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Poster Abstracts

A01 Progression-dependent transport heterogeneity of breast cancer liver metastases as a factor in therapeutic resistance

Ziemys, Arturas; Yokoi, Kenji; Kai, Megumi; Liu, Yan Ting; Kojic, Milos; Simic, Vladimir; Milosevic, Miljan; Holder, Ashley; Ferrari, Mauro

Metastatic disease is the major cause of mortality in most cancer patients. While many drug delivery strategies for anti-cancer therapeutics have been developed in pre-clinical studies using tumors implanted orthotopically in the primary site or ectopically into subcutaneous sites, the drug delivery properties of metastatic tumors have not been well investigated. Furthermore, therapeutic efficacy may hinge on efficient drug permeation into the tumor microenvironment, known to be heterogeneous, and thus potentially making drug permeation heterogeneous also. In this study, we show that doxorubicin does not permeate well into 4T1 liver metastases because of unfavorable and heterogeneous transport properties of these tumors. These drug extravasation results are distinct from analogous studies with 4T1 tumors growing in the primary site. A probabilistic tumor population model was developed to estimate drug permeation efficiency and kinetics of liver metastases. The parameter τ was introduced that integrates transport and structural properties of tumors and drugs based on in vivo and imaging studies. Our results demonstrate significant heterogeneity in metastases in terms of transport properties and doxorubicin delivery in the same animal model and even in the same organ. Diffusion into the extravascular space was found to be the key barrier for efficient drug delivery to metastases. The results also suggest that the level of heterogeneity depends on the stage of tumor progression and that differences in transport properties can define transport-based phenotypes of tumors, i.e. transport phenotypes. Therefore, therapeutics can permeate and eliminate a certain transport phenotype of metastases sparing tumors with more challenging transport properties that render these surviving tumors resistant to repeated treatments in view of drug delivery. We anticipate that our results may challenge current paradigms of drug delivery into metastases and highlight potential caveats for therapies that may alter tumor perfusion.

A02 Fructose Fuels Metabolic and Epigenetic Reprogramming of Liver Metastasis

Bu, Pengcheng; Chen, Kai-Yuan;Shen, Xiling

Cancer metastasis accounts for the majority of cancer-related deaths and remains a clinical challenge. Current in vitro models of metastasis tend to focus on testing the invasion and migration potential of cancer cells. However, recapitulation of the metabolic microenvironment of the metastatic organ has been largely overlooked, despite emerging evidence suggesting that the growth of micrometastases in the newly colonized organ may be a rate-limiting step for metastasis. Metastatic cancer cells generally resemble cells of the primary cancer, but they may be influenced by the microenvironment of the organs they colonize. Based on meta-analysis of extensive clinical datasets and in vivo orthotopic-metastasis models, we show that colorectal, breast, and pancreatic cancer cells undergo metabolic reprogramming after they metastasize and colonize the liver, a key metabolic organ. In particular, metastatic cells in the liver up-regulate the enzyme aldolase B (ALDOB) and ketohexokinase (KHK), which enhances fructose metabolism and provides fuel for major pathways of central carbon metabolism, nucleotide synthesis, and lipid synthesis during tumor cell proliferation. Fructose consumption is also shown to influence surrounding hepatic cells to create a more amicable milieu to metastatic tumor cells. Moreover, the metastatic cancer cells undergo specific epigenetic modification around metabolic enzyme genes involved in lipid and branched chain amino acids (BCAA) synthesis, hence perpetuating the reprogrammed metabolism. Targeting the fructose metabolism enzymes or reducing dietary fructose significantly reduces liver metastatic growth but has little effect on the primary tumor. Our upcoming collaboration with Pfizer enables us to test their newly developed, the first potent and specific small molecule inhibitor against the fructose enzymes in our patient-derived preclinical liver metastasis models. Our findings suggest that metastatic cells can take advantage of reprogrammed metabolism in their new microenvironment, especially in a metabolically active organ such as the liver. Manipulation of involved pathways may impact the course of metastatic growth. As part of our Cancer TEC U01 effort, we are developing new in vitro liver metastasis organ-on-a-chip models that better recapitulate the metabolic microenvironments of the primary and metastatic organs, which will provide a powerful tool for research and drug discovery.

A03 Cell-cell junction integrity and adaptive cellular contraction regulate gap formation in endothelial network

Cao, Xuan; Moeendarbary, Emad; Kamm, Roger; Shenoy, Vivek

Endothelial cell-cell junctions are essential for maintaining the endothelium integrity and have crucial roles in inflammation, wound healing and tumor metastasis. However, the mechanism behind how the endothelial network maintains its integrity remains unclear. In this study, we developed a chemomechanical model to elucidate how the two-way feedback loop between cell contractility (induced by activity of chemomechanical interactions such as

Rho signaling pathways) and intercellular junction binding status enables the endothelial network to maintain its integrity. Both our model and experimental results show that due to the larger intercellular forces generated by the endothelial cell contraction, the endothelial vertices have a higher risk of disruption than the borders. The disruption initiates at the vertex and develops along the border. The two-way feedback mechanism enables the endothelial cells to sense the variations in the junction binding status, adjust cellular contractility correspondingly, followed by the healing procedure. The model was tested and validated using experimental measurements on human umbilical vein endothelial cells (HUVECs) cultured on collagen substrates. Our model provides a theoretical framework to study endothelial barrier function under different physiological states and extracellular stimuli.

A04 Single-cell analyses reveal enhanced OXPHOS transcription for adaptation responses of prostate cancer cells in circulation

Lin, Chun-Lin; Tan, Xi; Hung, Chia-Nung; Osmulski, Pawel A; Chen, Meizhen; Gaczynska, Maria E.; Chen, Chun-Liang; Liss, Michael A.; Huang, Tim.

Circulating tumor cells (CTCs) undergo considerable adaptation for oxygen depletion and physical stress in the bloodstream. To further understand the underlying phenomena, we conducted genomic, transcriptomic, or biophysical analyses of 289 primary tumor cells shed in urine and 401 CTCs in blood of prostate cancer patients. Of 449 focal genomic alterations analyzed, increased or decreased copy-numbers of 113 recurrent regions were found in CTCs relative to primary tumor cells. These regions harbored amplified genes associated with oxidative phosphorylation and metabolism pathways that are exploited by hypoxic CTCs for energy fuels. In addition to epithelial-mesenchymal transition, upregulation of genes linked to apical junction can maintain membranous plasticity and adhesiveness of CTCs. Primary tumor cells with these pre-existing genomic alterations can be adaptively advantageous and selected for survival in circulation. These exfoliated cells are soft, deformed, and adhesive in shape, thus increasing their ability to tether to companion cells for distant colonization.

A05 A Model of Invasion and Intravasation from a Solid Breast Tumor into a Micro-Lymphatic Vessel

Usman Ghani, Allison K. Simi, Andreas P. Kourouklis, Siyang Han, Emily A. Margolis, Celeste M. Nelson, and Joe Tien

The invasion of tumor cells into surrounding tissue and their intravasation across an endothelium are two important steps in the metastatic cascade. To model these processes in culture, we created microscale solid tumors of MDA-MB-231 human breast carcinoma cells, near blind-ended vessels of primary human lymphatic endothelial cells (LECs). The tumors and lymphatics were formed within micromolded type I collagen gels and were separated by an initial minimum distance of 60-300 μm. Fluid pressure was applied to the lymphatic side on day 2 to promote tumor cell invasion. We found that tumors would invade the

collagen but not intravasate into a lymphatic if the lymphatic and tumor were separated by >150 μm. When the separation was <150 μm, invasion was followed by intravasation in ~40% of the samples within nine days after flow was applied. Surprisingly, the frequency of tumor cell escape into unseeded, blind-ended channels was similar to that into lymphatics. Our model successfully recapitulates invasion and intravasation from a solid breast tumor to a micro-lymphatic. The strongest determinants of intravasation efficiency are tumor-to-lymphatic separation distance and time in culture.

A06 Ensemble trajectory analysis of cell-state transition pathways in colonic tumors

Herring, Charles; Scurrah, Cherie; McKinely, Eliot; Kumar, Manu; Simmons, Alan; Banerjee, Amrita; Li, Wei; Lauffenburger, Douglas; Coffey, Robert;Lau, Ken

The proliferation of high throughput single-cell technologies that can profile thousands of cells has led to an explosion of studies utilizing these techniques. However, analyses that move beyond cell type identification from these high-resolution data are only emerging. Here, we present p-Creode, an computational tool for mapping cell-state transitions from multiplex single-cell data by leveraging an ensemble of trajectories to enable a probabilistic framework. We apply p-Creode to deconstruct the transitional program of different colorectal cancer models from single-cell RNA-seq data. We quantify the relative stability of these program, which illuminates the functions and behaviors of stem-like cells within the tumors. Our approach adds a layer of depth to characterizing the plasticity of cell states present colorectal cancer, and potentially for understanding heterogeneous responses to therapeutic interventions.

A07 Extracellular Matrix Density Triggers Phase Transition in Breast Cancer Spheroids

Ferruzzi, Jacopo; Koehler, Stephan A.; Kim, Jessica; Roblyer, Darren; Fredberg, Jeffrey J.; Zaman, Muhammad H.

