Poster Presentation Abstracts

Poster session 1 on Thursday, November 8th

Proteomics, post-translational modifications, and integrative analyses reveal heterogeneity of molecular mechanisms medulloblastoma subgroups

Presenter: Archer, Tenley

Tenley C. Archer, Tobias Ehrenberger, Filip Mundt, Maxwell P. Gold, Karsten Krug, Clarence K. Mah, Elizabeth L. Mahoney, Colin J. Daniel, Alexander LeNail, Divya Ramamoorthy, Philipp Mertins, D. R. Mani, Hailei Zhang, Michael A. Gillette, Karl Clauser, Michael Noble, Lauren C. Tang, Jessica Pierre-François, Jacob Silterra, James Jensen, Pablo Tamayo, Andrey Korshunov, Stefan M. Pfister, Marcel Kool, Paul A. Northcott, Rosalie C. Sears, Jonathan O. Lipton, Steven A. Carr, Jill P. Mesirov, Scott L. Pomeroy, Ernest Fraenkel

Boston Children's Hospital

There is a pressing need to identify therapeutic targets in tumors with low mutation rates such as the malignant pediatric brain tumor medulloblastoma. To address this challenge, we quantitatively profiled global proteomes and phospho-proteomes of 45 medulloblastoma samples. Integrated analyses revealed that tumors with similar RNA expression vary extensively at the post-transcriptional and post-translational levels. We identified distinct pathways associated with two subsets of SHH tumors, and found post-translational modifications of MYC that are associated with poor outcomes in Group 3 tumors. We found kinases associated with subtypes and showed that inhibiting PRKDC sensitizes MYC-driven cells to radiation. Our study shows that proteomics enables a more comprehensive, functional readout, providing a foundation for therapeutic strategies.

Metabolic reprogramming mediated through tumor-stroma crosstalk in lung adenocarcinoma

Presenter: Bouchard, Gina

Gina Bouchard, Weiruo Zhang, Irene Li, Amato Giaccia and Sylvia Plevritis

Stanford University

Cancer cells have a distinctive metabolic profile to meet the energy demand required for tumorigenesis. This metabolic reprogramming (MR) is associated with metastatic progression as well as drug resistance and is considered one of the new 'Hallmarks of Cancer'. Preliminary results from our group have identified the Hexosamine Biosynthesis Pathway (HBP) in MR of non-small cell lung cancer (NSCLC). The outcome of HBP is glycosylation, a post-translational modification that has been associated with cancer invasion and metastasis. Our group has shown that GFAT2, the rate-limiting enzyme of HBP, is overexpressed in NSCLC fibroblasts. This novel finding signifies that the stroma is highly involved in driving MR towards HBP, as opposed to cancer cells only like generally described. We hypothesize that fibroblasts originating from different sites (normal, tumor-adjacent and tumor) show different activation patterns, therefore affecting cancer cells differently. By utilizing co-cultures of lung cancer cells and cancer-associated fibroblasts (CAFs) freshly isolated from patient tumors, this study aims to characterize fibroblast heterogeneity in MR and its impact on cancer invasion and drug resistance using a single-cell approach. Our preliminary results indicate that fibroblasts originating from different sites are highly heterogeneous, have distinct morphologies, and behave differently in culture. Moreover, our results show that fibroblasts can be either pro- or anti- tumorigenic, depending on factors including but not limited to their activation state and spatial proximity with cancer cells. In summary, this study will allow us to better understand MR in the stroma leading to cancer invasion. By targeting MR towards HBP, our

ultimate goal is to affect malignant and stromal cells simultaneously, therefore optimizing NSCLC treatment.

Moving beyond genetic mutations to predict response to targeted therapies

Presenter: Carroll, Molly

Molly J. Carroll, Harin A. Patel, Carl R. Parent, C. David Page, Pamela K. Kreeger

University of Wisconsin-Madison

Inclusion criteria for clinical trials in cancer emphasize tumor type and the presence of specific genetic mutations; however, for some cancer subtypes druggable mutations are at very low frequencies. Recently, 'basket trials' have emerged as an option to test a therapy based on the presence of the mutation or biomarker independent of tumor type. Despite this approach's promise, its success is uncertain. Vemurafenib is currently used to treat BRAF V600E melanoma, but it was less effective in a basket clinical trial of non-melanoma cancers with BRAF V600E. We hypothesize that sensitivity to targeted therapies can be predicted using the expression and activity data of protein pathways within the tumor in conjunction with the mutation status of the targeted protein. To address this hypothesis we utilized orthogonal partial least squares regression (O-PLS) modeling to predict sensitivity to Vemurafenib (Quantitative Analysis of Pharmacogenomics in Cancer Portal) of 26 V600E BRAF mutated melanoma and non-melanoma cell lines from their expression of 232 proteins from Reverse Phase Protein Array (RPPA, MD Anderson Cancer Cell Line Project). Our model captured the heterogeneity in protein expression among 20 cell lines used to train the model and accurately predicted the area under the dose-response curve (AUC) for a test set of 6 cell lines (R2X= 0.753, Q2= 0.305, RMSE Prediction= 0.086). Investigation of the predictive proteins in our model identified increased activation of MAPK and apoptotic pathways in the more sensitive cell lines, while in resistant cell lines we identified increased expression and activation of components of the ErbB and PI3K pathway. Using multivariate modeling of protein expression/activity, our preliminary results illustrate the ability to predict sensitivity to Vemurafenib, and that identification of parallel activated pathways may indicate therapies to be used in conjunction with Vemurafenib in BRAF V600E tumors.

Validation of antibody panels for high-plex immunohistochemistry applications

Presenter: Confuorto, Nick

Douglas Hinerfeld, Kristi Barker, Heather Metz, Chris Merritt, Lucas Dennis, Philippa Webster, Joseph Beechem

Nanostring Inc.

Introduction: Characterization of the spatial distribution and abundance of key proteins within tissues enables a deep understanding of biological systems. However, it has proven difficult to perform such studies in a highly-multiplexed manner on FFPE tissue sections. There has been significant progress in developing technologies with expanded capabilities to analyze higher numbers of proteins, however, the validation of these technologies and their associated affinity reagents remains a significant barrier to adoption. We have developed a validation pipeline that ensures optimal sensitivity and specificity for high-plex antibody panels for the analysis of FFPE sections using the NanoString Digital Spatial Profiling (DSP) platform. The DSP is designed to simultaneously analyze up to 96 proteins by detecting oligos conjugated to antibodies that can be released via a UV-cleavable linker.

Methods: Antibodies targeting immuno-oncology proteins were tested for specificity and sensitivity by immunohistochemistry on FFPE human tissues, as well as human cell line pellets to evaluate binding specificity of both unconjugated and oligo-conjugated antibodies. The sensitivity and dynamic range were tested using FFPE cell pellets with target-specific positive and negative cells at different ratios. An

interaction screen was performed to evaluate potential deleterious effects of multiplexing antibodies, and a human tissue microarray (TMA) containing normal and cancer tissues was employed to assess assay robustness. The reproducibility of the panel on DSP was tested on serial FFPE tumor specimens by correlating the expression of all markers across 24 spatially-registered regions of interest (ROI) as well as the ability to reveal biological heterogeneity within lymphoid tissue by characterizing the expression of 40+ proteins in a spatial grid of 100um x 100um ROIs.

Results: Immunohistochemical analysis of unconjugated and oligo-conjugated antibodies displayed indistinguishable staining patterns on control tissues and cell lines. Mixed cell pellet assays revealed strong correlations between observed counts and positive cell numbers. Antibody interaction studies showed similar count values for antibodies alone or in combination, and TMA hierarchical clustering analysis demonstrated expected patterns of expression across tissue types. Analysis of all markers across 24 registered regions of interest across serial FFPE sections were highly correlated. Spatial analysis of lymphoid tissue revealed high levels of biological heterogeneity across multiple germinal centers.

Conclusion: These results demonstrate the validation and application of high-plex protein panels to accurately interrogate the immune biology within FFPE tissue using the NanoString DSP platform.

A network-based model to identify therapeutic targets in RAS-mutant cancer, minimal residual disease, and cancers driven by loss of tumor suppressors

Presenter: Costello, James

James C. Costello, Satoshi Washino, Gabriele Romano, Lawrence Kwong, Scott D Cramer

University of Colorado, Anschutz Medical Campus

Translating cancer -omic data into patient outcomes is an ongoing challenge. Successfully addressing this challenge is the basis for precision oncology. Cancer is a complex set of diseases, primarily driven by (epi)genetic aberrations. Even when a known, single cancer driving alteration can be directly and effectively targeted, it is inevitable that resistance will develop. Systems pharmacology attempts to address the challenges of therapeutically targeting cancer by modeling and studying cancer as a complex, integrative biological system. Here, we outline three critical challenges in precision oncology that we address using a network-based, systems pharmacology model, Transcriptional Regulatory Associations with Pathways (TRAP). The first challenge is to identify effective drug combinations. Using an NRAS-mutant melanoma mouse model, we predicted and validated the MEK and CDK4 drug combination. Results from these experiments have led to five clinical trials in RAS-mutant cancers. The second challenge is to target residual disease remaining after treatment. Using an inducible BRAFV600E mouse model, we found strong immune signals in residual tumors after extinction of BRAFV600E. We used TRAP to identify a central regulatory cytokine, which is known to be involved in T cell mobilization. This cytokine is strongly induced within 8 hours of BRAFV600E extinction, and then dissipates prior to residual disease; enforced expression of this cytokine eliminates the residual disease. The third challenge is to identify therapies for cancers driven by loss of tumor suppressors. Prostate cancers are largely driven by combination losses of tumor suppressors. Using the molecular subtype defined by loss of CHD1 and MAP3K7, which we previously showed to drive aggressive disease, we identified therapeutic targets using TRAP. We validated our predictions in vitro; future work will test promising candidates in mouse models. The sum of our results demonstrates that TRAP is an effective model to address many challenges we face in precision oncology.

Spontaneous age-associated epigenetic alterations are the underlying driver of oncogene-induced tumorigenesis

Presenter: Easwaran, Hariharan

Yong Tao, Byunghak Kang, Daniel A. Petkovich, Julie In, Genevieve Stein-O'Brien, Xiangqian Kong, Wenbing Xie, Nicholas Zachos, Shinji Maegawa, Stephen Brown, Ray-Whay Chiu Yen, Jai Thakor, Zhihao Lu, Yi Cai, Yuezheng Zhang, Izaskun Mallona Gonzalez, Miguel Angel Peinado, Cynthia A. Zahnow, Nita Ahuja, Elana Fertig, Jean-Pierre Issa, Stephen B. Baylin, Hariharan Easwaran

Johns Hopkins University

We addressed the precursor role of aging-like spontaneous promoter-DNA hypermethylation in initiating tumorigenesis. Using mouse colon-derived organoids, we show promoter hypermethylation spontaneously arises in cells mimicking the human 'aging-like' phenotype. The silenced genes activate the Wnt pathway causing a stem-like state and differentiation defects. These changes render aged organoids profoundly sensitive than young ones to transformation by BrafV600E producing the typical human proximal BRAFV600E-driven colon adenocarcinomas characterized by extensive, abnormal gene promoter CpG-island methylation or the methylator phenotype (CIMP). Conversely, CRISPR-mediated simultaneous inactivation of a panel of the silenced genes markedly sensitize to BrafV600E-induced transformation. Our studies tightly link aging-like epigenetic abnormalities to intestinal cell fate changes and predisposition to oncogene-driven colon tumorigenesis.

