior to cell agents. Each cell of same cell type behaves independently even though they start at the same point with same rules; due to stochasticity introduced in code, they soon desynchronize and follow individual paths. Agent based models are good to capture spatial long with temporal aspects of biological system. Cell-based models simulate the tissue (micro)environment for these agents/cells by integrating diffusion solvers in the background, thus modeling the secretion, movement, and consumption of chemical substrates within cells by having specific intracellular modules. Simulating intracellular components for each cells becomes computationally expensive when simulating thousands of cells. Intracellular networks can be modeled using a variety of methods like ordinary differential equations, partial differential equations or Flux balance analysis. All these methods are nonlinear and computationally expensive and thus simulating them for each cells account for considerable time in full simulation run. Deep Neural networks have a lot of interest in last decades after finding success in Computer vision and Computational Linguistics. Deep neural networks are function approximators and can approximate any nonlinear continuous function. In this work we have developed a multiscale multicellular simulation for colorectal cancer. Here each cell has a flux balance analyses components to solve for intracellular part of tumor cells. We replaced this FBA component with a deep neural network trained on data generated from running FBA independently with a range on initial values. After validating and evaluating this DNN can replace FBA inside each cell for intracellular model hence reducing run time for overall simulation. This work provides foundation to use deep learning for multifold computational acceleration for modeling of intracellular networks in multiscale multicellular simulations

61. *Modeling divergent HER2 growth dynamics reveals epithelial plasticity changes as a bottlenecking event in cancer progression*

Presenter: Ginzel, Joshua
Duke University

Joshua D. Ginzel (Duke University, Durham, NC)
Edwin J. Allen (Duke University, Durham, NC)
Lawrence S. Barak (Duke University, Durham, NC)
Bruce Rogers (Duke University, Durham, NC)
H. Kim Lyerly (Duke University, Durham, NC)
Joshua C. Snyder (Duke University, Durham, NC)

A significant challenge in breast cancer is the ability to differentiate between a lesion that will never progress in a patient's lifetime and one that will become an invasive, metastatic carcinoma. An understanding of the mechanisms that drive these divergent tumor trajectories is limited by a lack of preclinical tools. We have recently published a Crainbow mouse that is capable of modeling these distinct tumor trajectories by expressing three fluorescently barcoded isoforms of the protooncogene HER2 (Wildtype wt-HER2, exon 16 splice isoform d16-HER2, and c-terminal fragment p95-HER2). Previous work based on a small histopathological dataset from this mouse showed that not all tumors generated by HER2 isoform expression progressed to invasion and some appeared to stall as indolent lesions. Here we tested this observation by collecting and modeling thousands of tumor size, tumor genotype, and metastasis measurements across 20 timepoints using a whole mouse imaging approach. We used mathematical modeling and imaging to deduce distinct patterns of tumor progression inherent to each HER2 isoform. Oncogenic d16HER2 cells appeared more fit with a proliferative advantage, but many of these early proliferative lesions failed to progress to tumors. Conversely, p95HER2 cancer cells went through a period of apparent dormancy early with few detectable early lesions and limited proliferation, yet became the most tumorigenic. Whole lung imaging to assess the entire burden of metastatic cells in the lung showed that p95HER2 metastasizes long before a palpable tumor is detectable and the most proliferative d16HER2 tumors are unlikely to metastasize. Further study of previously described epithelial heterogeneity using mRNA FISH and whole gland imaging reveals marked plasticity within the epithelial compartment. The populations of epithelial cells that arise are a key attribute of the invasive tumors that don't undergo an obligatory proliferative phase. Our data demonstrate how modeling HER2 growth dynamics in HER2BOW reveals an epithelial plasticity program as a potential bottlenecking event in the progression of highly invasive tumors.