It is well established that cancer cell migration is rooted in the biomechanical and mechanobiological interactions between cells and the extracellular matrix (ECM). Migrating cells sense and respond to matrix structure and mechanics by displaying different modalities of invasion, including single and collective migration. Here, we show that tumor invasion can be viewed in terms of phase separation, wherein solid tumors possess a nearly jammed core from which cells escape via a phase transition. In addition to the expected differences in tissue invasion between non-tumorigenic and metastatic cells, we were surprised to find that drastically different migratory phenotypes can be obtained by modest changes in matrix density. Multicellular tumor spheroids were obtained from MCF-10A and MDA-MB-231 breast cancer cells, and subsequently embedded within three-dimensional (3D) collagen gels at different concentrations (2 and 4 mg/mL). Live imaging of spheroid invasion was performed using a spinning disk confocal setup, while optical clearing and multiphoton microscopy allowed us to investigate spheroid morphology and collagen

remodeling due to cell contractility and proliferation. Differences in collagen structure and mechanics were investigated combining high resolution microscopy, confined compression testing, and continuum biphasic modeling. We found that increasing collagen concentration impacts the hydraulic permeability (a metric directly related to matrix porosity) rather than the compressive stiffness of the matrix. Analyses of cellular migration and spheroid morphology revealed that tumor spheroids possess a solid-like core and, by varying collagen density, we can control their migratory response. In low density collagen, MDA-MB-231 cells escape individually from the core, adhere to collagen fibers, and migrate through the 3D porous network. Instead, MDA-MB-231 in high density collagen behave like MCF-10A cells in low density collagen, that is they migrate as a fluid-like multicellular collective and form protruding arms. Finally, MCF-10A cells in high density collagen tend maintain a spherical morphology with no signs of matrix invasion. These drastically different individual and collective modalities of migration from a cellular cluster are reminiscent, respectively, of the physical processes of sublimation and melting. If confirmed by further studies, this phase separation analogy could provide a new physical picture to study cancer invasion and metastasis.

A08 Phenotypic Drift as a Survival Strategy in BRAF-mutant Melanoma

Hayford, Corey; Paudel, Bishal; Harris, Leonard; Tyson, Darren; Al'Khafaji, Aziz; Johnson, Kaitlyn; Brock, Amy; Quaranta, Vito

Tumor heterogeneity has been known to contribute to diverse patient outcomes in response to targeted therapies. It has primarily been studied in the context of genetic mutations that are either pre-existing or acquired in response to drug treatment. Here, we have discovered signs of pre-existing differences in isogenic BRAF-mutant melanoma cell sublines, both on the proliferative and molecular levels. This non mutationally defined, pre-existing plasticity has led us to hypothesize that cancer cell populations hedge their bets amongst multiple phenotypic states in order to ensure population survival. In addition, we have evidence that these sublines begin to diversify into several proliferative states over time in drug naïve conditions, which we think is due to subtle changes in the molecular fingerprint of single cells. Using a novel guide RNA barcoding technology, in conjunction with single-cell RNA sequencing, we have begun to unravel distinct transitions between molecular states which we plan to correlate to proliferative phenotypes.

A09 Engineering Invasive Human Breast Tumors with Integrated Capillaries and Lymphatics

Joe Tien, Celeste M. Nelson, Derek C. Radisky, Kamil L. Ekinci, and Aziza Nassar

This U01 project seeks to develop and apply new methods of engineering vascularized tumors in vitro, in which the cellular, physical, and genetic composition of the tumor and its microenvironment can be controlled with high spatial and temporal resolution. The collaborative team consists of experts in biomaterials and tissue engineering (Tien), quantitative developmental and tumor biology (Nelson), mechanics (Ekinci), and clinical

tumor biology (Radisky) and pathology (Nassar). The core enabling technology, which we have been developing over the past sixteen years, is the use of three-dimensional (3D) micropatterned extracellular matrix hydrogels as scaffolds for directing the 3D organization of engineered tissues. The project is currently in Year 2 of the award. The main accomplishments of the project to date are: 1) We have created microscale human breast tumors that are located near a blind-ended lymphatic vessel or unseeded cavity in a type I collagen gel. Under appropriate interstitial pressure conditions, the tumor cells invade the surrounding matrix and escape into the lymphatic or cavity. We are currently determining the biophysical and biochemical signals that modulate tumor cell escape. 2) We have developed microscale vascularized adipose tissues that respond to lipogenic and lipolytic signals by growing or shrinking, respectively. These tissues will be used to provide fibrofatty stroma for the models in which we examine tumor cell invasion and escape. 3) We have shown that non-invasive spectroscopic imaging can map the gross biochemical composition of the microscale tumors and their surrounding interstitial fluid. This imaging technique will be used to provide longitudinal data for correlation with tumor cell behavior.

A10 Acidification or Glycolysis: Which came first?

Russell, Shonagh; Xu, Liping; Kam, Yoonseok: Damaghi, Mehdi; Lopez, Alex S.; Abrahams, Dominique; Epstein, Tamir; Ruiz, Epifanio; Lloyd, Mark C.; Wojtkowiak, Jonathan W.; Bui, Marylin; Gillies, Robert

Otto Warburg (1926) described that cancer cells ferment glucose (glycolysis), even in the presence of oxygen (Warburg Effect). Glycolysis produces minimal ATP but maximal acid and it is puzzling why cancers choose glycolysis when it is energetically unfavorable compared to oxidative phosphorylation. We argue the acidity generated by glycolysis benefits cancer by enhancing degradation of the extracellular matrix, metastasis, and inhibiting immune surveillance. Buffer therapy neutralizes tumor acidosis reducing metastasis, invasion, and promoting immune surveillance in some models. Thus, evolutionary selection of acid production impacts the success of cancer. To date, most cancer models assume acid production is a byproduct of upregulated aerobic glycolysis. However, we propose an alternative hypothesis that upregulated aerobic glycolysis is a consequence of increased acid production. To solve this, we investigated (to date) two model systems. We first used an artificial system, a proton pump, the yeast protein plasma membrane ATPase 1 (PMA1). We stably over-expressed PMA1 in a non-glycolytic, lowly invasive human breast adenocarcinoma cell line, MCF7. Over-expression of PMA1 resulted in a more aggressive, invasive, and glycolytic phenotype with increased metastasis observed in vivo. In these PMA1 over-expressing tumors, IHC identified a reduction in carbonic anhydrase IX (CA-IX) expression, compared to mock controls, suggesting that CA-IX is redundant with PMA1, as both facilitate the export of H+. Hence, our second model chosen was CA-IX, a membrane bound protein that reversibly hydrates extracellular CO2 into H+ and HCO3-. CA-IX is a negative prognostic indicator in multiple cancers with minimal expression in normal tissue. Over-expression of CA-IX in MCF-7 cells produced a stable glycolytic phenotype with increased acidification; increased glucose uptake; and increased

lactate production. In experimental metastasis studies the CA-IX expressing clones formed metastasis in the lung, whereas the mock and parental clones did not. Subsequently, buffer therapy to reduce acidosis in the tumor significantly reduced lung metastasis. We propose the enzymatic activity of CA-IX 'sucking in' glucose, drives glycolysis to ensure CA-IX can maintain acidification of the extracellular space and provide buffer to neutralize intracellular pH. Therefore, acid producing proteins alone can alter cancer metabolism and phenotype, resulting in poorer patient outcome.

A11 Interstitial fluid pressure signals through YAP to direct invasion of engineered human breast tumors

Kourouklis, Andreas; Siyang, Han; Allison, Simi; Tien, Joe; Nelson, Celeste;

Breast tumors have elevated interstitial fluid pressure (IFP), which in turn influences the migration of cells from the tumor to the surrounding tissue. To dissect the mechanotransduction pathways that regulate invasion in response to IFP, we have engineered 3D breast tumor models that allow application of defined pressure across a tumor of defined geometry. In brief, we embed MDA-MB-231 human breast cancer cells in a 120-μm-diameter cavity that is molded within a type I collagen gel. The multicellular aggregates are then subject to gradients of hydrostatic pressure through opposing reservoirs of culture media that are located at the base (Pbase) and the tip (Ptip) of the tumor. Because YAP has been implicated in mechanotransduction, we compared the effects of IFP on invasion from tumors of control MDA-MB-231 cells and those that were depleted of YAP (shYAP). Under IFP gradients that promote tumor invasion (Ptip > Pbase), we found that depletion of YAP reduced the frequency of collective cell invasions. We are currently defining the downstream targets of YAP that are affected by IFP, as well as the forces exerted by the tumor as it invades.

A12 Modeling the role of the atypical Notch ligand Dll3 in small cell lung cancer

Kim, Jun; Sage, Julien

Notch receptors interact with their neighboring cells (trans-interaction) by binding to their specific ligands on the plasma membrane. This triggers the associated downstream signaling proteins, which in turn, regulates Notch receptor and ligand expression, creating a feedback loop. Notch receptors have also been shown to bind to its coexpressed ligands (cis-interaction) as another mode of regulating signaling. Dysregulation of Notch pathway contributes to onset and maintenance of several diseases. In the context of small cell lung cancer (SCLC) our group and others have shown that Notch signaling affects tumor initiation, growth, and heterogeneity. Among Notch ligands, delta-like ligand 3 (DLL3) is a highly divergent member that has been shown to be overexpressed by SCLC cells with neuroendocrine phenotype and have been shown to be a therapeutic target for SCLC. We will describe our current effort to include DLL3 in canonical mathematical models of Notch signaling to predict the functional role of DLL3 in SCLC.

A13 Novel chemotherapy stable subpopulations are conserved across multiple Small Cell Lung Carcinoma Patient Derived Xenograft Models.