Inferring drug sensitivity in cancer cell lines during cancer progression

Presenter: Fleck, Julia

Julia L. Fleck; Ana B. Pavel; Christos G. Cassandras

Pontifical Catholic University of Rio de Janeiro

Precision medicine approaches rely on biomarker discovery, a process that involves identifying genetic abnormalities within a tumor. A number of recent massive-scale efforts have aimed at collecting, organizing and making publicly available multiple data types derived from genetic analysis of cancer cell lines or human tumor samples. Such data are typically collected at one point in time and may aid in uncovering common cancer progression pathways, as well as in classifying cancer patients into groups that will most likely benefit from a certain treatment approach. One of the main challenges in defining therapeutic targets is accounting for the heterogeneity of cancer. We have previously addressed this issue through a Mixed Integer Linear Program (MILP) that models the molecular progression of cancer, and have shown that by integrating somatic mutation and gene expression data, we can predict the temporal sequence of events from cross-sectional data in breast cancer. Here we build upon the MILP-based methodology and apply it to hundreds of heterogeneous cell lines from the Cancer Cell Line Encyclopedia (CCLE) in order to stratify known oncogenes and tumor suppressors into a number of phases of cancer progression, and predict their effect on gene expression. We thus infer a comprehensive set of mechanisms of cancer initiation and progression across a variety of cancer types and then analyze these mechanisms in light of drug sensitivity data from CCLE. By means of an initial study on the BRAF oncogene, a known predictor of drug sensitivity for several compounds used in cancer treatment, we verify that cells with a decreased number (≤ 3) of late-stage mutations generally respond better to drugs, which implies that successful treatment of tumors in advanced stages may involve tackling more than one genetic target simultaneously, e.g., through an appropriately defined combination of drugs. Part of our ongoing research involves gaining insight into the relative effectiveness of distinct drug combinations across different phases of cancer progression.

A hybrid CompuCell3D model of cancer migration in a metastatic remodeling extracellular matrix

Presenter: Versypt, Ashlee

Yen T. Nguyen Edalgo; Ashlee N. Ford Versypt

Oklahoma State University

One of the leading causes of cancer mortality is the acquired ability of malignant cancer cells to metastasize uncontrollably from a localized tumor to other parts of the body. Central to the metastatic migration is the mutual interaction between the cancer cell and the surrounding tumor microenvironment, particularly the extracellular matrix (ECM). During the early stage of metastatic invasion, remodeling enzymes including matrix metalloproteinases (MMPs) and lysyl oxidase (LOX) are secreted by cancer cells to degrade and crosslink collagen fibers in the ECM. Such structural alterations to collagen fibers induce changes in the overall physical and biomechanical properties of the ECM. However, questions regarding the underlying mechanism of how these changes facilitate the directional motility of cancer cells through the network of collagen fibers in the ECM remain unanswered. Employing a multiscale modeling approach, a hybrid discrete-continuous computational model is developed via the open-source software CompuCell3D to address the complex interplay between metastatic cancer cells and the ECM while the ECM undergoes chemical and physical remodeling. In this work, cancer cells are treated as discrete agents in a cellular Potts model while ECM components including collagen fibers and remodeling enzymes are modeled as a continuous system of coupled partial differential equations describing their concentrations. Results obtained from the model suggest that ECM fiber concentration is potentially a regulator of cell motility. The computational model of cancer migration addresses the influential role of remodeling enzymes MMPs and LOX and provides fundamental understanding of how ECM remodeling can affect the overall migration efficiency. Future extensions to models of this kind could potentially guide patient-specific and tissue-specific therapies by accounting for drug actions on inhibiting the effects of MMPs and LOX or altering the remodeling rate of the ECM to slow down metastasis.

Heterogeneous, multi-scale and patient-specific pharmacodynamic systems models for cancer with clinical applications

Presenter: Ghosh, Alok

Alok Ghosh, Dr. Ravi Radhakrishnan

University of Pennsylvania

Signaling pathways such as ErbB receptor mediated Ras-MAPK and PI3K-AKT and the tumor suppressor TP53 mediated DNA damage response pathways are frequently dysregulated in cancers of lung, breast, kidney etc. Mathematical modeling of these pathways provides powerful means of understanding the mechanisms of cancer progression and response to various treatment strategies. Although these pathways have been modeled individually before, to date there have been few attempts to couple them in a consistent manner. This is a challenging task due to differences in time scales, availability of experimental data and differences in modeling paradigms. Such an integrated model is of great clinical value due to its scope and its ability to test a great variety of clinically relevant scenarios. Here, we have developed a novel multiscale modeling framework that allows us to couple together systems models operating at different time scales and time resolution (continuous or discrete). Using this we were able to couple multiple pathways and to relate receptor level signaling and trafficking events to cell cycle events. microRNA expression data from actual patients were used to renormalize the initial expression levels of target mRNAs in the network thereby adding patient-specificity to our predictions. The heterogeneity of the tumor microenvironment was incorporated by adopting an ensemble-of-models approach averaging over multiple conditions of receptor expression, growth factor availability and nature of the memory coupling signaling and transcriptional modules. This modeling framework has been successfully applied

to lung cancer and nephroblastoma/Wilm's Tumor demonstrators where the model predictions in the form of cell kill and growth probabilities were used to obtain chemotherapeutic drug/radiation dosage specific Cell Kill Rates (CKR) using simple pharmacodynamic equations. The robustness of the models to internal and external perturbations were tested using global sensitivity analysis and determination of sensitivity eigenvalue spectra.

Systems biology understanding of the effects of lithium on cancer, and extension to other pharmaceuticals

Presenter: Jakobsson, Eric

Weihao Ge, Santiago Nunez-Corrales, Liudmilla Sergeevna Mainzer, Alexander E. Lipka, and Eric Jakobsson

University of Illinois at Urbana-Champaign

Lithium has many widely varying biochemical and phenomenological effects; therefore a systems biology approach is required to understand its action. Multiple lines of evidence suggest that lithium may inhibit cancer development. In this paper we take a systems approach by analyzing mutual enrichment between the interactomes of lithium-sensitive enzymes and the pathways associated with cancer. This work integrates information from multiple databases, most importantly STRING and KEGG pathways. We find that for the majority of cancer pathways the mutual enrichment between lithium sensitive interactomes and KEGG cancer pathways is many times greater than chance, with p-values <10E-3 as corrected by random resampling, reinforcing previous lines of published evidence from both epidemiology and laboratory studies. The intersections of the lithium-sensitive interactomes and the cancer-relevant pathways provide gene lists that form the core of putative lithium-sensitive cancer-specific protein-protein interaction networks. This approach is being integrated with software for inference of additive and epistatic effects among SNP's found to be correlated with phenotype, to enable comprehensive network reconstruction. While the work to date is aimed at understanding the action of lithium, the approach is readily extendable to reconstruction of networks associated with other presently used and potential anticancer drugs.

References:

Jakobsson, Eric, Orlando Argüello-Miranda, See-Wing Chiu, Zeeshan Fazal, James Kruczek, Santiago Nunez-Corrales, Sagar Pandit, and Laura Pritchet. 'Towards a Unified Understanding of Lithium Action in Basic Biology and its Significance for Applied Biology.' The Journal of membrane biology 250, no. 6 (2017): 587-604.

Ge, Weihao, and Eric Jakobsson. 'Systems Biology Understanding of the Effects of Lithium on Cancer.' bioRxiv(2018): 363077.

Angela H. Chen, Weihao Ge, William Metcalf, Eric Jakobsson, Liudmila Sergeevna Mainzer, and Alexander E. Lipka 'An assessment of true and false positive detection rates of stepwise epistatic model selection as a function of sample size and number of markers' Heredity, under review.

A novel approach for assessing diversity across single-cell landscapes

Presenter: Lau, Ken

Qi Liu, Charles A. Herring, Quanhu Sheng, Jie Ping, Alan J. Simmons, Bob Chen, Amrita Banerjee, Guoqiang Gu, Robert J. Coffey, Yu Shyr, Ken S. Lau

Vanderbilt University

Single-cell RNA-sequencing (scRNA-seq) has become a powerful tool for the systematic investigation of cellular diversity. As a number of computational tools have been developed to identify and visualize cell populations within a single scRNA-seq dataset, there is a need for methods to quantitatively and statistically define proportional shifts in cell population structures across datasets, such expansion or shrinkage, or emergence or disappearance of cell populations. Here we present sc-UniFrac, a framework to statistically quantify compositional diversity in cell populations between single-cell transcriptome

landscapes. sc-UniFrac enables sensitive and robust quantification in simulated and experimental datasets in terms of both population identity and quantity. We have demonstrated the utility of sc-UniFrac in multiple applications, including assessment of biological and technical replicates, classification of tissue phenotypes and regional specification, identification and definition of altered cell infiltrates in tumorigenesis, and benchmarking batch correction tools. sc-UniFrac provides a framework for quantifying diversity or alterations in cell populations across conditions, and has broad utility for gaining insight on how tissues are altered at the single-cell level.

Deciphering the extracellular inhibition of pro-angiogenic factors using a systems biology model

Presenter: Li, Ding

Ding Li and Stacey Deleria Finley

University of Southern California

Tumor angiogenesis is regulated by multiple pro- and anti-angiogenic factors. Anti-angiogenic agents target the interconnected network of angiogenic factors to inhibit neovascularization and tumor growth. Due to the complexity of this network, optimizing anti-angiogenic treatment requires detailed knowledge at the systems level. To better understand these interactions, we constructed a tissue-based model characterizing the extracellular network of four main angiogenic factors, including two pro-angiogenic ligands (Promoters): vascular endothelial growth factor (VEGF) and basic fibroblast growth factor-2 (FGF2), and two anti-angiogenic ligands (Inhibitors): thrombospondin-1 (TSP1) and platelet factor-4 (PF4). The model uses mass action kinetics to characterize the interactions and predicts the dynamics of 180 species, simulated by ordinary differential equations. With the model, we compared the impact of four different anti-angiogenic interactions: (a) Inhibitors directly sequester free promoters; (b) Inhibitors block promoters from binding to the co-receptors (Heparan sulfate proteoglycans); (c) Inhibitors block the coupling of co-receptors and pro-angiogenic receptors; (d) Inhibitors compete for binding sites in the basement membrane and extracellular matrix. The model predicts a counterintuitive situation, in which the secretion of inhibitors can increase the level of promoters in tumor tissue and might activate pro-angiogenic signaling pathways. Additionally, we predicted the effects of different therapeutics, including targeting pro-angiogenic ligands, co-receptors or pro-angiogenic receptors. This study provides a framework to investigate the inhibition of pro-angiogenic factors mediated by anti-angiogenic species in the extracellular space. We found that various inhibitory interactions have differential impacts on the formation of pro-angiogenic signaling complexes. The effect of endogenous anti-angiogenic factors is a mixture of the outcomes of multiple inhibitory interactions. Thus, our model helps comprehensively understand the impact of anti-angiogenic factors on pro-angiogenic signaling. In addition, the knowledge generated from our model can be translated to study the response to mimetics of anti-angiogenic factors and guide the design of therapeutics.