62. *HSC depletion and expansion of inflammatory GMP cells are clinically relevant features of disease progression in chronic myelomonocytic leukemia (CMML)*

Presenter: Ferrall-Fairbanks, Meghan C.
University of Florida

Meghan C. Ferrall-Fairbanks (University of Florida, Gainesville, FL)
Abhishek Dhawan (Moffitt Cancer Center and Research Institute, Tampa, FL)
Brian Johnson (Moffitt Cancer Center and Research Institute, Tampa, FL)
Hannah Newman (Moffitt Cancer Center and Research Institute, Tampa, FL)
Virginia Volpe (Moffitt Cancer Center and Research Institute, Tampa, FL)
Christopher Letson (Moffitt Cancer Center and Research Institute, Tampa, FL)
Markus Ball (Moffitt Cancer Center and Research Institute, Tampa, FL)
Anthony Hunter (Winship Cancer Institute of Emory University, Atlanta, GA)
Maria Balasis (Moffitt Cancer Center and Research Institute, Tampa, FL)
Traci Kruer (Moffitt Cancer Center and Research Institute, Tampa, FL)
Nana-Adjoa Ben-Crentsil (Moffitt Cancer Center and Research Institute, Tampa, FL)
Jodi Kroeger (Moffitt Cancer Center and Research Institute, Tampa, FL)
Robert Balderas (BD Biosciences, San Jose, CA)
Rami Komrokji (Moffitt Cancer Center and Research Institute, Tampa, FL)
David Sallman (Moffitt Cancer Center and Research Institute, Tampa, FL)
Jing Zhang (University of Wisconsin-Madison, Madison WI)
Rafael Bejar (University of California San Diego Health, La Jolla, CA)
Philipp M. Altrock (Max Planck Institute of Evolutionary Biology, Ploen, Germany)
Eric Padron (Moffitt Cancer Center and Research Institute, Tampa, FL)

Myeloblast expansion is a hallmark of disease progression in chronic myeloid neoplasms and comprises CD34+ hematopoietic stem and progenitor cells (HSPC). How this compartment is shaped by disease progression is unknown. To address this important feature of disease progression, we transcriptionally and immunophenotypically mapped CD34+ HSPCs at single-cell resolution for 66 samples from 45 patients with CMML. Using single-cell RNA-sequencing and high parameter flow cytometry, we show that CMML CD34+ HSPCs can be classified into three differentiation trajectories: monocytic (mono-bias), megakaryocyte-erythroid progenitor (MEP), and normal-like. These groups were associated with distinct clinical genomic characteristics and were congruent with patient-specific bulk sequencing. For example, MEP biased cases had statistically higher hemoglobin and mono-bias cases were associated with adverse survival, inflammatory clinical correlates, and RAS pathway mutations. Importantly, we identified significant depletion of HSC across CMML that was most pronounced in the mono-bias group. This was validated by flow cytometry in 26 CD34+ enriched samples, which showed HSC numbers decreased as myeloblasts expanded and disease progressed. Mono-biased differentiation trajectories were enriched by inflammatory GMP-like cells expressing CD120b involved in cytokine receptor signaling pathways. Cytokine receptor diversity was an adverse feature across all HSPC subtypes and was elevated in CD120b GMPs. Hypomethylating agents decreased inflammatory GMP-like cells in CMML patient sequential samples. NRAS competitive transplants and LPS-treated PDX models recapitulated mono-biased CMML, suggesting that hematopoietic stress precipitates the mono-biased state. Our data suggests that HSC depletion is a characteristic of myeloblast expansion during disease progression. Further, even in a disease with homogenous hematopoietic output (monocytosis), progenitor expansion of HSPCs can occur in three distinct skewed differentiation trajectories. The mono-biased state is associated with poor outcomes and can be recapitulated by modeling stress-induced hematopoiesis in CMML. Deconvolution of HSPC compartments in other myeloid neoplasms and identifying therapeutic strategies to mitigate the monocytic-biased differentiation trajectory should be explored.