Lehman, Jonathan; Leelatian, Nalin; Harris, Bradford; Hoeksema, Megan; Yong, Zoug; Staub, Jeremy; Senosain, Maria; Doxie, Deon; Irish, Jonathan; Massion, Pierre

Introduction: Small cell lung cancer (SCLC) is a high grade neuroendocrine carcinoma of the lung responsible for up to 25% of lung cancer deaths and the 6th leading cause of cancer death. SCLC initially responds well to chemotherapy, but inevitably recurs even after initial complete responses. The etiology of this relapse is likely secondary to tumor heterogeneity and/or chemotherapy resistance subpopulations reconstituting tumor. Characterization of these resistant subpopulations could yield novel therapeutic targets for SCLC treatment. Mass cytometry uses metal labeled antibodies to profile expression and phosphorylation of multiple proteins in a single cell and offers the opportunity to identify new subpopulations as targets for novel therapies in SCLC. Methods: Nude mice with SCLC patient derived xenografts (PDXs) were treated with one cycle of carboplatin/etoposide or saline injection. PDX samples were stained with a 26-30 marker panel and an intercalator dye to identify nucleated cells. This panel measured phospho-signaling, neuroendocrine, immune, and mesenchymal cell markers, and functional markers including ki67 and cleaved caspase 3. Mouse cells, including leukocytes, were excluded using mouse MHC1 gating and Histone H3 was used to identify nucleated cells Single cell protein expression and phosphorylation was analyzed using viSNE, manual gating, as well as unsupervised clustering approaches with SPADE to identified multiple subpopulations with neuroendocrine and non-neuroendocrine features. Subpopulations were compared across multiple patient derived xenograft (PDX) models with and without chemotherapy treatment. Results: Patient derived Xenograft (PDX) tumors across 3 distinct models including with and without a single cycle of chemotherapy treatment released viable tumor and stromal cells suitable for cryopreservation and mass cytometry. Chemotherapy treated tumors had dramatic changes in subpopulation distribution compared to matched mock treated tumor. This included enrichment in EPCAM+, CD24+, CD44- progenitor like subpopulations. Similar patterns of population shift were observed in multiple models. Of note, chemotherapy stable subpopulations were conserved across 5 different PDX models including SOX2+ and Oct ¾+ tumor populations. These subpopulations sorted similarly in multidimensional space in multiple PDX models suggesting conserved origins. Conclusions: Mass cytometry was able to identify multiple NE and non-neuroendocrine cell populations from SCLC PDXs and characterize their signaling include rare subpopulations with stem like signaling factors of interest. Chemotherapy treated PDX had differential subpopulation distribution with enrichment of progenitor like cells with chemotherapy treatment similar to previous work in mouse genetic SCLC. However, rare conserved chemotherapy stable subpopulations enriched in stem-like signaling factors were identified across 5 PDX models including those with and without chemotherapy treatment. This work raises the possibility that chemotherapy stable subpopulations may contribute to progenitor populations which lead

to relapse. Future work in this lab will focus on characterizing the stem like features of these subpopulations and their phenotypic dynamics and signaling.

A14 Deciphering evolution of resistance to targeted therapies

Robert VanderVelde, Nara Yoon, Jakob Scott and Andriy Marusyk

Background/Rationale: Therapies that target oncogenic kinases elicit dramatic clinical responses in susceptible tumors, but ultimately fail in advanced cancers. Massive research efforts in the field of molecular oncology rapidly advance our understanding of proximal mechanisms of resistance, but because of evolvability and heterogeneity of tumors, these studies are unlikely to provide more than incremental improvements. On the other hand, evolutionary informed therapies hold promise to dramatically advance our ability to control tumors without digging deeper into molecular mechanisms or developing new targets/drugs. However, development of these therapies is contingent on adequate understanding of the underlying evolutionary dynamics, including the impact of non-cell autonomous interactions and microenvironmental protective niches - an area of knowledge which still remains terra incognita. Objective: To interrogate the evolutionary dynamics of the development of acquired resistance to therapies targeting oncogenic tyrosine kinases, focusing on ALK inhibitors in lung cancers. Results: To start closing the glaring gap of knowledge, we decided to focus on drugs, that inhibit abnormal tyrosine kinase activity in ALK+ lung cancers (ALK TKIs) - a poster child of success and failure of targeted therapies. Using an in vitro cell line model, we found that resistance develops from pre-existent drug-tolerant populations, which evolve in the presence of the drugs, via incremental acquisition of multiple individual resistance mechanisms, which gradually and cooperatively improve fitness. Cancer associated fibroblasts, one of the major cellular component of tumor microenvironment, substantially reduce sensitivity of treatment naive tumor cells to most of the ALK inhibitors in a spatially dependent manner, thereby limiting selective advantage of resistant cells and impacting the evolutionary dynamics. Conclusions: Acquisition of resistance to ALK TKI involves intermediate, partially resistant intermediates, which might 'lubricate' ability of populations of tumor cells to evolve full resistance. While evolution must involve substantial stochastic component, our results indicate significant impact of deterministic therapy-specific selective pressures in shaping emergence of distinct resistance mechanisms. Finally evolution of resistance is shaped by microenvironmental contexts that modify fitness differences between therapy sensitive and resistant cells.

A15 Obesity-Associated Mammary Extracellular Matrix Promotes Breast Cancer Stem Cell Characteristics

Tan, Matthew L.; Druso, Joseph E.; Shimpi, Adrian A.; Wittmann, Katharina; Reizes, Ofer; Rubin, Mark A.; Inghirami, Giorgio; Hopkins, Benjamin D.; Cantley, Lewis C.; Fischbach, Claudia

Obesity is epidemiologically linked with poor clinical prognoses and high mortality rates in breast cancer patients. Metastatic tumor growth is the primary cause of death in patients diagnosed with cancer, and circulating tumor cells commonly display cancer stem cell (CSC) characteristics. While obesity-associated systemic factors such as cytokines, adipokines and hormones have been shown to affect tumorigenesis, recent evidence suggests that the abnormal extracellular matrix (ECM) found in obese tissues may also play a role in directing cancer cell behavior. Here, we hypothesized that obesity-associated ECM may independently induce CSC characteristics in breast cancer cells. To recapitulate obesity-associated ECM characteristics in vitro, we generated cell-derived matrix (CDM) from control and obesity-associated adipose-derived stromal cells. When compared to growth on control CDM, triple-negative MDA-MB-231 breast cancer cells grown on obesity-associated CDM displayed elevated stem cell characteristics such as the expression of NANOG, OCT-4 and SOX-2, as well as enhanced aldehyde dehydrogenase (AlDh) activity. Interestingly, breast cancer patient-derived tumor xenograft spheroids also showed elevated AlDh activity when cultured on obesity-associated, as compared to control, CDM. Using a NANOG-GFP reporter MDA-MB-231 breast cancer cell line, we further analyzed the mechanisms by which stemness was induced by obesity-associated CDM. Luminex multiplex assays showed alterations in key signaling pathways in MDA-MB-231 cells expressing high levels of NANOG, including increased levels of phosphorylated ERK1/2, c-Jun, STAT3 and STAT1. We've also demonstrated that cancer cell stemness is associated with an altered metabolic phenotype, where MDA-MB-231 cells expressing high levels of NANOG displayed increased HIF-1 α expression, glucose consumption, and lactate production. Taken together, these results suggest that the ECM in obese tissues has independent contributions to regulating stem-like behavior of breast cancer cells and that metabolic alterations may play a role in this process, thus opening new potential avenues of therapeutic considerations.

A16 Understanding intratumoral heterogeneity and overcoming cellular plasticity-mediated therapeutic resistance

Tyler Risom, Ellen Langer, Kristof Torkenczy, Andrew Adey, Joe Gray and Rosalie Sears

Intratumoral heterogeneity resulting from cell-intrinsic and cell-extrinsic factors is a major cause of therapeutic resistance in breast cancer. We have characterized the expression of luminal, basal, and mesenchymal markers in basal-like breast cancers and have shown that this subtype of breast cancer has high differentiation-state heterogeneity and that distinct classes of targeted therapeutics eliminate or enrich specific differentiation-state subpopulations resulting in increased homogeneity of residual cells. We have found that BET inhibitors are effective combination therapies with these state-aggregating drugs and have hypothesized that their synergy is due to inhibition of the epigenetic changes necessary for transition to a resistant differentiation state. Using high-content imaging and single cell ATAC-seq to access differentiation state and map the chromatin accessibility landscape we found that basal-like breast cancer cells treated with BEZ235, a PI3K/mTOR inhibitor, transition toward a luminal differentiation state with distinct chromatin

architecture. Moreover, combination with the BET inhibitor JQ1 inhibits the differentiation state changes and chromatin remodeling induced by BEZ235, and this results in complete cell kill. We are continuing to analyze RNA-seq along with single cell ATAC-seq data on cells treated with state aggregating drugs +/- BET inhibitors as well as other epigenetic inhibitors to identify global changes in chromatin accessibility and transcriptional networks controlling the response to targeted treatments. The tumor microenvironment also contributes to the response of tumors to therapeutics. Thus, in addition to understanding the cell-intrinsic mechanisms that influence heterogeneity, plasticity, and therapeutic efficacy, we are interrogating cell-extrinsic factors that affect these phenotypes to determine whether and how they contribute to resistance. For this, we are generating complex, heterotypic, scaffold-free and highly manipulable in vitro tumor tissues in which breast cancer cells are surrounded by stromal cell types including fibroblasts, endothelial cells, and mesenchymal stem cells using a 3D tissue bioprinter. As these printed tissues mature over 1-3 weeks, the cells self-organize, lay down matrix, and respond to extrinsic signals. Together with xenograft and genetically engineered mouse models, these tissues are being used to understand the effects of individual stromal cell types and cell-extrinsic factors on the baseline differentiation state heterogeneity and therapeutic efficacy.