Heterogeneous stromal fibroblast subpopulations associated with progression of invasive lung adenocarcinoma

Presenter: Li, Irene

Stanford University

In solid tumors, tumorigenesis requires coordinated interactions by multiple cell types in the stroma. While often overlooked as bystanders, fibroblasts have been shown to be essential mediators of malignant cells' interactions with their surrounding microenvironment, contributing to tumor growth by exacerbating metabolic reprogramming, encouraging fibrosis, and triggering pro-invasive cancer signaling networks. To decipher the complex role of fibroblasts in the tumor microenvironment, we analyzed bulk RNAseq and matched clinical data from 96 LUAD patient tumor samples. We found that expression of the previously understudied gene GFPT2 is prognostically significant and overexpressed in

LUAD-associated fibroblasts of glucose-avid tumors, as determined by clinical FDG-PET/CT maximum standard uptake values (SUVmax). GFPT2 is the rate-limiting enzyme in the hexosamine biosynthesis pathway, an alternative glycolytic pathway that produces essential post-translational protein modifications. Moreover, in recent scRNAseq analyses of patient tumors that identified 5 heterogeneous clusters in LUAD-associated fibroblasts, we find that GFPT2 is differentially expressed among these clusters, indicating that specific fibroblast subtypes may direct metabolic reprogramming within the LUAD microenvironment. Utilizing in vitro co-culture models of MRC5 normal fibroblast and HCC827 LUAD cells, we demonstrated that GFPT2 expression fluctuates after fibroblasts and cancer cells are able to physically interact. To investigate other mechanisms by which LUAD-associated fibroblasts may contribute to a tumor-permissive microenvironment, we investigated the presence of collagen-rich fibrotic areas in FFPE LUAD tumor microarray, finding that GFPT2 expression co-localizes with areas of local fibrosis. In our clinical samples, GFPT2 expression also correlates with the presence of ground-glass opacity nodules, a CT radiological feature which has been associated with preinvasive, in-situ LUAD lesions. Taken together, these findings indicate that specific fibroblast subtypes may be associated with early molecular events that precede invasive LUAD. Our results show that further investigation in the role of GFPT2 may help further decipher the functional heterogeneity among LUAD-associated fibroblast subtypes and identify specific microenvironment-targeted features to aid in LUAD detection, diagnostics and therapeutics.

Targeting the root of non-genetic cancer relapse using an expressed barcode library

Presenter: Oren, Yaara

Yaara Oren, Heidie Cabanos, Aaron Hata, Joan Brugge, Aviv Regev

Broad Institute

Despite a favorable initial response, many cancer patients will experience recurrence of disease within months to years of diagnosis. Recurrence largely arises as a result of the growth of residual cancer cells that remain after treatment. The ability of a subset of cells to survive is attributed frequently to genetic heterogeneity, however recently it was shown that in multiple cancer types relapse can arise due to the presence of persister cells. Persisters are a subpopulation of transiently drug-tolerant cells that are able to survive therapy through reversible, non-mutational mechanisms. Tumor dormancy, stochastic cell state shifts and stem cell-like populations are amongst the mechanisms hypothesized to underlie persister phenotype. However, given the lack of high-throughput methods to concurrently track cell state and lineage, it is not currently feasible to distinguish the relative contribution of each of these factors. To address this need, we generated the Watermelon library. The Watermelon library is a high-complexity expressed barcode library that enables to simultaneously trace the lineage as well as the transcriptional and proliferative state of each cell in the population during drug treatment. We have applied the watermelon system to study the mechanisms underlying cell-fate decisions in response to EGFR tyrosine kinase inhibitors. We combine time lapse imaging with single-cell RNA sequencing to uncover the role that probabilistic phenotypes and transient states play in non-genetic relapse. We anticipate that this unique library, which can be applied in many different systems, would facilitate a better understanding of the cellular and molecular pathways that affect non-inherited drug resistance.

Melanoma-infiltrating macrophages differentiate from bone-marrow precursors into two F4/80+ populations with differential expression of functional markers

Presenter: Pizzurro, Gabriela

Yale University

Melanoma is a challenging disease as it progresses rapidly, and traditional treatments are rarely effective and do not improve survival. Macrophages from different tissues may have an origin from embryonic or bone marrow (BM) precursors. Tumor-associated macrophages (TAMs) can either mediate direct anti-tumor activity and antigen presentation to T cells or favor tumor development. In the present work, we studied and characterized the origin of the TAMs in a murine melanoma model and its relationship with their tumor-promoting functions. Using the injectable YUMM1.7 melanoma cell line, we analyzed the TAM populations in different mouse models for BM ablation and transplant. From the infiltrating CD11b+ population in intradermal YUMM1.7 tumors, we identified three populations according to their F4/80 expression: F4/80high, F4/80low and F4/80neg. In a conditional c-myb knock-out mice, which can induce BM precursor impaired function, we determined that all F4/80+ populations originate from BM cells. In the tumor, there were more infiltrating F4/80high TAMs. The skin exhibited both tissue-resident and BM-derived macrophages, with a predominant F4/80low population. We corroborated this result with a model of BM chemical ablation with busulfan. A major proportion of TAMs co-expressed the chemokine receptors CCR2 and CX3CR1, with differences in the F4/80-expressing populations. The BM-derived TAM populations also showed different expression of functional markers, such as Ly6C, MHCII, iNOS, CD206, Arginase-1, suggesting a differential role in tumor progression. Using these models, we characterized the origin of TAM infiltration and potential mechanisms of action of these sub-populations. A better understanding of the interactions between these TAM populations and tumor cells will help determine new strategies for the treatment of this complex disease.

Elucidating the HNSCC lymph node invasion by CODEX multidimensional imaging

Presenter: Samusik, Nikolay

Samusik, N, Black, S.M., Goltsev, Y., Chang S., Sunwoo, J., Plevritis, S.K., Nolan, G.P.

Stanford University

CODEX is a novel multiplexed immunofluorescence imaging platform that uses DNA-tagged antibodies to visualize >50 of antigens in the same slice of tissue. We have developed and vigorously validated a 54-marker human onco-immune panel. The panel comprises 38 classical immune subtype markers (CD85j, CD15, CD21, CD4, CD66, CD8, CD11c, CD54, CD7, TCRyd, CD38, CD25, Foxp3, CD40, CD45RA, CD19, CD154, CD3, CD69, CD57, CD34, IgM, CD36, CD1c, CD45, CD90, CD117, CD5, CD2, CD56, CD9, CD49f, CD16, CD123, HLA-ABC, CD127, HLADR, CD39), along with activation and immune checkpoint markers (PD1, PD-L1, PD-L2, CD40L, ICOS, CTLA-4), markers of metastasis (MMP3, 9), epithelium/stroma/basal lamina/endothelium (cytokeratin, FAP, Collagen V, podoplanin, CD31), metabolism (GLUT1, HIF1a), proliferation (CD1. which we applied to study the immune infiltrates in fresh-frozen samples of human primary HNSCC, as well as matched metastatic lymph nodes. A total of 22 HNSCC samples (17 primary, 5 matched metastatic lymph nodes) were analyzed. Using a purpose-made suite of algorithms, CODEX images were turned into single-cell expression data, where each cell is registered in XYZ coordinates and is matched with a 54-marker phenotypic profile. Automated mapping of multivariate cell phenotypes with X-shift algorithm identified systematic differences in immune repertoires between HNSCC node-negative and node-positive samples. We also, for the first time, provided an in-depth digital description of tumor-associated germinal centers, which

based or prior studies, are associated with a positive prognosis and are hypothesized to be a hallmark of systemic cancer immunity.

A mechanism for epithelial-mesenchymal plasticity in populations of cancer cells

Presenter: Tripathi, Shubham

Shubham Tripathi, Mohit Kumar Jolly, Herbert Levine, and Michael W. Deem

Rice University

Intratumoral heterogeneity, observed across cancer types, has been implicated in the failure of anti-cancer therapies. Cancer cells within a tumor can exist in distinct phenotypic states and exhibit phenotypic plasticity, transitioning stochastically between different states. Recent studies have suggested that epithelial-mesenchymal plasticity plays a critical role in the metastatic aggressiveness of cancers and in the emergence of treatment resistance via triggering of invasion and stemness programs. Molecular mechanisms underlying such behavior remain uncharacterized. Here, we considered a mathematical model of the epithelial-mesenchymal transition (EMT) decision making circuit which consists of the transcription factors SNAIL and ZEB and of the microRNAs miR-34 and miR-200. Using computer simulations of a population of cancer cells, each carrying a copy of the EMT circuit, we showed that the spontaneous transitions of cancer cells between epithelial, mesenchymal, and hybrid epithelial-mesenchymal states are a consequence of stochastic distribution of molecules among the daughter cells at the time of cell division. Our model accurately captures the experimental time scales over which cancer cells transition between states. The model characterizes the mechanism underlying the inherent plasticity of hybrid epithelial-mesenchymal cells along with their ability to rapidly give rise to a population consisting of both epithelial and mesenchymal cells. The model also captures the relative stability of epithelial and mesenchymal phenotypes. We further used our model to describe how GRHL2 stabilizes the population of cells exhibiting the hybrid epithelial-mesenchymal phenotype. We also tested how the epithelial-mesenchymal plasticity of cancer cells is altered in the presence of TGF-β and retinoic acid signaling. The predictions from our model were in agreement with previous experimental observations. Finally, we characterized the sensitivity of plastic behavior of the cancer cell population to the division and death rates of cancer cells to identify conditions that attenuate the behavior associated with metastatic aggressiveness in cancers.