63. Tissue-specific biomaterials to study cancer dormancy

Presenter: Peyton, Shelly
UMass Amherst

Hyuna Kim (University of Massachusetts, Amherst MA)
Lauren Barney (University of Massachusetts, Amherst MA)
Lauren Jansen (University of Massachusetts, Amherst MA)
Ninette Irakoze (University of Massachusetts, Amherst MA)
Alyssa Schwartz (University of Massachusetts, Amherst MA)

Improved experimental model systems are critically needed to better understand cancer progression and bridge the gap between lab bench proof-of-concept studies, validation in animal models, and eventual clinical application. Many methods exist to create biomaterials, including hydrogels, which we use to study cells in contexts more akin to what they experience in the human body. Our lab has multiple approaches to create such biomaterials, based on combinations of poly(ethylene glycol) (PEG) with peptides and zwitterions. In this presentation, I will discuss our synthetic approaches to building life-like materials, how we use these systems to grow cells and understand how a cell's environment, particularly the extracellular matrix regulates cancer cell growth, dormancy, and drug sensitivity.

64. Modeling glioblastoma stem cell heterogeneity with a dynamic causal model of the cell signaling network

Presenter: Cochran, Brent
Tufts University School of Medicine

Emilee Holtzapple (University of Pittsburgh, Pittsburgh, PA)
Natasa Miskov-Zivanov (University of Pittsburgh, Pittsburgh, PA)
Brent H. Cochran (Tufts University School of Medicine, Boston, MA)

Glioblastomas and glioblastoma stem cells are heterogeneous with respect to mutations, gene expression, and response to drugs. To make predictive responses of individual GBM stem cell lines to kinase inhibitors, we have constructed a causal model of glioblastoma stem cell signaling. The core model was built starting from pathways identified from TCGA mutation data ith the addition of the Jak/STAT, Hedgehog, and Notch pathways. Elements and relations between them were validated and extended using the PCNet interaction database and the INDRA database which includes machine read extractions from the biomedical literature. The result is a high confidence executable model consisting of 169 elements (proteins, genes, RNAs) and 295 regulatory logic rules between the elements. Stochastic simulations of the model provide dynamic (quantile) changes in time and responses to perturbations. The output simulates activity of individual nodes as well as cell cycle progression, apoptosis, and differentiation. To simulate the responses of individual cell lines to kinase inhibitors, the model was initialized using DNA sequencing data, RNA-seq, and reverse phase protein array (RPPA) data from each cell line. Comparing the results of the simulations to the drug responses of 11 different kinase targets in 3 cell lines, the model was 88% accurate in predicting effects on growth and survival. The model was further tested by comparing the effects of Raf inhibition of each of the cell lines in the model to the results observed in the RPPA data which overlap by 127 elements. In this case, there was less than 65% concordance between the model and the data for individual nodes. Discrepancies between the model predictions and the data are being investigated to determine whether the model logic or extent needs to be revised to improve the model. This modeling approach is a step toward developing algorithms for personalized therapeutics for GBM.

65. *Multiplexed Imaging of Signaling and Metabolism at the Single Cell Level*

Presenter: Coskun, Ahmet F.
Georgia Institute of Technology

Ahmet F Coskun (Georgia Institute of Technology, Atlanta, GA)
Shuangyi Cai (Georgia Institute of Technology, Atlanta, GA)
Mayar Allam (Georgia Institute of Technology, Atlanta, GA)
Thomas Hu (Georgia Institute of Technology, Atlanta, GA)

The spatial organization of cells in tissues and subcellular networks can be considered a quantitative metric in determining health and disease states. Single-cell analyses of molecular profiles with in-situ detection methods dissect spatial heterogeneity of distinct cell types. Such detailed cellular “digital maps” shed light on the spatial regulation mechanisms of many disorders. The next challenge in spatial biology is to link the cellular functional responses to the cell identities and phenotypes in their native three-dimensional (3D) environments. To achieve this important goal, image-based multiparameter molecular profiling has the potential to decode high-dimensional dynamics of signaling and metabolism at the subcellular and molecular level in complex tissues and organs. In this talk, I will introduce multiplex imaging modalities (genomics, proteomics, and metabolomics) to decipher the spatial and temporal decision-making of single cells at macromolecular resolution in engineered organoids and human tissues for spatially resolved cancer systems biology and subcellular precision oncology applications. Automated machine learning algorithms in this single-cell big data impact biomedical practice and clinical care. Specifically, this presentation will cover single cell spatial metabolomics analysis, using imaging mass spectrometry and cytometry to dissect the tumor microenvironment, and single cell spatial signaling analysis to map out the signaling protein networks in-situ in single cancer cells and tissues to decipher the resistance to targeted therapies using multiplexed imaging of transcripts and proteins. In the last part of the presentation, digital technologies interfacing cellular interactive media will be presented using the virtual reality of 3D spatial omics.