A17 Mathematically Universal and Biologically Consistent Astrocytoma Genotype Encodes for Transformation and Predicts Survival Phenotype

Katherine A. Aiello, Sri Priya Ponnapalli, and Orly Alter

DNA alterations have been observed in astrocytoma for decades. A copy-number genotype predictive of a survival phenotype was only discovered by using the generalized singular value decomposition (GSVD) formulated as a comparative spectral decomposition. Here we use the GSVD to compare whole-genome sequencing (WGS) profiles of patient-matched astrocytoma and normal DNA. First, the GSVD uncovers a genome-wide pattern of copy-number alterations, which is bounded by patterns recently uncovered by the GSVDs of microarray-profiled patient-matched glioblastoma (GBM) and, separately, lower-grade astrocytoma and normal genomes. Like the microarray patterns, the WGS pattern is correlated with an approximately one-year median survival time. By filling in gaps in the microarray patterns, the WGS pattern reveals that this biologically consistent genotype encodes for transformation via the Notch together with the Ras and Shh pathways. Second, like the GSVDs of the microarray profiles, the GSVD of the WGS profiles separates the tumor-exclusive pattern from normal copy-number variations and experimental inconsistencies. These include the WGS technology-specific effects of guanine-cytosine content variations across the genomes that are correlated with experimental batches. Third, by identifying the biologically consistent phenotype among the WGS-profiled tumors, the GBM pattern proves to be a technology-independent predictor of survival and response to chemotherapy and radiation, statistically better than the patient's age and tumor's grade, the best other indicators, and MGMT promoter methylation and IDH1 mutation. We conclude that by using the complex structure of the data, comparative spectral

decompositions underlie a mathematically universal description of the genotype-phenotype relations in cancer that other methods miss.

A18 GSVD- and Tensor GSVD-Uncovered Patterns of DNA Copy-Number Alterations Predict Survival in Response to Platinum in Adenocarcinomas

Katherine A. Aiello, Matthew W. Bradley, Rui Luo, Sri Priya Ponnapalli, Heidi A. Hanson and Orly Alter

More than a quarter of lung, uterine, and ovarian adenocarcinoma (LUAD, USEC, and OV) tumors are resistant to platinum drugs. Patterns of copy-number alterations that predict survival in response to platinum were recently discovered by using the tensor GSVD to compare Agilent microarray platform-matched profiles of patient-matched normal and primary OV DNA. Here we use the GSVD to compare whole-genome sequencing (WGS) and Affymetrix microarray profiles of patient-matched normal and primary LUAD, USEC, and OV DNA. First, the GSVD uncovers patterns similar to one Agilent OV pattern, where a loss of the chromosome arm 6p combined with a gain of 12p encode for transformation. Like the Agilent OV pattern, the WGS LUAD and Affymetrix LUAD, USEC, and OV patterns are correlated with shorter survival in response to platinum. Like the tensor GSVD, the GSVD separates the tumor-exclusive genotype from experimental inconsistencies, including the WGS technology-specific effects of guanine-cytosine content variations. Second, by identifying the shorter survival phenotype among the WGS- and Affymetrix-profiled tumors, the Agilent pattern proves to be a technology-independent predictor of survival in response to platinum, independent of stage, the best other indicator at diagnosis. Third, the pattern, like no other indicator, predicts survival of OV patients experiencing progression-free survival following platinum-based treatment of the primary tumor throughout the course of the disease. We conclude that comparative spectral decompositions, such as the GSVD and tensor GSVD, underlie a mathematically universal description of the relations between a primary tumor's genome and a patient's overall survival, which other methods miss.

A19 Stochastic Profiling for mRNA Seq Data

Amrhein, Lisa; Theis, Fabian J. ; Janes, Kevin A.: Fuchs, Christiane

Acute Myeloid Leukemia (AML) is the most common acute leukemia affecting adults, where incidence increases with age. Even after complete remission (~70% of the patients) is achieved, leukemic cells likely remain in numbers too small to be detected with current diagnostic techniques. If no further postremission or consolidation therapy is given, almost all people with AML will eventually relapse. Almost always this relapse is lethal. AML patients frequently carry a mixture of different cancer cell types, so-called subclones, which evolve over time, so that the mixture at relapse is different from the one at diagnosis. Understanding clonal evolution and identifying rare subclones, especially for those mutations causing relapse, is still an open challenge. We aim to parameterize transcriptional heterogenity from RNA-Seq counts taken from small groups of cells (e.g. 10-cells). To that end, we will extend our Stochastic Profiling Method previously proposed for microrray data

(Janes et al., Nature Methods 2010; Bajikar et al., PNAS 2014). This technique infers single-cell regulatory states by mathematically deconvolving n-cell measurements. This averaging-and-deconvolution approach allows us to quantify single-cell regulatory heterogeneities while avoiding the technical measurement noise of single-cell techniques.

A20 Multiscale Biophysical Modeling of Receptor Activation, Signaling and Trafficking in Cancer

Alokendra K. Ghosh, K. K. Sreeja, Bin Wu, Zachary Graber, Tobias Baumgart, Wei Guo, Ravi Radhakrishnan

We describe applications of multiscale biophysical modeling in the subcellular regime (i.e. length time scale of Angstroms to microns and timescale of pico seconds to seconds) in oncology/cancer biology. We outline the computational horizons in cancer and describe our recent efforts in describing the mutational landscape/diversity in epithelial tumors and their relationship to drug sensitivity and resistance in targeted therapies involving kinase inhibitors. Specifically, we describe molecular, network, as well as biophysical modeling approaches in predicting the effect of mutation/altered mechanics on signaling and trafficking. A theme we have explored in relation to tumors of the soft tissues is 'Can Soft Signals Turn Oncogenic'. There are emerging links between the stiffness of the tissue microenvironment and the tumorogenicity in several tumors of soft tissues, thereby bringing to light the importance of how cells transduce mechanical signals to alter signals and cell fate. We focus on molecular and subcellular mechanisms of curvature induction and sensing in cell membranes by a novel class of membrane remodeling proteins. Consistent with in vitro biophysical as well as cellular experiments, the curvature sensing/generating proteins can also be shown to be exquisite sensors of membrane tension thereby representing an important class of transducers of mechanical signals that are crucial for cell survival and intracellular signaling mediated by exosome production and secretion. We present theory-guided design of quantitative biophysical experiments to measure physical variables such as membrane tension in live cells. We also present a modeling framework to investigate morphological transitions in cell membrane using soft-matter approaches and show how the framework can be extended to include interactions from the extracellular matrix as well as from the cytoskeletal remodeling. We demonstrate how membrane morphologies such as protrusions can serve as signaling hubs to initiate and sustain survival as well as proliferative pathways in single cells that are initiated solely by physical stimulus and without any external biochemical cues. We also present evidence that such pathways can educate multiple cells in the tumor microenvironment through altered intracellular trafficking in the form of mechanosensitive release and capture of exosomes. We describe applications to melanoma, hepatocellular carcinoma, and breast cancers including opportunities for directly profiling human subjects who are cancer patients. In our second theme, we developed integrated systems modeling frameworks, useful in clinical cancer modeling due to their ability to incorporate a wide variety of patient data and tumor heterogeneity. Due to differences in time and length scales of individual processes, such an integration is a challenging task. We have combined cell proliferative pathways mediated by

MAPK and PI3K/AKT with p53 mediated DNA damage pathways to generate patient specific predictions for different cancer types. MAPK, PI3K-AKT and the p53 mediated DNA damage response pathways have important implications for different cancers. These pathways have been modeled individually before. However, there have been very few attempts to integrate them into a combined cellular model due to significant challenges arising from differences in time scales and type of modeling paradigm. We show that such an integrated model is of great clinical value due to its scope and its ability to test a great variety of clinical scenarios. Specifically, we have incorporated the miRNA expression data for various patients to re-normalize the initial expression levels of corresponding mRNAs to add patient-specificity to our predictions. The heterogeneity of the tumor microenvironment is 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. We have successfully applied this modeling framework to lung cancer and nephroblastoma demonstrators, where the model predictions for the efficacy of combination therapies are evaluated by computing an effective Cell Kill Rate (CKR) for multiple treatments. ACKNOWLEDGMENT: These works are supported in part by NIH/NCI through the Physical Sciences in Oncology Network U54CA193417, Cancer Systems Biology Consortium U01CA227550, and by EU FP7-ICT-2011-9-600841.

A21 Quantitative Assessment of Protein Activity in Orphan Tissues and Single Cells Using the metaVIPER Algorithm

Hongxu Ding, Eugene F. Douglass Jr., Adam M. Sonabend, Angeliki Mela, Sayantan Bose, Christian Gonzalez, Peter D. Canoll, Peter A. Sims, Mariano J. Alvarez and Andrea Califano

We and others have shown that transition and maintenance of biological states is controlled by master regulator protein, which can be inferred by interrogating tissue-specific regulatory models (interactomes) with transcriptional signatures, using the VIPER algorithm. Yet, some tissues may lack molecular profiles necessary for interactome inference (orphan tissues), or, as for single cells isolated from heterogeneous samples, their tissue context may be undetermined. To address this problem, we introduce metaVIPER, a novel algorithm designed to assess protein activity in tissue-independent by integrative analysis of multiple, non-tissue-matched interactomes. This assumes that transcriptional targets of each protein will be recapitulated by one or more available interactome. We confirm the algorithm's value in assessing protein dysregulation induced by somatic mutations, as well as in assessing protein activity in orphan tissues and, most critically, in single cells, thus allowing transformation of noisy and potentially biased RNA-Seq signatures into reproducible protein-activity signatures.

A22 Quantifying the development of resistant phenotypes and therapeutic strategies to avoid them.