Mathematical model of simultaneous tumour growth at two distant sites

Presenter: Wilkie, Kathleen

Farjana Aktar and Kathleen Wilkie

Ryerson University

Experimental data demonstrates that simultaneous injection of cancer cells at two distant sites often results in one large and one small tumour. Given that inflammation has been shown to promote tumour growth, we hypothesize that an imbalance of inflammatory cells between the two tumour sites may explain the growth rate discrepancy. To explore this, we develop a mathematical model for tumour growth subjected to a pro-inflammatory, tumour-promoting immune response. We use a multi-compartment ODE system with competition in immune recruitment between the two cancer sites. Simulated annealing is used to parameterize the model to control data (one tumour burden). We then explore the two tumour scenario through model simulations designed to perturb model parameters relating to potential biological mechanisms of action. We also explore the model and parameter space through stability and parameter sensitivity analyses.

Elucidating the role of fibroblasts in HER2-targeted therapy resistance in breast cancer

Presenter: Zervantonakis, Ioannis

Ioannis Zervantonakis, Jia-Ren Lin, Laura Selfors, Peter Sorger, Gordon Mills, Joan Brugge

Harvard Medical School

Introduction: HER2 overexpressing (HER2+) breast cancer accounts for 15-20% of all breast cancer cases, and although HER2-targeted therapies offer improved patient outcomes, advanced disease is rarely cured. Although several studies have demonstrated the role of tumor cell growth factor pathways in HER2-therapy resistance, the roles of extrinsic signals stemming from the tumor microenvironment remain poorly understood. Here, we investigate the role of fibroblasts in HER2-therapy resistance by utilizing high-throughput screening, cyclic immunofluorescence, proteomic measurements and in vivo xenograft models.

Results and Discussion: We measured the dose-response of HER2+ breast cancer cell lines to the FDA-approved HER2-targeted agent lapatinib under monoculture and direct co-culture with breast fibroblasts. Direct co-culture with fibroblasts induced lapatinib resistance in 4/7 HER2+ breast cancer cell lines. Exposure to fibroblast conditioned medium in a subset of the HER2+ breast cancer cell lines could phenocopy the effects of direct fibroblast co-culture suggesting that paracrine factors are sufficient to induce HER2-therapy resistance. Factors secreted by fibroblasts upregulated pro-survival signaling in EFM192 cells noted by high RAS/MAPK activity, reduction in pro-apoptotic proteins (BIM and BAX) and increased anti-apoptotic protein activity (MCL-1). Using a drug combination screening approach we identified that SRC inhibition reduced tumor cell viability under coculture with fibroblast and did not affect fibroblast viability or tumor cell viability under monoculture. Finally, our in vivo results of an enriched fraction of Ki67+ tumor cells in lapatinib-treated tumors at the tumor-fibroblast in vivo interface compared of untreated tumors suggest that stroma-rich areas mediate drug resistance in vivo.

Conclusion: Our studies highlight the critical role of fibroblasts in mediating drug resistance in a subset of HER2+ breast cancers that is mediated by activation of anti-apoptotic pathways. Furthermore, we identified a new combination therapy using SRC inhibitors to restore HER2-therapy sensitivity in breast cancer models that exhibit fibroblast-mediated resistance.

Poster session 2 on Friday, November 9th

Quantifying intraleukemic heterogeneity through single cell RNA sequencing

Presenter: Philipp M. Altrock

Meghan C. Ferrall-Fairbanks, Markus Ball, Eric Padron, and Philipp M. Altrock

Moffitt Cancer Center and Research Institute

Acute Myeloid Leukemia (AML) is the most common adult leukemia with an incidence of about 20,000 cases yearly and a 5-year survival of only 27.4%. Accounting for 2% of all cancer-related deaths, this aggressive malignancy is hallmarked by proliferation of immature myeloid cells in the bone marrow and life-threatening ineffective hematopoiesis. Intraleukemic heterogeneity (ILH) serves as a reservoir for resistance evolution, which often occurs due to selection of minor cellular sub-clones. However, little is known regarding whether targeted therapies (single or combination) against AML alone can ever be successful and if left untreated, the median survival of AML is measured in weeks. There exists a critical need to understand the mechanisms of mutationally-directed therapy resistance evolution. Our goal is to study molecular predictors, evolution and therapeutic consequences of ILH in hematologic malignancies. Here, we focused on individual AML patient bone marrow mononuclear cell samples, in comparison to healthy subjects, to describe heterogeneity. We use a generalized diversity measure to quantify ILH, which can characterize disease stage at the level of sub-population structures derived from single cell RNA sequencing, and we hypothesize that this measure can be used to better quantify AML tumor evolution. We developed a pipeline that can be used to analyze single cell RNA seq. data, via multi-sample normalization, clustering and mathematical interpretation. We verified this platform with clinical data. This approach enabled us to distinguish between leukemic states based on high-dimensional single cell patient samples. Our analysis shows how structured single cell RNA sequencing data can become useful when clinical sampling is combined with computational analyses and mathematical modeling.

Optimizing drug combinations to account for tumor heterogeneity

Presenter: Anchang, Benedict

Benedict Anchang, Alborz Bejnood, Kara L. Davis, Sylvia K. Plevritis

Stanford University

Currently, cancer drug combinations primarily focus on mutational heterogeneity between primary tumors and do not account for single-cell variations that can give rise to drug resistance. Moreover, even with the increasing number of potential FDA approved chemotherapies and targeted drugs including immunotherapies, methods are needed to identify better combination therapy that leverages intratumor and intertumor heterogeneity. Despite advances in single-cell technologies that capture intratumor heterogeneity, there are limited drug combination strategies that utilize single-cell platforms or integrate data from single-cell and bulk systems. We recently published an algorithm called 'DRUG-NEM', to optimize combination therapy for an individual patient by analyzing distinct single-cell drug perturbation responses on a tumor sample. DRUG-NEM is composed of three steps: (1) identify the subpopulations that makeup the tumor and may respond differently to treatment (2) reconstruct a drug-nested-effects model that integrates the drug effects across all subpopulations to capture sub-setting relationships among individual drug effects and (3) systematically score potential drug combinations to identify the regimen with the minimum number of drugs that produces the maximal desired intracellular effect. We present 2 applications of DRUG-NEM on how it uses intratumor and intertumor heterogeneity to optimize for combinations of targeted and chemotherapies on an individual and cancer subtype level respectively. The first application is based on CyTOF data generated on de-identified malignant research samples from 30 ALL pediatric patients before and after exposure to 3 targeted FDA approved single

drugs (Dasatinib, Tofacitinib and BEZ235). We found that the most common combination treatment strategy (Dasatinib and BEZ235) might not be optimal for all 30 ALL patients, with 2 of the 30 likely responding best to Tofacitinib alone. Using in-vitro survival assays, we validated that DRUG-NEM prediction of BEZ235 and Dasatinib as a potential synergistic combination on ALL cell lines. The second application is based on published curated data generated from combining patient-specific molecular measurements and 1197 x 152 patient-drug pair response information across 28 solid cancer subtypes from The Cancer Genome Atlas (TCGA). We observed that different drugs have the same or very close treatment start and end time for some patients implying possible effects of drug combinations in the study. The goal of the study is to identify the optimal chemotherapies across and within all cancer subtypes using DRUG-NEM. By replacing the subpopulation-step data with cancer-subtype-patient data within the DRUG-NEM framework, we observed that treating patients with regimens including cisplatin had a better prognosis compared to combinations without cisplatin within and across cancer subtypes. We further identified molecular signatures including DDB1, DLL4, miR-30c, miR-30e implicated in DNA repair, tumor growth and cisplatin resistance mechanism as significant predictors of drug-patient response. In summary, DRUG-NEM is a framework optimized for single-cell technologies to guide drug combination strategies. It can further stratify patient cohorts by incorporating complementary molecular data to ultimately achieve more effective therapy for the individual patient or a cohort of cancer patients with similar genetic background.

Structural variation detection by proximity ligation from FFPE tumor tissue

Presenter: Costa, Helio

Christopher J. Troll, Nicholas H. Putnam, Paul D. Hartley, Brandon Rice, Marco Blanchette, Sameed Siddiqui, Javkhlan-Ochir Ganbat, Martin P. Powers, Christian A. Kunder, Carlos D. Bustamante, James L. Zehnder, Richard E. Green, Helio A. Costa

Stanford University

Background: The clinical management and therapy of many solid tumor malignancies is dependent on detection of medically actionable or diagnostically relevant genetic variation. However, a principal challenge for genetic assays from tumors is the fragmented and chemically damaged state of DNA in formalin-fixed paraffin-embedded (FFPE) samples. From highly fragmented DNA and RNA there is no current technology for generating long-range DNA sequence data as is required to detect genomic structural variation or long-range genotype phasing.

Methodology: We have developed a high-throughput chromosome conformation capture approach for FFPE samples that we call 'Fix-C', which is similar in concept to Hi-C. Fix-C enables structural variation detection from archival FFPE samples. We applied this method to 15 clinical adenocarcinoma and sarcoma positive control specimens spanning a broad range of tumor purities.

Results: In this panel, Fix-C analysis achieves a 90% concordance rate with FISH assays - the current clinical gold standard. Additionally, we are able to identify novel structural variation undetected by other methods and recover long-range chromatin configuration information from these FFPE samples harboring highly degraded DNA. Significance: This powerful approach will enable detailed resolution of global genome rearrangement events during cancer progression from FFPE material and inform the development of targeted molecular diagnostic assays for patient care.

Hierarchical organization endows the kinase domain with regulatory plasticity

Presenter: Creixell, Pau

Koch Institute for Integrative Cancer Research at MIT

The functional diversity of kinases enables specificity in cellular signal transduction. Yet general rules for how the kinase domain allows the more than 500 members of the human kinome to receive specific regulatory inputs and convey information to appropriate substrates - all while using the common signaling currency of phosphorylation - remain enigmatic. Here, using co-evolution analysis and quantitative live-cell assays, we reveal a deep hierarchical organization of the kinase domain that facilitates the orthogonal evolution of regulatory inputs and substrate outputs while maintaining catalytic function. Three quasi-independent functional units in the kinase domain (known as protein sectors) encode for catalysis, substrate specificity and regulation, and these distinct subdomains are differentially disrupted by somatic cancer mutations and harnessed by allosteric inhibitors. We propose that this functional architecture endows the kinase domain with inherent regulatory plasticity.

Drug sensitivity relationship profiles inform design of new combination drug regimens

Presenter: Dalin, Simona

MIT

Chemotherapy resistance is a major obstacle to curing cancer patients. Since the 1950s, clinicians have employed combination drug regimens as a tactic to increase survival rates, however the current overall 5-year survival rate across all cancers is only 68% (1). Recent work has suggested that the efficacy of drug combinations is due to a population averaging effect - in each individual patient, only one drug is effective (2,3). In light of this, rational design of new combination regimens would benefit from information on cross-sensitivity relationships between drugs. Several groups have published drug sensitivity profiles to hundreds of drugs for thousands of cell lines (4,5). By comparing sensitivity to pairs of drugs within each cell line, we can identify drug pairs that are commonly cross-resistant, cross-sensitive, or have no interaction. In addition to informing design of future combination drug regimens, this information could shed light on drug mechanism of action because several types of 'omics data are available for these cell lines. For example, one would expect drug pairs with similar mechanisms of action to be cross-resistant. Drug pairs like these with unexpected lack of cross-resistance may actually be acting through different pathways. This hypothesis can be explored through studying variables in the 'omics data that diverge with sensitivity to the pair of drugs. Looking at the relationship between sensitivity to pairs of drugs will lay the groundwork for designing more effective combination drug regimens, as well as shed new light on drug mechanisms of action.