66. *Melanoma co-opts homeostatic signaling to promote liver-specific metastasis*

Presenter: Rogava, Meri
Harvard Medical School/ Columbia University

Meri Rogava (Columbia University Irving Medical Center, New York, NY, United States)
Clemens Hug (Harvard Medical School, Boston, MA, United States).
Johannes Melms (Columbia University Irving Medical Center, New York, NY, United States)
Stephanie Davis (Harvard Medical School, Boston, MA, United States).
Amit Dipak Amin (Columbia University Irving Medical Center, New York, NY, United States)
Bryan Ngo (Memorial Sloan Kettering Cancer Center, New York, NY, United States).
Michael Lee (Columbia University, New York, NY, United States)
Patricia Ho (Columbia University Irving Medical Center, New York, NY, United States)
Yiping Wang (Columbia University Irving Medical Center, New York, NY, United States)
Stephen Tang (Columbia University Irving Medical Center, New York, NY, United States)
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David Liu (Dana-Farber Cancer Institute, Boston, MA, United States).
Thomas Tüting (Magdeburg University Clinic,Magdeburg, Germany).
Martin Röcken (Tuebingen University Hospital, Tuebingen, Germany).
Thomas K. Eigentler (Charité University Hospital, Berlin, Germany).
Wei-Yu Chen (Weill Cornell Medicine, New York, NY, United States).
Ethan Earlie (Weill Cornell Medicine, New York, NY, United States).
Ashley Laughney (Weill Cornell Medicine, New York, NY, United States).
Samuel F. Bakhoum (Memorial Sloan Kettering Cancer Center, New York, NY, United States).
Andrei Molotov (Columbia University, New York, NY, United States).
Akiva Mintz (Columbia University, New York, NY, United States).
Lewis C. Cantley (Dana-Farber Cancer Institute, Boston, MA, United States).
Peter K. Sorger (Harvard Medical School, Boston, MA, United States).
Benjamin Izar (Columbia University, New York, NY, United States)

Liver metastasis (LM) occurs frequently and is associated with a poor prognosis and reduced therapy response in several cancers, including in patients with melanoma and lung cancer. To identify drivers of metastatic niches, we used a syngeneic mouse melanoma model which recapitulates genomic, metastatic and therapy response patterns seen in patients. We performed a large-scale in vivo CRISPR-Cas9 knockout screen and identified perturbations that promote LM, but not primary tumor growth or metastasis to other organs. The “top hit” in this screen associated with LM was loss Pip4k2c. Loss of Pip4k2c in melanoma cells led to increased insulin-induced activation of the PI3K/AKT pathway, that was Pip4k2c allosteric domain dependent. Treatment with different PI3K inhibitors abrogated the pathway, but was partly bypassed in the presence of insulin. In vivo loss of Pip4k2c produced a significantly increased LM burden compared to parental cells, that was rescued upon Pip4k2c reconstitution. We reasoned that Pip4k2cKO cells preferentially colonized the liver by co-opting the insulin-rich milieu in this organ. To test this, we generated Pip4k2cKO/InsrshIR and showed that Insr was required but not sufficient to enhance LM burden. Given the promising in vitro activity of PI3K inhibitors, we next tested whether these could abrogate enhanced LM. Interestingly, LM burden increased in mice bearing Pip4k2cKO melanoma cells upon PI3K inhibition compared to vehicle group. We reasoned that paradoxical activation due to host-mediated increase in glucose and insulin in response to PI3K inhibitor, promoted liver metastasis. Breaking this loop with either ketogenic diet or with SGLT2 inhibitor circumvented increased host responses and resulted in reduced LM burden in combination with PI3K inhibition. In summary, we identify a novel mechanism of metastatic liver organotropism and pharmacological and dietary combinations to reduced liver metastatic burden. Given the expanding use of PI3K inhibitors, our findings may have important clinical implications.