Griffiths, Jason; Chi, Feng; Bild, Andrea; Adler, Fred

Drug resistant phenotypes can emerge rapidly, driven in part by stress induced plasticity in cell development. Signaling pathways enabling resistant phenotypes are under strong selection, often becoming over activated during disease progression. However, resistant states are reversible, and therapies inhibiting the development of resistant phenotypes can maintain the effectiveness of chemotherapy. Successful management of induced resistance will rely upon a quantitative understanding of the rate, cost and benefit of its development, as well as measurement of the efficacy of therapeutic agents at reversing resistance phenotypes. I will discuss a combination of experiments, tracking the development of resistant phenotypes over time, and a novel mathematical modeling framework to capture the heterogeneous development of resistance under differing treatment strategies. Application of this approach can allow measurement of the rate of inducible resistance, its impact on cellular birth and death processes and the effectiveness of resistance inhibiting therapies. These insights can guide the formation of precision treatment regimens to stall the induction of drug resistant cancers.

A23 Heterogeneous multi-scale framework for cancer systems models and clinical applications

Ghosh, Alokendra; Radhakrishnan, Ravi

ErbB receptor mediated Ras-MAPK and PI3K-AKT and the p53 mediated DNA damage response pathways have important implications for different cancer types. These pathways have been modeled individually before. However, there have been very few attempts to integrate them into a combined cellular model due to significant challenges arising from differences in time scales and type of modeling paradigm. Such an integrated model will be of great clinical value due to its scope and its ability to test a great variety of situations which would be directly useful to a clinician. Here, we have successfully integrated these pathways taking into account the differences in time scales. We have also incorporated the miRNA expression data for various patients to re-normalize the initial expression levels of corresponding mRNAs to add patient-specificity to our predictions. The heterogeneity of the tumor microenvironment is 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 demonstrators where the model predictions in the form of a Cell Kill Rate (CKR) has been used as an input to phenomenological tumor growth models.

A24 Mechanistic Hypothesis Generation and Multimodel Inference for Cancer Signaling Networks

Kochen, Michael; Lopez, Carlos

Quantitative physicochemical models of biological systems can be constructed when enough mechanistic detail has been elucidated through experimentation and analysis. In reality, our knowledge of these interaction mechanisms is never perfect, and often sparse. Ambiguities

in interpretation and conflicting experimental results impede the construction of accurate, detailed, quantitative models that can predict biological behaviors. To address these data limitations, multiple competing models can be constructed and a favored model chosen through model selection techniques. For physicochemical models, model selection involves sampling over a high dimensional parameter space to find the most robust model topology given an experimental data set. These methods are computationally intensive, and when combined with the arduous task of manual model construction, selection becomes feasible only when comparing small numbers of hypothetical models. Here we introduce an automated workflow for the construction and ranking of mechanistic hypotheses for ill-defined biological signaling networks. Our software, HypBuilder, is a Python based tool that first generates a collection of mechanistic model hypotheses using a node-edge graph and a number of user-defined criteria as inputs. These hypotheses are ranked using a nested sampling algorithm that calculates the probability of a model given available data. Once ranked, various groups of molecules and reactions are broken out and analyzed, producing leads for further experimentation. We evaluate the feasibility and accuracy of the method using several hundred model hypotheses of Mitochondrial Outer Membrane Permeabilization (MOMP) regulation, the commitment step in apoptosis execution.

A25 Variational Autoencoding Phenotypic Response to Microenvironment Perturbation

Schau, Geoffrey F.; Dane, Mark A; Thibault, Guillaume; Gray, Joe W. ; Heiser, Laura M. ; Chang, Young Hwan

Recent advancements in cellular imaging analyses have significantly improved single-cell analysis and enable quantification of phenotypic differences among a variety of cell populations in response to diverse chemical or genetic treatments. Although significant progress in single-cell imaging segmentation, feature extraction, and cytoprofile analysis has been reported, defining and characterizing microenvironment-dependent multi-cellular spatial organization has remained an unmet challenge. In this study, we employ variational autoencoders (VAE) to learn latent space representations of multi-cellular growth patterns across high-throughput microenvironment microarray imaging-based screens of human mammary epithelial cells in response to microenvironment perturbations. We demonstrate that learned features match expert annotation of cell colony organization, which indicates the utility of deep learning systems to meaningfully characterize tissue growth patterns in a fully unsupervised manner. These results can inform biological understanding of how microenvironment perturbations affect cellular organization in both cancer and disease. Our results suggest that VAE may provide an important and useful approach for studying multi-cellular phenotypic responses to microenvironment and drug treatment perturbations. These analyses represent a preliminary exploration into the utility of learning systems to capture biologically meaningful spatial organization features with which to characterize tissue growth patterns with the high-throughput screening experiments. Future investigations will extend this approach to incorporate generative adversarial network (GAN) architectures to further refine the decoding mechanism for

more accurate manifold inference and provide a more powerful tool for researchers to characterize tissue growth in response to microenvironment perturbation.

A26 Rapid insights from high throughput proliferation datasets using Thunor

Lubbock, Alexander L. R.; Harris, Leonard A.; Quaranta, Vito; Tyson, Darren R.; Lopez, Carlos F.

Numerous experiments have assessed the effects of drugs on cell proliferation in vitro, including large datasets produced using high throughput techniques. However, the visualization and analysis of these data has generally been performed using bespoke or proprietary software, thus limiting wider usage, development, and reproducibility. Here, we introduce Thunor, an open source software platform for data management, analysis, and visualization of drug effects on cell proliferation. Thunor has a graphical web interface, enabling the ability to rapidly 'browse' and analyze large datasets without programming skills, while also utilizing a Python core library for simple integration into computational workflows. From user-uploaded cell count data, Thunor automatically calculates viability and drug-induced proliferation (DIP) rates, fits dose response curves, and calculates derivative statistics (IC50, EC50, Hill coefficient, etc.). It includes a tagging system for grouping sets of cell lines or drugs together, e.g. by cell line mutation or tissue of origin, or by drug molecular target. Thunor also includes a paneled plot interface with multiple plot types: time courses, dose response curves, dose response parameter box/bar/scatter plots, and quality control analyses; users can optionally select or aggregate data via tags prior to plotting. Data are stored in an indexed relational database, and Thunor's web interface uses encrypted connections and has group-based access control to optionally share datasets and tags with other users. When combined, these capabilities can generate rapid insights through relevant visualization and statistical analysis. For example, one could plot IC50s of drugs grouped by molecular target on lung cancer cell lines, statistically testing for differences between targets using one-way ANOVA. Thunor's easy to use sharing capabilities help foster intra- and inter-lab collaboration on cell proliferation datasets. The software and an online demo are available at www.thunor.net.

A27 Prediction of cell line-specific recruitment of signaling proteins to the insulin-like growth factor 1 receptor

Erickson, Keesha; Rukhlenko, Oleksii; Shahinuzzman, Md; Slavkova, Kalina; Lin, Yen Ting; Suderman, Ryan; Stites, Edward; Anghel, Marian; Posner, Richard; Barua, Dipak; Kholodenko, Boris; Hlavacek, William

In cellular networks composed of interacting biomolecules, it is unclear how information is processed to generate appropriate and specific responses to signals. For example, receptors that detect distinct ligands and regulate distinct cellular activities commonly interact with overlapping sets of downstream signaling proteins. Here, we use mechanistic modeling to investigate the downstream signaling of the insulin-like growth factor 1 (IGF1) receptor (IGF1R), a receptor tyrosine kinase. In normal cells, IGF1R signaling regulates

differentiation, proliferation, motility, and anti-apoptosis; however, elevated IGF1R signaling has also been strongly linked to the progression of cancer. Models capable of predicting IGF1R signaling pathway behavior could aid in the realization of effective treatment strategies for IGF1R-driven disease. We formulated and analyzed 40 cancer cell line-specific mathematical models, which account for recruitment of 18 different binding partners to six sites of receptor autophosphorylation in IGF1R. The models were parameterized using available protein copy number and site-specific affinity measurements. We find that recruitment is influenced by the protein abundance profile of a cell, with different patterns of recruitment in different cell lines. Furthermore, in a given cell line, we find that pairs of IGF1R binding partners may be recruited in a correlated or anti-correlated fashion. This work predicts that receptor signaling pathway selectivity is the consequence of expression levels of recruited signaling proteins and the competition among them for receptor binding domains.

A28 Gene expression is regulated by crowding dynamics in the nuclear nanoenvironment

Anne Shim, Luay Almassalha, Hiroaki Matusda, Rikkert J. Nap, Vadim Backman, Igal Szleifer

Altogether, a system of macromolecular crowding, encompassing both small proteins and large subsections of chromatin, highly concentrates the nuclear environment and influences the thermodynamics and efficiency of gene expression. Of important consequence, processes like chromatin translocation and reorganization, protein diffusion, and DNA loop extrusion cause macromolecular crowders to be highly dynamic. These dynamics further affect the efficiency of nuclear processes by altering the kinetics and availability of necessary machinery. However, little is known about how crowding dynamics integrate with gene expression. Therefore, we consider how transcription kinetics are altered as a result of their existence within a time-evolving, crowded nanoenvironment. We conducted a parametric study, whereby temporal changes in crowding density were nominated from experimental measurements and incorporated into a computational model of transcription. We show that transcription kinetics are not only governed by the local average crowding density, but also depend critically on the time scale and variation of crowding dynamics. In total, this work demonstrates that macromolecular crowding may play an even greater role in regulating transcription kinetics than previously understood, as it presents crowding dynamics within the bulk nuclear nanoenvironment as a novel regulatory framework for gene expression.