Systems biology of combination therapy in ovarian cancer

Presenter: Franz, Alexandra

Dana-Farber Cancer Institute and Harvard Medical School

Ovarian cancer is the worldwide leading cause of death of woman from a gynecologic malignancy. High-grade serous ovarian cancer (HGSOC) is the most common and most aggressive subtype of ovarian cancer. Unfortunately, patients with HGSOC respond poorly to chemotherapy and have poor overall survival. Major challenges for improving patient outcome arise from the high degree of genomic instability and the resulting inter- and intratumor genetic heterogeneity. Despite extensive efforts, few recurrent genetic alterations have been identified so far that show promise as individual therapeutic targets. However, the combination of multiple targeted drugs has not yet been explored systematically. To improve our understanding of the pathomechanisms and to design effective combination therapies,

we will integrate large-scale cellular and molecular profiling with mathematical modeling. We will perform (1) systematic drug perturbations in both HGSOC cells as well as normal cells-of-origin and comprehensively profile perturbed cells using mass spectrometry (proteins, phospho-proteins, metabolites) and imaging (growth, apoptosis). Based on these multivariate profiles, we will (2) derive computational network models to simulate the effects of numerous so-far unseen perturbations and predict cancer vulnerabilities as potential targets for combination therapies. Predicted combination vulnerabilities will be (3) tested with available small-molecule inhibitors or pooled, dual-CRISPR/Cas9 knockout screens to validate computational models and rank the most effective combinations for pre-clinical testing. This experimental-computational approach promises to uncover a multitude of cancer vulnerabilities that are not directly accessible with classical experimental screens, and will lead to the nomination of novel combination targets for clinical evaluation.

Characterizing mutation heterogeneity within and between oncogenes

Presenter: Grzadkowski, Michal

Oregon Health & Science Institute

Although the mutation profiles of some oncogenes are concentrated in a few hotspots, other genes have profiles that are considerably more diverse, with mutations taking on multiple forms spread out over many genomic locations. We sought to find evidence of genes in breast cancer whose mutations could be subdivided into two or more groupings, each characterized by a unique downstream expression perturbation profile. This turned out to be the case for at several known driver genes, including GATA3, PIK3CA, and MAP3K1. Furthermore, we discovered cases of modules of genes that contained subdivisions of mutations with similar perturbation effects despite the genes themselves having divergent perturbation effects when considered as a whole. The discovery of groupings of mutations within and between genes with expression signatures profiles separable from that of their parent genes points toward a more complete transcriptomic characterization of perturbation profiles in tumours.

Defining the network architecture coordinating double strand break repair and p53 dynamics

Presenter: Hanson, Ryan

Ryan L Hanson and Eric Batchelor

National Cancer Institute

In response to DNA double-strand breaks (DSBs) the tumor suppressor p53 exhibits undamped pulses in expression. Previous studies have demonstrated that these pulses play a critical role in regulating expression dynamics of downstream target genes and shifting these dynamics can have a significant impact on p53-mediated cell fate decisions. While it has been shown that DNA damage activated kinases shape these dynamics, the roles of specific DSB repair pathways in regulating p53 dynamics remain poorly characterized. Improving our understanding of the network architecture coordinating DSB repair and the p53-mediated stress response is crucial for expanding our knowledge of both cancer progression and potential therapeutic treatments, as these pathways are commonly mutated in cancers and targeted by chemotherapies. In this study, we targeted several DSB repair pathways through disruption of crucial repair regulators, including LigIV and PARPs associated with non-homologous end joining (NHEJ) and alternative non-homologous end joining (alt-NHEJ), respectively. Using live-cell fluorescence microscopy, we observed distinct alterations to normal p53 pulsatile dynamics in response to DSBs in conjunction with these repair pathway disruptions. In a cancer cell line, the PARP inhibitor Rucaparib increased the period of p53 pulses, and the NHEJ inhibitor SCR7 disrupted pulse synchronization. Both perturbations altered expression of several downstream cell fate mediators. In contrast, in non-transformed primary-like cells, PARP inhibition eliminated p53 pulses in response to DNA damage and generated a distinct change in cell viability. Our results suggest that perturbing p53 dynamics through selective targeting of specific DNA repair pathways in conjunction with DNA damaging therapies

provides a method for generating distinct p53-mediated cell fate responses in cancer cells versus healthy cells.

Determining the impact of p53 expression dynamics on target gene promoter activation in single cells

Presenter: Harton, Marie

National Institutes of Health, National Cancer Institute

The p53 tumor suppressor is a transcription factor that responds to various cellular stress signals and subsequently regulates distinct cell fate pathways, including cell cycle arrest, apoptosis, and senescence. Single cell studies revealed that p53 expression undergoes complex, stimulus dependent dynamics. In response to DNA double strand breaks p53 levels increase in a series of discrete pulses of fixed amplitude, duration and frequency, while in response to UV they increase in a single pulse with a dose-dependent amplitude and duration. Further studies have demonstrated that eliminating p53 pulses changes target gene expression and alters cell fate. Although it is well-established that stress signals are encoded in p53 expression dynamics, the mechanisms by which cells decode p53 dynamics into diverse target gene responses are underexplored. Here, we investigated whether cells can harness differences in p53 target promoter activation to produce distinct target gene expression patterns from a single dynamic p53 input. We developed a chemical perturbation method to independently manipulate the amplitude, duration, and frequency of the p53 pulse and simultaneously monitor target promoter activation in living single cells. Using this method, we quantified changes in the activation of two canonically regulated p53 target promoters in response to p53 pulse modulation. We identified specific signal processing features that distinguished the p53 target promoters, including: amplitude threshold, duration sensitivity, and dynamic filtering of frequency-modulated inputs. To pinpoint parameters responsible for the variation in the timing and magnitude of target promoter activation, we developed a mathematical model of promoter activation. Using this model, we discovered that only parameters involved in the recruitment of transcriptional machinery could significantly change both the activation timing and magnitude across multiple p53 pulse modulated inputs. Our study demonstrates that p53 target promoters can produce diverse target gene expression patterns from a single dynamic p53 input, suggesting that target promoter activation may be important for facilitating cell-fate decisions.

Systematic integration and network modeling of multi-omics data to derive driver signaling pathways for drug combination prediction

Presenter: Huang, Lei

Lei Huang, David Brunell, Clifford Stephan, James Mancuso, Xiaohui Yu, Bin He, Timothy C. Thompson, Ralph Zinner, Jeri Kim, Peter Davies and Stephen T.C. Wong

Houston Methodist Cancer Center and Research Institute

Driver signaling pathways with genes that show simultaneous mutation, methylation, copy number, and expression alterations are likely to play key roles in tumor progression. Drug combinations that disrupt or suppress multiple driver signaling pathways with synergistic or synthetic lethal relationships may reduce drug resistance and increase therapeutic choices. Due to the astronomical number of potential drug combinations, smart, systematic methods are needed to efficiently select the most promising combinatorial drug candidates by quantifying drug-induced targeting effects on driver signaling pathways. We have developed a computational systems biology tool DrugComboExplorer to predict driver signaling pathways, synergistic effects of drug combinations, and their potential mechanisms of action by integrating the knowledge embedded in vast amounts of publicly available pharmacogenomics and omics data. This tool processes multiple types of omics data (DNA-seq, DNA copy number, DNA methylation and RNA-seq) from individual cancer patients using an integrated pipeline of algorithms that includes bootstrap aggregating-based Markov random field, weighted co-expression network analysis, and supervised regulatory network learning to identify driver signaling networks. Furthermore,

DrugComboExplorer uses a functional systems pharmacology approach to predict effective drug combinations by capturing drug combination (15,593,320 available drug combinations) induced synergistic targeting effects on driver signaling networks from gene expression profile of 5,585 drug-treated cancer cells. Our studies on specific subtypes of prostate cancer and OCI-LY3 B-lymphoma demonstrated how synergistic drug combinations inhibit multiple driver signaling pathways. We have conducted in-vitro cell viability assays to evaluate predictive performance. Compared to existing approaches, DrugComboExplorer showed higher prediction accuracy in terms of both the percentage of experimentally validated effective drug combinations among the top predicted combinations and probability concordance index. These results demonstrate that the network-based in-silico drug efficacy screening approach enabled by DrugComboExplorer reliably prioritizes synergistic drug combinations for cancer and uncovers potential mechanisms of drug synergy, warranting further study in individual cancer patients to derive personalized treatment plans.

Modeling malignant myelopoiesis to increase efficacy of targeted leukemia therapy

Presenter: Iniguez, Abdon
University of California, Irvine

Chronic myeloid leukemia (CML) is a blood cancer in which there is dysregulation of maturing myeloid cells (granulocytes) driven by a chromosomal mutation which creates the fusion gene, BCR-ABL1. Although there has been much progress in the treatment of CML by the application of tyrosine kinase inhibitors (TKI), there are still unmet clinical needs. For example, there is still a small cohort of patients who, for reasons that are still unknown, do not respond to TKI treatment. Further, a significant proportion of patients who appear to have a complete molecular remission while on TKIs experience a relapse of CML when TKI treatment is discontinued. Mathematical modeling of CML hematopoiesis can provide insight on these processes. Here, we explore how more physiologically accurate, data-driven mathematical models of CML hematopoiesis that incorporate feedback control and lineage branching can provide such insight. Although it is recognized that feedback plays a role in CML hematopoiesis, the interactions are poorly understood. In many cases we don't know which cell types are providing and receiving the feedback, what signals are used, and what aspects of proliferative cell behavior they influence. Here, we propose to use mathematical modeling to help sort this out. We develop an automated method for model selection that integrates biological observations to select plausible classes of feedback models. We first apply this approach to normal hematopoiesis and identify models that have desired system properties, e.g., stable equilibria, and make predictions about system behavior upon perturbation. New experiments by our collaborators are shown to validate model predictions and are used to further refine the models. When extended to incorporate CML hematopoiesis, our initial assessment shows that feedback/branching models are more robust and have a better fit to alternative patterns of patient response than simple linear models.