67. An Engineered Model of Pancreatic Cancer to Assess the Effect of Tissue Architecture and Fibroblast Content on Cellular Phenotype and Behavior

Presenter: Cavidad, Jose
University of Toronto

Jose L. Cadavid (U. of Toronto), Simon Latour (U. of Toronto, Mt. Sinai Health System), Ferris Nowlan (U. of Toronto, Mt. Sinai Health System), Ileana L. Co (U. of Toronto), Natalie Landon-Brace (U. of Toronto), Bradly G. Wouters (U. of Toronto), Barbara T. Grünwald (University Health Network), Mark Nitz (U. of Toronto), Hartland Jackson (U. of Toronto, Mt. Sinai Health System, Ontario Inst. of Cancer Research), Alison P. McGuigan (U. of Toronto)

Purpose/Objective: Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest types of cancers and its treatment has not improved significantly over the past decade. PDAC tumors contain a high abundance of fibroblasts and feature regions of hypoxia, which drive cancer cells resistance to therapy. Additionally, the spatial configuration of cells in the tumor microenvironment has been shown to affect cancer cell phenotype. Yet, traditional tissue culture models rely on cancer monocultures placed in a homogeneous oxygen-rich environment, thus limiting their capacity for modelling PDAC. Therefore, we set out to optimize and characterize a novel PDAC model that incorporates fibroblasts and features hypoxia. This model allows for manufacturing and analysis of tumour tissues with diverse architectures.

Methodology: To do this, we leveraged the Tissue Roll for Analysis of Cellular Environment and Response (TRACER) model developed in our group. In TRACER, cells suspended in a hydrogel are infiltrated into a cellulose scaffold that is then rolled around an aluminum mandrel, thus creating a 3D layered tissue structure with flexible configurations. Cells can then be retrieved from different TRACER layers, allowing for snapshot analysis of cell phenotype as a function of their position, hence microenvironment, in TRACER. Importantly, we have previously shown that patient derived cancer organoids (PDOs) can be incorporated in TRACER and lead to hypoxic gradients in the system. We therefore optimized the culture conditions that allowed us to reproducibly incorporate fibroblasts in TRACER and to culture them with PDOs to create layered cocultures. We used qPCR and flow cytometry to assess the behaviour of fibroblasts in our system when stimulated with key cytokines. Finally, we used mass cytometry to analyze how hypoxia, proliferation, and cell phenotype were influenced by stromal content and tissue architecture in TRACERs built with different coculture configurations.

Results: We determined the appropriate hydrogel composition to achieve homogeneous and reproducible spreading of fibroblasts in TRACER. Importantly, these cells are not significantly affected by the cellulose scaffold and can be polarized to expected phenotypes through the addition of relevant cytokines. We then devised a coculture strategy that allowed us to manufacture tissues with different number of layers with fibroblasts or PDOs (i.e. tissue architecture) that remained stable for at least three days in culture. Applying mass cytometry on cells retrieved from TRACER showed that different tissue architectures lead to different profiles of hypoxia in the system. This was accompanied by differential gradients in cell proliferation and in a graded phenotypic response in different fibroblast and epithelial markers. Importantly, these phenotypes were different from the ones found in PDOs or fibroblast monocultures, highlighting heterotypic interactions in the layered TRACER model.

Conclusion/significance: Embracing the heterogeneity and complexity of tumors is essential for devising effective and robust treatments. We have shown that our tumor model recapitulates key features of PDAC tumors and represents a simple and reproducible platform for mechanistic, systems-wide, studies of the complex PDAC microenvironment and its influence on cell behaviour.