A29 Immune interconnectivity of anatomically distant tumors as a potential mediator of systemic responses to local therapy

Walker, Rachel; Enderling, Heiko

Complex interactions occur between tumor and host immune system at each site in the metastatic setting, the outcome of which can determine behavior ranging from dormancy to rapid growth. An additional layer of complexity arises from the understanding that

cytotoxic T cells can traffic through the host circulatory system. Coupling models of local tumor-immune dynamics and systemic T cell trafficking allows us to simulate the evolution of tumor and immune cell populations in anatomically distant sites following local therapy and thus computationally evaluate immune interconnectivity. Results suggest that the presence of a secondary site may either inhibit or promote growth of the primary, depending on the capacity for immune recruitment of each tumor and the resulting systemic redistribution of T cells. Treatment such as surgical resection and radiotherapy can be simulated to estimate both the decrease in tumor volume at the local treatment-targeted site, and the change in overall tumor burden and tumor growth trajectories across all sites. Qualitatively similar responses of distant tumors to local therapy (positive and negative abscopal effects) to those reported in the clinical setting were observed. Such findings may facilitate an improved understanding of general disease kinetics in the metastatic setting: if metastatic sites are interconnected through the immune system, truly local therapy does not exist.

A30 Linking Wnt to patterns of metabolism in colon cancer using math models and single cell sequencing

Chen, George; Yan, Huaming; Pervolarakis, Nicholas; Lee, Mary; Wang, Kehui; Puttock, Eric; Tifrea, Delia; Kessenbrock, Kai; Edwards, Robert A; Hughes, Christopher CW; Lander, Arthur; Lowengrub, John S; Waterman, MarianL

Metabolic reprogramming of cells in tumors is a broadly observed survival feature of carcinogenesis. For example, aerobic glycolysis (Warburg metabolism) is commonly thought to enable tumors to survive and proliferate in the face of limited nutrients. Whether this reprogramming occurs broadly and uniformly in tumors, and whether it spontaneously develops or is directed by a network of oncogenic signals is not fully known. We observe that glycolytic, Warburg metabolism is heterogeneous in xenografted colon tumors. The heterogeneity appears as a self-organizing spatial pattern consisting of an array of cell clusters most prominent at the leading edge of tumors. These clusters, or 'spots', consist of 6-7 cells with strong biomarker levels of glycolysis surrounded by cells with lower levels that indicate less glycolysis. We also observe a matching, directly correlated spotted pattern of high-to-low Wnt signaling. To understand these patterns, we developed a mathematical model that incorporates Wnt signaling and Warburg metabolism. Our model utilizes Reaction-Diffusion equations and recapitulates the xenograft patterns. Two model predictions - that interfering with Wnt signaling triggers an increase in Wnt ligand diffusion and that glycolysis and Wnt inhibitors act synergistically in colon cancer - were validated in in vitro assays and in in vitro vascularized microtumors. Single cell sequencing of xenograft tumors identified six cell subtypes, including a Wnt-signaling-high population, from which the highly glycolytic cell clusters may emerge, and a putative cancer stem cell population. A strong principal component factor in the identification of these distinct subtypes was population-specific expression patterns of ligands and receptors for different signaling networks. Currently, we are developing a signaling interactome between the subtypes and

the mouse stromal microenvironment with the goal of further understanding the biological and clinical implications of these patterns and how they emerge as the tumor develops.

A31 In silico tumor organoids: delineation of microenvironmental and drug effects

Katarzyna Rejniak

In silico tumor organoids, the three-dimensional computational models capable of recreating the morphology and micronevironmental conditions of in vivo tumors and organoid cultures were used to test how different microenvironmental conditions affect tumor cell response to anti-cancer drugs. We will present techniques for creating in silico organoids and their integration with experimental data from breast cancer cell lines, as well as novel methods for quantifying and categorizing organoid morphologies and predict their responses to therapeutics. By explicitly including the fibril structure of the extracellular matrix (ECM), we were able to examine the ECM properties that promote breast cancer microinvasions, and derive hypotheses on relative importance of microenvironmental factors on tumor development under chemotherapeutic treatments. Thus the in silico organoids provide an invaluable tool for hypotheses testing, and present an opportunity to explore experimental conditions beyond what is physically feasible in laboratory experiments.

A32 A systematic analysis of regulatory networks underlying pancreatic ductal adenocarcinoma reveals new molecular subtypes

Laise, Pasquale; Maurer, H. Carlo; Elayda, Ela; , Tuveson David; Olive, Kenneth P.; Califano, Andrea

Pancreatic ductal adenocarcinoma (PDA) is one of the most aggressive cancers. It constitutes the fourth leading cause of cancer mortality with a 5-year survival rate of 5%. This is probably due to the fact that, despite the molecular heterogeneity of PDA, all patients are currently treated with standard therapies and without significant improvement in survival rate. In the last years, genome-wide expression analyses have identified multiple molecular subtypes of PDA. Unfortunately, despite a concordance rate in terms of 'good-prognosis' vs. 'poor-prognosis' prediction, there is no convergence between these gene expression classifiers and therefore no significant contribution to the effective treatment of PDA. In our study, we used a systems biology approach to investigate the molecular classification of PDA by performing a systematic analysis of PDA regulatory networks, and stratifying patients according to the activity of their transcriptional regulatory programs. Briefly, we first generated context-specific regulatory networks from multiple PDA data sets using ARACNe; then, we used metaVIPER, a new algorithm specifically designed for the integrative analysis of multiple networks, to assess the activity of ~6000 proteins in each patient's tumor sample. A completely unsupervised and independent clustering analysis based on protein activity identified two main clusters of patients in each data set. Interestingly, a master regulator analysis, independently

performed in each data set, showed a complete convergence of differentially activated proteins that distinguish these two clusters across all the data sets. We further validated our protein activity signature and the identity of two clusters in a PDA laser capture microdissected (LCM) epithelial data set. Finally, in line with results from the LCM data, the application of our signature to a PDA single cell dataset distinguished two main clusters of epithelial cells, suggesting these clusters as potential epithelial PDA subtypes. Interestingly, virtually all patients have cells falling in both the clusters pointing to the coexistence of both the subtypes in the same patient. Our results provide new insights into the molecular classification of PDA and may be used to define new therapeutic strategies.

A33 Cancer cell induced activation of associated fibroblasts is PDGFR-mediated

Majumder, Anurima; Boutcheung-Djidjou, Martial; Kim, Jae-Young; Sumi, Natalia; Rix, Uwe; Haura, Eric B.

Recent studies have shown that tumorigenesis is not a cell autonomous process and is actively shaped by the tumor microenvironment (TME). The effect of cancer associated fibroblasts (CAFs), a critical component of the TME, on the growth, progression and drug sensitivity of the tumor itself is well studied. However, there is limited understanding about key signaling pathways activated in CAFs by cancer cells (CCs). In this study, we aim to better understand the bi-directional signaling between CCs and CAFs at a systems level. We first characterized tyrosine phosphoproteomes of EGFR mutant PC9 lung CCs and WI-38 lung fibroblasts in co-culture using mass spectrometry-based phosphoproteomics (pY). Cell type specific proteome labeling by amino acid precursors (CTAP) was used to distinguish two proteomes in co-culture. The pY data shows differential regulation of receptor tyrosine kinase (RTK) signaling by co-culture in both CCs and CAFs. We focused our attention first on using a conditioned medium (CM) system to explore which potential ligands can mediate some of the CC-induced changes in kinase signaling detected in fibroblasts from the pY experiment. Treatment of fibroblasts with PC9 CM induced a transient but robust increase in phosphorylation of effectors of tyrosine kinase (TK) signaling, such as AKT and ERK, in CAFs. This activation was blocked by RTK inhibitors Sunitinib, Cabozantinib and Foretinib. Chemical proteomics and subsequent siRNA experiments show that the CC CM-mediated AKT activation in fibroblasts is driven by PDGFR. CM-induced PDGFR activation results in activation of fibroblasts as evidenced by its increased proliferation and migration capacity that can be blocked by the PDGFR inhibitor Sunitinib. Overall, our data shows that CCs transiently trigger PDGFR-mediated TK signaling in fibroblasts thereby activating them. We next aim to understand the effect of this activation on oncogenic signaling and behavior of CCs. A better understanding of this bi-directional crosstalk may allow identification of targeted therapies against activation of CAFs that support tumor growth.

A34 Profiling Heterogeneous Gene-expression States in Luminal Breast Tumors at Diagnosis

Shambhavi Singh, Lixin Wang, Kathy Repich, Dylan Schaff, Jennifer Harvey and Kevin A. Janes

The most common form of breast cancer is the luminal subtype, expressing the estrogen receptor (ER). Luminal breast cancers fall into Luminal A or Luminal B categories based on whole-tumor gene-expression profiles that indicate favorable or poor prognosis. Discouragingly, luminal sub-classifications have not translated into clinically actionable diagnostic markers like ER, for which anti-estrogen therapy is recommended even if as few as 1% of tumor cells are ER positive. Finer separation of the Luminal A/B subtypes may be infeasible because conventional gene-expression measurements are population averages that mask cell-to-cell variations. At the single-cell level, intra-tumor heterogeneity can arise as cancer cells switch between differentiation or regulatory states in a rapid, reversible, and context-dependent manner. Assessing intra-tumor heterogeneity of gene regulation requires precise transcriptomic measurements of a very small number of cells isolated from within the tumor context. To overcome these challenges, we have developed a method for in situ RNAseq-based profiling that quantifies gene expression accurately and sensitively in 10-cell samples randomly isolated by laser-capture microdissection. Applying this method to diagnostic breast tumor biopsies, we can apply fluctuation analyses to quantify intra-tumor regulatory states that may relate to progenitor status, proliferative potential, and potential therapeutic interventions. In our first application of this method to profile the in situ regulatory heterogeneity of a luminal biopsy sample, we detected 6908 genes on average per 10-cell sample, a substantial increase from tissue-based scRNA-seq methods that usually detect 2000-5000 genes. The fidelity of isolating breast carcinoma cells by laser capture was confirmed with luminal marker genes such as KRT8 and FOXA1 and the absence of markers for basal cells, immune cells, and endothelial cells. In this first tumor sample, we identified 95 candidate genes whose regulation is predicted to be heterogeneous. The gene list includes CLDN4, MUC1, and VIM, which are implicated in breast cancer progression and invasiveness, supporting the utility of the approach. 10-cell sequencing of tumor biopsies suggests transcriptional programs of co-fluctuating genes within tumors. Stratifying tumors by intra-tumor cell states may suggest cellular mechanisms underlying the variable prognoses of patients within the luminal subtype.