Systems biology analysis of cancer metabolism

Presenter: Jia, Dongya
Dongya Jia, Mingyang Lu, Kwang Hwa Jung, Jun Hyoung Park, Linglin Yu, José N. Onuchic, Benny Abraham Kaipparettu, Herbert Levine
CTBP, Rice University

Metabolic reprogramming is a hallmark of cancer. It has been becoming clear that both glycolysis and oxidative phosphorylation (OXPHOS) play critical roles in various types of cancer. However, it remains elusive how cancer cells balance glycolysis and OXPHOS to facilitate metabolic plasticity and malignancy. Through coupling a gene regulatory network model with the metabolic pathways it controls, we establish a theoretical framework to study the interplay between glycolysis and OXPHOS. Our model demonstrates a direct association between the activities of AMPK and HIF-1, master regulators of OXPHOS and

glycolysis respectively, with the activities of three metabolic pathways: glucose oxidation, glycolysis and fatty acid oxidation (FAO). Moreover, our model predicts that cancer cells are able to acquire a hybrid metabolic state characterized by high AMPK/HIF-1/OXPHOS/glycolysis activities. Guided by the model, we develop metabolic pathway signatures to quantify the activities of glycolysis, FAO and the citric acid cycle of tumor samples by evaluating the expression levels of enzymes involved in the corresponding processes. By applying the pathway signatures and our previously defined AMPK/HIF-1 signatures, we confirmed their association and the existence of a hybrid metabolic phenotype at both the tumor level and the single cell level. The association of AMPK/HIF-1 activity with metabolic pathway activity, predicted by the model and verified by analyzing the gene expression and metabolite abundance data of patient samples and single cells, was further validated by in vitro studies of aggressive triple negative breast cancer cell lines. In summary, we demonstrate a direct association of the AMPK/HIF-1 activity with metabolic pathway activity and investigate the existence of a aggressive hybrid metabolic phenotype.

Investigation of the effect of cell density on early stage growth dynamics in cancer utilizing ecological principles

Presenter: Johnson, Kaitlyn

The University of Texas at Austin

While many models of tumor growth have focused on the late stages of tumor expansion, the earliest stages of tumor growth have been less well characterized. However, growth dynamics at very low tumor cell densities are of utmost clinical importance, as these determine whether a nascent lesion expands or remains indolent. We hypothesize that early stage tumor growth dynamics resemble ecological principles that guide the expansion of a species in its environment. Specifically, we ask whether the growth dynamics of tumor initiation can be described by the Allee effect, in which a species growth rate scales with its population density. To explore this hypothesis, we use an in vitro model system to measure growth rates of cells seeded at very low cell densities. Each time course is fit to a stochastic model of a random birth-death process to evaluate if initial cell density affects the estimated birth and death rates. We then fit the growth measurements from all initial cell densities to both the model of a random birth-death process and the simplest Allee effect model in which the probability of birth increases with cell number. Stochastic parameter estimation is performed in a computationally efficient manner using a moment approach. Simulations of both birth-death processes and birth-death-Allee processes validate the parameter estimation techniques. Model selection metrics are used to determine if the Allee model is a significant improvement on the classic birth-death model. To investigate if the mechanism of growth rate scaling with initial cell density is driven by cooperation between diverse cell types, we measured growth rates of clonal or multiclonal low cell density cultures. This combined experimental and computational framework may shed light on an alternative model of early stage tumor growth dynamics that could have implications for new therapeutic avenues aimed at targeting cooperation between cancer cells.

Inhibition of glutathione synthesis is synthetically lethal with glucose deprivation

Presenter: Joly, James

James H Joly, Nicholas A Graham

University of Southern California

Oncogene-directed metabolic reprogramming can render cancer cells dependent on metabolic substrates including glucose for survival. As such, the vulnerability of cancer cells to glucose deprivation presents an attractive opportunity for therapeutic intervention. However, some cancer cells, even highly glycolytic ones, are resistant to glucose deprivation. The molecular mechanisms by which cancer cells evade glucose

deprivation-induced cell death remain unknown. Here, using mass spectrometry-based metabolomics, we identified accumulation of L-cystine and L-cysteine and depletion of glutathione to be metabolic markers of glucose deprivation-induced cell death. Consistent with these observations, treatment with inhibitors of the glutamate/cystine antiporter system xc- rescued glucose deprivation sensitive cells. Building upon these findings, we probed the rate limiting enzyme of glutathione synthesis, glutamate-cysteine ligase (GCL), and found that the modifier subunit (GCLM) but not the catalytic subunit (GCLC) was limiting in glucose deprivation sensitive cells. Because starving cancer cells of glucose is not possible in vivo, we tested small molecule co-targeting of GLUT1 and GCL to mimic the inhibition of glutathione synthesis upon glucose deprivation. Indeed, inhibition of GLUT1 and GCL proved synthetically lethal in glioblastoma cells dependent on glucose for survival. These findings will serve as a basis for rational design of novel anti-metabolic treatment strategies co-targeting glucose deprivation and glutathione synthesis.

Defining early determinants of pancreatic tumorigenesis

Presenter: Juarez, Eleonora

Eleonora Juarez, Brittany Allen-Petersen, Ellen Langer, Mara Sherman, Andrew Adey, Rosalie Sears

Oregon Health and Science University

Pancreatic ductal adenocarcinoma (PDAC) is a devastating disease with a 5-year survival rate of 8%. Limited understanding of the processes that contribute to PDAC incidence and progression have hindered intervention and detection efforts. Therefore, studies focused on early events in pancreatic cancer and on identifying intervention targets are of the utmost importance. Premalignant Pancreatic Intraepithelial Neoplasms (PanINs) are the most common precursor to PDAC. Low grade PanINs are common, increase in frequency with age, and do not usually progress to PDAC. In contrast, high grade PanIN lesions are uncommon and are found almost exclusively in pancreata with invasive PDAC. The full spectrum of PanIN lesions can be found within a single pancreas in mouse models of pancreatic cancer and patient tumors, providing a unique landscape of neoplasm evolution within the same germline and host environment. Moreover, detailed characterization of PDAC progression has been limited by 1) low neoplastic cellularity, only 5-20%, and 2) early development of a dense fibrotic stroma, both of which reduce tumor-specific signals. Finally, PDAC is characterized by progressive accumulation of four common genetic alterations, and a plethora of infrequently mutated genes, none of which are currently targetable. The lack of targetable genetic alterations describing early PDAC development suggests that epigenetics plays a key role in modulating the cellular pro-neoplastic pathways underlying PDAC initiation and progression. We hypothesize that detailed investigations into chromatin regulatory networks may identify novel early detection and intervention targets. Here we applied our Single-cell Combinatorial Indexing ATAC-seq (sciATAC-seq) methodology, which maps the epigenetic chromatin landscape with single-cell level resolution, to overcome the low neoplastic cellularity, deconvolute the heterogeneous pancreatic cellular populations, and report promising follow-up targets modulating PDAC tumorigenesis.

Identifying altered intercellular signaling networks in cancer: WNT-inducible signaling pathway protein 1 (WISP1) as an illustrative example

Presenter: Klinke, David

West Virginia University

Tissue homeostasis is a dynamic process organized by multiple cell types whose relative abundance and functional disposition can change with time. To aid in understanding this dynamic process, mathematical modeling and simulation can provide insight into mechanisms that underpin system response by integrating experimental observations with prior knowledge. Our prior knowledge of biological

mechanisms, as represented by key players and their interrelationships, is based largely on normal biology. Yet, pathophysiology can change these mechanisms and create significant gaps in our understanding. In the case of cancer, cycles of mutation and selection could enable malignant cells to re-wire modes of intercellular communication within a tissue. Such network re-wiring could provide a short-term fitness advantage but place long-term survival of the organism at risk. To identify whether the intercellular network becomes re-wired in cancer, we use a multi-faceted approach. This approach combines identifying novel mechanisms using in vitro models of a cellular network, testing whether these novel mechanisms inhibit immune-mediated control of tumor growth using pre-clinical mouse models, and confirming clinical relevance of these mechanisms using human 'omics and clinical outcomes data from patient populations. Mathematical modeling and simulation is a common thread interweaved with the research foci. To illustrate this approach, I will trace the discovery of WNT1 inducible signaling pathway protein 1 (WISP1) as a suppressor of anti-tumor immunity and as a promoter of metastasis by inducing epithelial-mesenchymal transition. Specifically, we used in vitro co-culture assays and proteomics to identify that WISP1 suppresses immune cell response to Interleukin-12, an important cytokine that regulates anti-tumor immunity. In humans, WISP1 is upregulated at the invading front of melanoma and in tissue samples obtained from patients with invasive breast cancer. On-going work suggests that WISP1 suppresses anti-tumor immunity in vivo and promotes tumor metastasis by inducing epithelial-mesenchymal transition.

BioFlow: predicting systemic effect of large-scale genome perturbation

Presenter: Kucharavy, Andrei

Andrei Kucharvy, Joel Bader

Johns Hopkins University

Developing novel personalized therapies for cancer is a complex task, hindered by the entanglement of cancer pathways, differences in the genetic make-up of individual cancers, and the variety of molecular mechanisms of cancer progression. Overcoming these obstacles requires a systems biology approach to integrate high-throughput data characterizing cancers as well as experimental models with existing biological knowledge. To perform such integration, we introduce a new mathematical formalization of biological organisms. We model a biological system as a graph of directly interacting molecular entities that carry out distributed computations representing biological processes. Within this model, every function in a biological system is associated with an information flow pattern in that graph, supported by underlying nodes or edges. Disruption or perturbation of nodes and edges in the network by mutations, copy number variations, or drugs leads to changes in information flow and hence to the disruption or enhancement of functions of interest. We build a master graph of molecular interactions by integrating existing repositories of biological knowledge. We validated our approach on K14 and TWIST1-associated pathways, which were previously reported to drive cancer cell dissemination and metastases in genetically engineered mouse models. The method was effective for prioritizing candidate genes for functional validation within a large list of differentially expressed genes. It was further able to identify additional potential targets that were not transcriptionally regulated. This application revealed major information-routing hubs among differentially expressed genes driven by K14 or TWIST1 and connected them to well-known cancer genes. We anticipate that this approach could eventually lead to insights into personalized treatments and mechanisms of drug resistance. An open-source implementation of the method is available.

Determining pathway activity from multi-omics data

Presenter: McDermott, Jason

Jason McDermott, Osama Arshad, Vincent Danna, Anupriya Agarwal, Brian Druker, Bing Zhang, Karin Rodland

Pacific Northwest National Laboratory

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The availability of high-throughput multi-omic datasets for different cancer types from such efforts as The Cancer Genome Atlas (TCGA) and the Clinical Proteomic Tumor Analysis Consortium (CPTAC) promise to revolutionize prediction of cancer outcomes based on molecular-level data. A key outcome of such data is determination of signaling pathway activity, however it remains unclear how to interpret such data to achieve this goal and predict the end effect of signaling pathway activity, phenotypic outcome. In the current work we analyze a large set of matched proteomic and phosphoproteomic data from multiple different tumor types from CPTAC projects for signaling activity. We show that for some kinases, the combination of kinase protein levels and substrate phosphopeptide abundance accurately predicts activity. We show how these predictions can be connected with phenotypic outcome such as overall survival and drug resistance in ovarian and colorectal cancer, and acute myeloid leukemia. Finally, we apply machine learning methods to combine multi-omics data to assess pathway activity and its relationship outcome.

Dissecting FcγR regulation through a multivalent binding model

Presenter: Meyer, Aaron

Ryan A. Robinett, Ning Guan, Anja Lux, Markus Biburger, Falk Nimmerjahn, Aaron S. Meyer

University of California, Los Angeles

Antibodies (Abs) are crucial and central regulators of the immune response. Those of the IgG isotype interact with FcγR receptors on effector cells. IgGs transduce effector function through multiple cell types (e.g. macrophages, monocytes, etc) and through multiple processes (e.g. antigen presentation, cytokine response, phagocytosis, etc). IgGs are particularly versatile agents for cancer treatment on account of their immunotherapeutic effects as well as those of direct antigen binding. Indeed, IgG molecules comprise a broad range of approved cancer immunotherapies and targeted therapies, many of which are known to rely in part on effector cell response. At the same time, the multiplicity throughout-of constant region composition, FcγR receptors, cell populations, and antigen binding in combination-make precisely understanding, measuring, and manipulating effector function a yet-elusive goal. In this study, we show that a model of multivalent receptor-ligand binding can effectively account for the contribution of IgG-FcγR affinity and antigen-Ab complex valency. This model is able to quantitatively predict both binding and activation of individual effector populations upon stimulation with synthetic antigen-Ab complexes. This model favors the role of inhibitory FcγRs in biasing effector response to individual IgG isotypes over providing a threshold to response. Applying this model to an in vivo immunocompetent murine model, we are able to predict the ability of an anti-tumor antibody to eliminate melanoma lung metastases better than previously used metrics, as well as identify the cell populations driving response. In total, these results enable both rational design of IgGs for a desired effector function and the deconvolution of effector function mechanism on the molecular and cellular scale.

Host irradiation promotes aggressive tumors by affecting anti-tumor immunity

Presenter: Moore, Jade

Jade Moore, Lin Ma, Haoxu Ouyang, Coral Omene, Mary Helen Barcellos-Hoff

University of California, San Francisco

Anti-tumor immunity represents a target for both prevention and targeted therapy in aggressive mammary carcinoma. We used a radiation-genetic mammary chimera to evaluate the effect of radiation

on host biology in the development of mammary cancer. Here we investigated the hypothesis that host irradiation modifies anti-tumor immunity, both local and systemic, to shape the diversity of mammary cancers. Using gene expression profiling and quantitative pathology, we stratified tumors from irradiated versus sham-irradiated mice. Gene expression profiling invoked greater inflammation and decreased immune surveillance signatures in tumors from irradiated hosts. Consistent with this, tumors arising in irradiated animals had elevated cyclooxygenase-2 (COX2) gene expression and protein by immunostaining. Quantitative pathology showed that tumors arising in irradiated hosts were enriched in COX2-associated immunosuppressive myeloid cells. Tumors from irradiated hosts were also characterized by pathways associated with immune suppression as evidenced by decreased expression of cytokines and chemokines necessary for migration and recruitment of immune cells. Quantitative pathology showed that tumors arising in irradiated hosts lacked lymphocytes. Recently, human cancers have been classified by patterns of immune cell infiltrate that are called inflamed, excluded and desert. Strikingly, tumors arising in non-irradiated mice were either inflamed or excluded whereas tumors arising in irradiated hosts were either excluded or deserts; the latter of which were the fastest growing tumors. To test whether immunomodulation was critical, mice were fed caffeic acid phenethyl ester (CAPE) in standard chow. Tumors arising in CAPE-treated irradiated mice lacked tumor immune signatures and the rapid tumor growth rate evident in irradiated controls. These data suggest ionizing radiation exposure has a systemic effect that alters the anti-tumor immunity and establishes a highly significant correlation between inflammatory responses and suppression of anti-tumor immunity associated with aggressive tumors as a consequence of irradiated host biology.

Engineering a kinase toolkit for the production of recombinant, tyrosine phosphorylated proteins

Presenter: Nasir, Amjad

Amjad Nasir, Kathy Schaberg, Roman Sloutsky, Kellie Stoka, and Kristen Naegle

Washington University in St. Louis

Tyrosine phosphorylation regulates key cellular processes in eukaryotes including protein synthesis, cell division, and signal transduction. Protein tyrosine kinases (PTKs) are responsible for adding phosphate groups to target tyrosine residues (Y>pY) while protein tyrosine phosphatases (PTPs) counteract PTKs by removing phosphate moieties. Although Considerable work has been done in characterizing PTKs and PTPs as well substrate proteins, advancing the understanding of tyrosine phosphorylation in the context of cell processes is challenging because: 1) specific forms of phosphorylated proteins are difficult to make, 2) PTPs are highly active enzymes making the process transient and 3) phosphomimics, with Y>E mutations, fail to recapitulate the shape and charge of phosphotyrosines.We have developed a kinase toolkit using a bacterial expression system. The premise of this work is that phosphorylation can be driven by bringing a kinase and its substrate in close proximity, by engineering a secondary protein interaction between an SH3 domain and its polyproline ligand sequence (PxxP). We hypothesize that we can control the pattern of stoichiometries of substrate phosphorylation by altering the affinity of the secondary interaction and selecting amongst kinases with varying innate catalytic specificity. Our toolkit utilizes human SH2 domains as substrate in the development of this technology as: 1) SH2 domains are differentially phosphorylated in human cancers and 2) their function is testable (they bind phosphotyrosine-containing proteins). Substrate vectors include the choice of four PxxP sequences with varying affinity to the ABL SH3 domain fused to a constitutively active PTK (current kinases include ABL, BTK, SRC, EGFR, FAK and EPHA4). A multitude of kinases and their respective substrates can be studied using this technology and we anticipate developing the toolkit to include other post-translational modifications as well.

Overcoming adaptive drug resistance in acute myeloid leukemia using a systems biology approach

Presenter: Przedborski, Michelle

Michelle Przedborski, David Sharon, Mohammad Kohandel, Steven Chan

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Leukemia is the second most frequent blood cancer diagnosis in North America, and the most common childhood cancer. The rate of new cases of leukemia in Canada increased by 15% from 2014 to 2016 and is expected to continue to increase due to an aging population. While research since the 1990s has led to a substantial increase in the 5-year survival rate, it is now only 58% overall, and a dismal 26.8% for acute myeloid leukemia (AML) in particular. Patient prognosis can be improved with targeted therapies, whose development have recently been enabled by a better understanding of the the genomic landscape of AML. One such therapy is Venetoclax (ABT-199), which is a BH3 mimetic that selectively inhibits the anti-apoptosis protein Bcl-2, sensitizing cancer cells to apoptosis; however, ABT-199 monotherapy is often accompanied by the development of drug resistance. It was recently discovered that this acquired drug resistance can be overcome by treatment with ABT-199 in combination with the antibiotic Tedizolid. Tedizolid inhibits mitochondrial translation, leading to the activation of the integrated stress response (ISR), which is believed to be the mechanism behind the combination treatment. Here we use a systems biology approach to acquire a deeper understanding of this molecular mechanism and, in particular, of the role of the ISR in the commitment to apoptosis. Specifically, we develop a mathematical model of the ISR coupled to the apoptosis pathway and simulate the effects of combination ABT-199 and Tedizolid treatment on the coupled pathway. We validate the model using genetics data (RNA sequencing), proteomics data (Western Blot), and CRISPR knockout screen cell counts, and perform a sensitivity analysis to measure the robustness of the model to perturbations in the parameter values.

Chronic myeloid leukemia incidence based estimates of hematopoietic stem cell numbers per person

Presenter: Radivoyevitch, Tomas

Tomas Radivoyevitch, Brian Hobbs, Yasunobu Nagata, Jacob Scott, Asha Kallianpour, Takuya Sakaguchi, Jaroslaw P. Maciejewski and Yasushi Miyazaki

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Chronic myeloid leukemia (CML) risks may equal the number of hematopoietic stem cells (HSC) per person N multiplied by the risk per HSC. If N is regulated to increase only if below a threshold needed to sustain a functional hematopoietic system, N would then typically lie in a dead-band of acceptable values [Radiat Res 157 106-109 (2002)] that may have evolved to be not too high to avoid hematologic malignancy risks and not too low to have sufficient reserves in times of severe needs. This is consistent with broad variation in N across individuals, low N after marrow transplants, and N varying across races and cities. If N can be manipulated, perhaps via TET2 activity, monitoring and controlling N could become an important public health objective. Our goal is to estimate N by combining CML incidence data with BCR and ABL data. Using intron and genome sizes we estimated N by analyzing CML incidence and translocations as functions of radiation dose [Math Biosci 162, 85-101 (1999)] and age [Radiat Environ Biophys 38, 201-206 (1999)]. As CML risk is fixed by epidemiological data, increases in risks per cell due to BCR-to-ABL tethering [Chromosoma 108, 426-435 (1999)] imply lower estimates of N [Radiat Environ Biophys 40, 1-9 (2001)]. Assuming tethering, and sex differences in CML incidence being due to N rather than latency [Radiat Environ Biophys 53, 55-63 (2014)], we present city- and sex specific A-bomb survivor estimates of N. CML is suppressed in Nagasaki survivors, and thus so too is N. The reason is not known. Using our R package SEERaBomb [Leukemia 30, 285-94 (2016)] we provide evidence of chemotherapy reducing radiation-induced CML risks. HSC killing within a dead-band might

explain how chemicals lower CML risks. Space-time correlations needed to form BCR-ABL may explain why chemicals do not elevate them.

A systems approach identifies a role for RUNX1 signaling pathway in endocrine resistant breast cancer

Presenter: Shajahan-Haq, Ayesha
Virginia Tech and Georgetown University

About 70% of all breast cancer tumors are estrogen receptor positive (ER+) and are treated with antiestrogen therapies. While the inevitability of developing resistance to these therapies remains uncontested, little is known about the mechanism and prevention of resistance. Our ultimate goal is to use mathematical modeling to optimize dynamic therapies that decrease proliferation and stave off resistance. In this initial study, we used MCF7 cells as a model of ER+ breast cancer and estrogen deprivation as a surrogate for antiestrogens. We also developed long-term estrogen deprived MCF7s (LTEDs) that proliferate similarly to untreated MCF7s but are resistant to antiestrogens. We collected time-course data for gene and protein expression (NanoString Pan Cancer panel, a non-amplification based digital method) over 6 weeks to capture early molecular adaptations of deprived MCF7s that are also present in LTEDs. Principal component and correlation analyses showed a critical role for RUNX1-mediated signaling. RUNX1 and its obligate partner, CBFbeta, are transcription factors that are mutated in ER+ endocrine therapy resistant human breast tumors. Treatment with a small molecule inhibitor, which disrupts RUNX1-CBFbeta binding, showed significant decrease in MCF7 proliferation. In LTEDs, where RUNX1 levels are dramatically increased, efficacy of the inhibitor significantly decreased versus MCF7s. Furthermore, immunoprecipitation showed decreased interaction of RUNX1-CBFbeta in LTED versus MCF7 cells. This knowledge allowed us to hypothesize a molecular mechanism for resistance that can be tested experimentally. To begin building a dynamic model, we measured a 7-day time course of RUNX1 and estrogen related proteins. The model is built around ER signaling and the cell cycle, and simulates protein and proliferation changes in response to deprivation and antiestrogen (ICI182,780) treatment. Future work will involve extending the model to longer time scales and using it for treatment optimization. Targeting RUNX1 function could be a plausible strategy to inhibit endocrine resistance.