A35 Predictive understanding of adaptive resistance in BRAF-mutant cancers

Gerosa, Luca; Sanchez, Gabriela, Chidley, Chris; Muhlich, Jeremy; Sang, Kyun Lim; Chen, Jia-Yun; Sorger, Peter K.

Cancer cells treated with targeted inhibitors of oncogenic pathways can escape treatments through homeostatic changes in their signaling networks, a phenomenon termed 'adaptive resistance'. Our limited ability to predict the response of signaling pathways to drug perturbations is a key obstacle to design drug strategies that can prevent adaptive resistance. Here, we use a system-level approach based on experimental and modelling

cycles to build predictive models of drug adaptation in colorectal, thyroid and skin cancers bearing BRAF_V600E, a mutation that is present in 10% to 50% of these cancers and is responsible for hyper-activation of the pro-growth RAF/MEK/ERK signaling pathway. We hypothesize that adaptive resistance in these cancers is governed by their lineage-specific receptor dynamics and feedback regulation strengths. By incorporating the biochemistry of ERK signaling and the mechanisms of action of various targeted drugs into an Ordinary Differential Equation model, we reproduced the adaptive response of these cancers to targeted inhibitors. To extend and validate the models, we are currently generating time-course, single-cell data using multiplexed immunofluorescence and live-cell imaging. Preliminary results suggest that integrating single-cell data within kinetic modelling can deliver a predictive understanding of novel mechanisms of adaptive resistance to targeted therapy.

A36 Inorganic Phenotypes Distinguishing Tumorigenic and Nontumorigenic Breast Cancer

Haimei Chen, Friederike Hoeg, Eric Wagner, Wenan Qiang, Massimo Cristofanilli, Thomas V. O'Halloran

Quantitative changes in element signatures drive cell cycle changes [Que, Nature Chemistry, 2015] and are shown here to be emerging biomarkers and possible prognostic factors for tumorigenicity. The imbalance of the essential elements Cu, Zn and Fe are reported to be associated with carcinogenesis [M. P. Silva, et al., BMC Res Notes, 2012]. Other metals such as Na, Mg, K and Ca play important roles in cell cycle, DNA repair or chromatin scaffold maintenance [Richardson, D. R., Curr Med Chem, 2005]. Here, we present a robust methodology for measuring the inorganic signature or metallome of human cancer cells using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) wash-free assays. We find significant inorganic signatures that distinguish tumorigenic/malignant and non-tumorigenic cell lines. Using gene expression data from breast cancer patients (TCGA-BRCA project) and human breast cancer cell lines [Messier TL, Oncotarget, 2016], we are looking for distinct expression patterns that give rise to the emerging metal signature. These findings will help to determine whether these inorganic signatures are also evident on the transcriptome level, and, if so, they can be used as a new predictive marker or hallmark for tumorigenicity.

A37 Diverse AR-V7 cistromes in castration-resistant prostate cancer are governed by HoxB13

Zhong Chen, Dayong Wu, Jennifer M. Thomas-Ahner, Changxue Lu, Pei Zhao, Qingfu Zhang, Connor Geraghty, Pearlly S. Yan, William Hankey, Benjamin Sunkel, Xiaolong Cheng, Emmanuel S. Antonarakis, Qi-En Wang, Zhihua Liu, Tim H.-M. Huang, Victor X. Jin, Steven K. Clinton, Jun Luo, Jiaoti Huang, Qianben Wang

The constitutively active androgen receptor (AR) splice variant 7 (AR-V7) plays an important role in the progression of castration-resistant prostate cancer (CRPC). Although

biomarker studies established the role of AR-V7 in resistance to AR-targeting therapies, how AR-V7 mediates genomic functions in CRPC remains largely unknown. Using a ChIP-exo approach, we show AR-V7 binds to distinct genomic regions and recognizes a full-length androgen-responsive element in CRPC cells and patient tissues. Remarkably, we find dramatic differences in AR-V7 cistromes across diverse CRPC cells and patient tissues, regulating different target gene sets involved in CRPC progression. Surprisingly, we discover that HoxB13 is universally required for and colocalizes with AR-V7 binding to open chromatin across CRPC genomes. HoxB13 pioneers AR-V7 binding through direct physical interaction, and collaborates with AR-V7 to up-regulate target oncogenes. Transcriptional coregulation by HoxB13 and AR-V7 was further supported by their coexpression in tumors and circulating tumor cells from CRPC patients. Importantly, HoxB13 silencing significantly decreases CRPC growth through inhibition of AR-V7 oncogenic function. These results identify HoxB13 as a pivotal upstream regulator of AR-V7-driven transcriptomes that are often cell context-dependent in CRPC, suggesting that HoxB13 may serve as a therapeutic target for AR-V7-driven prostate tumors.

A38 Drug-induced receptor tyrosine kinase overexpression drives resistance to vemurafenib in melanoma cell lines

Atanasova, Mariya; Lauffenburger, Douglas; Sorger, Peter

Approximately 50-60% of melanoma patients carrying a BRAFV600E mutation show initial response to the BRAF kinase inhibitors vemurafenib or dabrafenib. However, the response is fractional and resistance invariably develops, making long-term progression-free survival the outstanding clinical challenge of targeted therapies in melanoma. We have shown that over the course of four weeks of vemurafenib treatment, cell lines exhibit pronounced changes in the abundance of several receptor tyrosine kinases (RTKs), such as EGFR, ERBB2, MET, and AXL. Inhibition of these upregulated receptors results in significantly increased cytotoxicity, suggesting that signaling through these RTKs provides survival advantage to cells in the presence of vemurafenib. Interestingly, the magnitude of the effect of RTK inhibitors varies depending on the time of addition after treatment with vemurafenib. Our results point to a selective temporal sensitivity to RTK inhibitors, which develops as a result of vemurafenib treatment and may prove to be clinically relevant.

A39 Proteomics, post-translational modifications, and integrative analyses reveal heterogeneity of molecular mechanisms within medulloblastoma subgroups

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

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. Kinome analyses identified kinases, some of which are targeted by FDA-approved drugs that are associated with specific subtypes. Our study shows that proteomics enables a more comprehensive, functional readout, providing a foundation for new therapeutic strategies in medulloblastoma.

A40 Analyzing the Physical and Functional Protein Interaction Landscape of Breast Cancer

Kim, Minkyu; Kim, Kyumin; Soucheray, Margaret; Swaney, Danielle; Zheng, Fan; Park, Jisoo; Tutuncuoglu, Beril; Gordon, David; O'Leary, Patrick; Coppe, Jean-Philippe; van 't Veer, Laura; Ashworth, Alan; Ideker, Trey; Krogan, Nevan

A key unanswered question in cancer genetics is how different mutations, dispersed across a multitude of genes, elicit similar pathology and patient outcomes. The answer may lie in understanding the molecular networks and protein complexes (i.e. signaling pathways, chromatin architecture, etc) in cancer and mapping mutated genes into the complexes and pathways in which they function. Determining how systematic interaction networks are wired in cancer cells and how different mutations perturb these networks will guide the search for new cancer genes and provide a platform for integrating patient data to make biological and clinical predictions more accurate. The goal of this study is to uncover the comprehensive protein-protein interaction networks and pathways in various breast cancer subtypes to better understand how mutated cancer genes and genomes hijack and re-wire pathways and complexes during the course of breast tumorigenesis. Here we catalog protein-protein interactions for 40 genes recurrently mutated in breast cancer, using affinity purification and mass spectrometry. To identify co-associated proteins, cDNA clones expressing each protein were tagged with 3xFLAG at either N or C-terminus and introduced into MCF10A (non-tumorigenic 'healthy' control), MCF7 (luminal A subtype), and MDA-MB-231 (claudin-low) cells using doxycycline-inducible lentiviral vectors. For proteins with prevalent pathogenic mutations (e.g. PIK3CA-H1047R, BRCA1-C61G), mutant cDNA clones were also analyzed in parallel. Our interaction network reveals subtype and mutation-specific protein-protein interactions, many of which are not previously reported. Given that genes encoding components of a protein complex or a biological pathway often share similar phenotype upon genetic perturbation, we genetically knocked out genes interacting with DNA damage response (DDR) proteins using CRISPR/Cas9, and found multiple novel interacting genes whose knockout results in significant PARPi (olaparib) and/or cisplatin sensitivity. This result not only functionally validates the physical protein interactions, but also demonstrates that our interactome mapping approach can helps

identify new druggable vulnerabilities in cancer cells. We anticipate the breast cancer interactome study will uncover aberrant pathways and protein complexes uniquely operating in breast cancer cells, and thus pinpoint proteins that may potentially serve as distinct biomarkers or therapeutic targets for tumors having the same or similar subtypes and/or genomic mutations.