Systematic modeling communications between stromal and cancer cells in tumor microenvironment

Presenter: Sheng, Jianting
Jianting Sheng, Tsz-Lun Yeung, Hyejin Choi, Fuhai Li, Xiaohui Yu, Vivek Mittal, Samuel Mok, Stephen TC Wong.
Houston Methodist Hospital

It is long known that tumors, particularly solid tumors, are complex tissues composed of not only masses of proliferating cancer cells, but also multiple distinct cell types in the stromal tumor microenvironment. Recent studies show that dysregulation of certain ligands and exosomal miRNAs promote tumor growth, stimulate progression, and confer chemoresistance. Despite these findings focusing on specific factors, an in-depth understanding of the complex intercellular signaling pathways in tumor microenvironment using systematic approach is in urgent need to help the design of novel therapeutic strategies. In this study, we aim to establish an advanced systems biology modeling program to investigate both receptor-mediated and exosome-mediated crosstalk between stroma and cancer cells within the tumor microenvironment, together with a java based software, Cell-Cell Communication Explorer (CCCExplorer) for modeling, predicting and visualizing crosstalk signaling networks in various cancer types. We have found that activation of TGF-β-dependent and TGF-β-independent Smad signaling in CAFs was associated with poor patient survival by applying CCCExplorer on transcriptome profiles generated from microdissected cancer-associated fibroblasts(CAFs) and ovarian cancer cells. In addition, several known and novel tumor-stroma paracrine and tumor autocrine crosstalk-signaling pathways were

found in non-small cell lung cancer based on RNA deep-sequencing analysis of individually sorted myeloid lineage and tumor epithelial cells. On the other hand, we have identified several miRNAs and long non-cdoing RNAs that are enriched in CAF derived exosomes. Further analysis showed that multiple signaling pathways were altered in ovarian cancer cell lines treated with CAF derived exosomes compared to the treatment of exosomes derived from normal fibroblasts (NF). Using cell type-specific expression profiles for the identification of protein and non-coding RNA mediated communications between stroma and cancer cells would shed light on our understanding of cancer initiation, progression and drug resistance, and has the potential for development of therapeutic strategies that target tumor-stroma interactions.

Substrate-based kinase activity inference identifies global signaling differences between K-Ras alleles

Presenter: Strasser, Samantha

Samantha Dale Strasser, Emily J. Poulin, Joao A. Paulo, Alina Starchenko, Yi-Jang Lin, Douglas K. Brubaker, Brian A. Joughin, Rebecca DeStefanis, Phaedra C. Ghazi, Jessica J. Gierut, Steven P. Gygi, Douglas Lauffenburger, Kevin M. Haigis

Massachusetts Institute of Technology, Harvard Medical School, Beth Israel Deaconess Medical Center, Massachusetts General Hospital

Phosphoproteomic data generated by global mass spectrometry (MS) contain high-content information on protein phosphorylation, a process central to cellular signaling. We employ this data to provide fresh insights into new therapeutic approaches for mutant K-Ras cancers. As the biological function of the vast majority of phosphorylation sites remains unknown we developed Substrate-based Kinase Activity Inference (SKAI), a methodology to infer kinase activity from phosphoproteomic data. We draw upon prior knowledge of kinase-substrate interactions to construct custom lists of kinases and their respective substrate sites, termed kinase-substrate sets. Using these sets within the Gene Set Enrichment Analysis (GSEA) framework, we infer kinase activity from global phosphoproteomic data comparing mouse tissues engineered to express A146T or G12D mutant K-Ras. Results identify many differences between the signaling pathways regulated by each allele. This provides a foundation to direct future studies that investigate allele specific therapeutic targets.

Pediatric Cancer Systems Biology at UT Southwestern Medical Center

Presenter: Welf, Erik

Erik Welf, David Saucier, Dagan Segal, Bo-Jui Chang, Reto Fiolka, James Amatruda, Gaudenz Danuser

UT Southwestern Medical Center

At UT Southwestern Medical Center, we are building a research program for which the primary goal is to understand the mechanisms of functional adaptation in Ewing sarcoma as a prototypical model for childhood cancers and to determine how this functional adaptation influences metastatic propensity at the single-cell level. Ewing sarcoma presents an opportunity to study functional adaptation outside of typical genomic evolution because Ewing sarcoma is caused by a single gene fusion. Despite this genomic simplicity, Ewing sarcoma cells are surprisingly diverse and functionally plastic. To study this functional plasticity, we are applying an integrated approach that combines single-cell genomic and transcriptomic analyses with live cell imaging and high-dimensional computational analysis to connect cellular mechanisms with cell fate in vivo. We will present our preliminary efforts at characterizing single cell transcriptomic heterogeneity in Ewing sarcoma and identifying cell subtypes with altered signaling pathways that support metastatic dissemination. To complement in vitro cell studies, we use a zebrafish xenograft model to evaluate metastatic efficiency in different populations of cells and in different zebrafish tissues. By virtue of its optical transparency, the zebrafish embryo offers the ability to image cells at subcellular resolution in vivo, allowing us to test the hypothesis that specific cell signaling states determine cell fate. We are developing 3D analytical approaches to characterize the complex 3D signaling

and morphological patterns exhibited by cells in vivo, and we use high resolution light sheet fluorescence microscopy to measure cell signaling in xenografted cells. Finally, we are developing algorithms to integrate all of the data sets generated by our team, including genomic, transcriptomic, cell signaling, and cell fate in order to identify the cellular programs that facilitate metastatic dissemination.

NetBID, a novel systems biology approach, identifies Hippo signaling as a 'hidden' driver selectively programming CD8α+ dendritic cell activity

Presenter: Yu, Jiyang

Xingrong Du, Jing Wen, Yanyan Wang, Peer W. F. Karmaus, Alireza Khatamian, Haiyan Tan, Yuxin Li, Cliff Guy, Thanh-Long M. Nguyen, Yogesh Dhungana, Geoffrey Neale, Junmin Peng, Hongbo Chi & Jiyang Yu

St. Jude Children's Research Hospital

CD8α+ dendritic cells (DCs) present antigens to CD8+ T cells and play a pivotal role in activating cytotoxic T cell responses to viruses, bacteria and tumors. Although lineage-specific transcriptional regulators of CD8α+ DC development have been identified, the molecular pathways that selectively orchestrate CD8α+ DC function remain elusive. In order to identify subset-specific regulators between CD8α+ and CD8α- DCs, we developed a systems biology approach, data-driven Network-based Bayesian Inference of Drivers (NetBID), by integrating transcriptomic, whole proteomic and phosphoproteomic data. Specifically, we computationally reconstructed a DC-specific signaling Interactome (DCI) from a collective cohort of gene expression profiles of total DCs using information theory-based approaches. Next, we superimposed the data-driven DCI with transcriptome, as well as whole and phosphoproteomes of CD8α+ and CD8α- DCs profiled via tandem-mass-tag (TMT) and mass spectrometry technologies. Our NetBID analysis reveals a marked enrichment of the activity of Hippo pathway kinases in CD8α+ DCs relative to CD8α- DCs, although the expression levels of the individual molecules show little changes. For functional validation, we applied genetic models to disrupt Hippo signaling in DCs via the CD11c-Cre deletion system. We found that CD8α+ DCs deficient in Hippo signaling are impaired in presenting extracellular proteins and cognate peptides to prime CD8+ T cells in vivo and in vitro, but show no defects in priming CD4+ T cells. In contrast, the mutant CD8α- DCs are functionally intact in priming CD8+ and CD4+ T cells. Moreover, mice with DC-specific deletion of Hippo signaling show impaired anti-tumor immunity in vivo. Our results highlight the power of NetBID in identifying 'hidden' drivers in the immune system, which can be further applied to any similar biological problems. Reference 1. Du et al, (Yu & Chi). Hippo/Mst signalling couples metabolic state and immune function of CD8alpha(+) dendritic cells. Nature. 2018;558(7708):141-5. PMID: 29849151.

Network modeling of drug resistance mechanisms and drug combinations in breast cancer

Presenter: David Wooten on behalf of Jorge G. T. Zanudo

Jorge G. T. Zanudo, Reka Albert, and the SU2C-The V Foundation-NSF Drug Combinations Convergence Team

Pennsylvania State University/Dana-Farber Cancer Institute

Durable control of invasive solid tumors is thwarted by the lack of knowledge of effective drug combinations and of the acquired and intrinsic resistance mechanisms of drugs. In an effort to tackle this problem, the SU2C-NSF-TVF Drug Combination Convergence Team is using mechanistic models of cancer cell signaling based on therapeutic and cell line data in order to identify elements within cancer cells that might eventually be exploited through therapeutic combinations. Here we present a comprehensive mechanistic network model of signal transduction in ER+ PIK3CA-mutant breast cancer. Focusing on PI3K inhibitors, the model recapitulates known resistance mechanisms and predicts other possibilities for resistance: loss of RB1, FOXO3, P27, or PRAS40. To test these predictions, we analyzed genome-wide CRISPR screens of two breast cell lines in the presence of PI3K inhibitors and found that

the predicted genes were significantly enriched in the screens. Some of these resistance genes (e.g. loss of RB1) were found to be cell-line specific and follow-up experiments in RB1-KO cells confirmed the cell-line-specific nature of PI3K-inhibitor resistance. The model also reveals known and novel combinatorial interventions that are more effective than PI3K inhibition alone. For example, the model predicts that the combination of PI3K inhibitors with inhibitors of anti-apoptotic proteins MCL1 or BCL2 would be effective. Follow up experiments in cell lines confirmed that MCL1 inhibitors enhance the effect of PI3K inhibitors and that this combinatorial effect is cell-line-specific, similarly to what was found in the resistance genes case. In conclusion, the model predicted drug resistance mechanisms and effective drug combinations, some of which were verified experimentally and found to be cell-line-specific. Next iterations of the model will incorporate the identified discrepancies, the newly identified resistance mechanisms to drugs of clinical interest, and the results from cell death and BH3 profiling experiments in response to these drugs.