A41 Susceptibility to targeted therapy depends on cell cycle phase

Chang, Jeremy; Wu, Lani; Altschuler, Steven; Krogan, Nevan

The Human Epidermal Growth Factor Receptor (HER) family controls cell proliferation, and members of the family are genetically altered in nearly a fifth of all cancers. Cell culture models of HER family-driven cancers commit apoptosis in response to HER family inhibitors. However, these inhibitors have limited clinical efficacy, and the mechanism by which they cause apoptosis is unknown. Using time lapse fluorescent microscopy, we monitored the HER2-addicted breast cancer cell line SK-Br-3 following treatment with the HER2 inhibitor lapatinib. We discovered that only cells in G1 or early S phase are susceptible to HER2 inhibition and commit apoptosis, whereas cells in late S or G2 phase are not susceptible. We used time lapse mass spectrometry to understand the molecular underpinnings of this cell cycle dependency. Moreover, we identified a similar cell cycle dependency in PC-9, an EGFR-addicted lung cancer cell line, suggesting that this phenomenon extends to all HER family members. A full understanding of this mechanism may suggest strategies to improve the clinical efficacy of HER family targeted therapies.

A42 Comprehensive Proteomic Analyses of Cetuximab Treatment in Head and Neck Cancer Reveals Mechanisms of Resistance

Mehdi Bouhaddou, Neil Bhola, Margaret Soucheray, Rachel O'eefe, Toni Brand, Nevan J Krogan, Danielle L Swaney, Jennifer Grandis

The 21st century has seen a boom in genomic approaches to studying biology, with therapeutically-relevant applications to cancer biology. Efforts such as The Cancer Genome Atlas (TCGA) and The Cancer Cell Line Encyclopedia (CCLE) have comprehensively catalogued individual cancer genes in a diverse array of cancer types. Interestingly, these studies have concluded that although the mutational overlap between distinct cancer types is scarce, mutations largely converge on a recurrent set of hallmark signaling pathways. This suggests that a network-based proteomic-level analysis of cancer cells will reveal greater similarity between genetically diverse cancer contexts. In this vein, a recent effort, the Cancer Cell Map Initiative (CCMI) – a multi-investigator and multi-site project – seeks to comprehensively map the protein networks driving disease across distinct cancer types. Such a map may reveal network-level vulnerabilities indiscernible from genomic studies alone. Here, we present a focused application of the CCMI project to cetuximab resistance in head and neck squamous cell carcinoma (HNSCC). Cetuximab, a monoclonal antibody inhibitor of EGFR, is currently the only FDA approved targeted therapy for the treatment of HNSCC, to which resistance develops quickly. To better understand the mechanisms of

resistance, and how to overcome it, we generated a cetuximab-resistant HNSCC cell line and performed a variety of mass spectrometry based proteomic analyses to compare it to the parental line, including global proteomic abundance, global phosphoproteomics, and targeted AP-MS on TCGA-identified prevalent HNSCC genes. We find dramatic rewiring of cancer signaling networks, exemplified by marked changes to the phosphoproteome and significant alterations to protein-protein interaction networks. We highlight these changes and use our maps to identify novel strategies to overcome cetuximab-induced drug resistance in HNSCC.

A43 Abnormal Nuclear Morphologies in Cancer: Role of Chromatin Regulators

Andrew C. Tamashunas, Vincent J. Tocco, James H. Matthews, Hendrik Luesch, Jonathan D. Licht, Richard B. Dickinson, Tanmay P. Lele

While the nucleus of a normal, healthy cell has a smooth and uniform ellipsoidal shape, the nucleus of a cancer cell commonly has irregularities in its contour including invaginations, folds, and lobes. These nuclear irregularities are prognostic and diagnostic markers of cancer, but the mechanisms by which these nuclear irregularities manifest in cancer remain poorly understood. Recent research efforts have focused on cytoskeletal forces onto the mechanically 'soft' cancer nucleus as potential causes of abnormal shapes. However, intranuclear forces, such as those generated from chromatin remodeling, may also be important but have received less attention. Here we screened a library of chromatin regulators to discover proteins that are required for maintenance of normal nuclear shapes. Nuclear shapes were quantified using an elliptical Fourier analysis and top candidates were selected for further mechanistic studies. Features in the nuclear lamina (such as holes, grooves, and folds) were identified by an automated texture analysis. A library of epigenetic pharmacological agents was also screened and common agents that affect nuclear shape and lamin structure, in both model breast epithelial cells and metastatic breast cancer, were identified. Our results identify key epigenetic genes that may be involved regulating nuclear morphology.

A44 MICROMECHANICS AND STRUCTURE OF METAPHASE CHROMOSOMES AND THE CELL NUCLEUS

Biggs, Ron; Stephens, Andrew, Banigan, Ed; Adam, Adam; Goldman, Robert; Marko John F.

MICROMECHANICS AND STRUCTURE OF METAPHASE CHROMOSOMES AND THE CELL NUCLEUS I will discuss studies of the mechanics and structure of metaphase chromosomes extracted from cells using glass micropipettes. Using a combination of mechanical, biochemical and genetic approaches we have shown that the metaphase chromosome is a 'chromatin gel', without a contiguous protein scaffold, and that condensin plays a major role as a 'crosslinker', mainly via SMC2 siRNA experiments. I will also describe our use of the same general approach to analyze the mechanics of mammalian cell nuclei. We have found that the nucleus shows two distinct mechanical responses: an initial chromatin-dominated elastic behavior, followed by a stiffer, lamin-dominated regime at larger extension. We have

found that the low-force chromatin-based elasticity can be modulated by changing histone acetylation and methylation using epigenetic drugs, and that the morphology of the nucleus is impacted by these changes. Finally I will show how physiochemical cues outside the cell can trigger intracellular signalling that increases histone methylation and heterochromatinization, stabilizing the nucleus against blebbing and other shape dysfunction. These results suggest a general mechanical self-defense scheme for the cell nucleus, based on increased heterochromatinization and consequent stiffening of the nucleus. A.D. Stephens, E.J. Banigan, S.A. Adam, R.D. Goldman, J.F. Marko, Chromatin and lamin A determine two different mechanical response regimes of the cell nucleus, Mol. Biol. Cell 28, 1984-1996 (2017). A.D. Stephens, P.Z. Liu, E.J. Banigan, L.M. Almassalha, V. Backman, S.A. Adam, R.D. Goldman, J.F. Marko, Chromatin histone modifications and rigidity affect nuclear morphology independent of lamins, Mol. Biol. Cell 29, 220-233 (2018).

A45 A mutation in the core of histone H2B represents a new class of oncogenic drivers

Richard A. Bennett, Aditya Bele, Christine M. Will, Eliza C. Small, Behnam Nabet, Rajarshi Ghosh, Adrian Grzybowski, Tao Yu, Qiao Zhang, Alberto Riva, Greta M. Wodarcyk, Vadim Backman, Tanmay Lele, George C. Schatz, Alex Ruthenberg, Jan Liphardt, Jonathan D. Licht

By examination of the cancer genome anatomy project database we have identified a new class of missense mutations in the body of histones H2A, H2B, H3 and H4 that frequently occur in cancer. In contrast to previously reported histone alterations that affect the covalent modification of histone tails, the most frequent mutations observed in cancer were predicted to disrupt nucleosome stability. To test this we characterized a mutation of histone H2B at amino acid 76 from glutamate to lysine (H2B-E76K) that was frequently observed in bladder, lung and breast cancers and predicted to disrupt a critical salt bridge between H2B and H4 in the histone octamer. Nucleosome assembly assays performed with recombinant histones demonstrated that the H2B-E76K mutant formed dimers with H2A but was unable to form stable histone octamers with H3 and H4, resulting in nucleosomes more sensitive to micrococcal nuclease treatment than wild type (WT) counterparts in vitro. Expression of the equivalent H2B mutant in yeast restricted growth at higher temperature and caused both increased sensitivity of chromatin to micrococcal nuclease and defective nucleosome-mediated gene repression. Significantly, in the normal mammary epithelial cell line MCF10A, expression of H2B-E76K increased cellular proliferation and cooperated with PIK3CA to promote colony formation compared to cells expressing wild-type H2B. MCF10A cells expressing H2B-E76K displayed significant drift in gene expression especially of genes regulating cell growth. Furthermore, incorporation of H2B-E76K into chromatin of MCF10A cells caused significant changes in chromatin accessibility as demonstrated by increased sensitivity to micrococcal nuclease digestion, increased mobility of the H2A-H2B dimer in FRAP experiments and increased chromatin accessibility at promoter regions by ATAC-seq. Taken together these data reveal a new mechanism of epigenetic dysfunction in cancer by demonstrating that mutations in the core

histones frequently occur in cancer to destabilize the nucleosome, deregulate chromatin accessibility and drive cellular transformation.

A46 Elucidating the impact of cancer cell migration through confined environments on DNA damage and genomic instability.

Shah, Pragya; Lammerding, Jan

Cancer metastasis i.e., the spreading of cancer cells from primary tumors to distant sites in the body, is responsible for over 80% of cancer related deaths. During this process, cancer cells often migrate through very small interstitial spaces about 1-2 μm in size, i.e., much smaller than the cell diameter. Deformation of the nucleus, which is the largest and stiffest organelle in the cell, can form a rate-limiting step during such confined 3D migration. As cancer cells squeeze through confined spaces, they incur extensive nuclear deformation, and in some cases undergo nuclear envelope rupture and DNA damage. We are investigating the cause of the observed DNA damage and the DNA repair mechanisms that are active in migrating cancer cells that promote their viability. Using a panel of different cancer cell lines, we found that in some cancer cell lines, DNA damage arises only after nuclear envelope rupture, while in other cell lines, nuclear deformation, even in the absence of nuclear envelope rupture, is sufficient to induce DNA damage. Furthermore, inhibiting specific DNA damage response and repair kinases can modulate the nuclear deformability, promoting their invasiveness through confined environments. We are currently elucidating the mechanism behind the differences seen in the cause of DNA damage in migrating cancer cells and the possible link between DNA damage response pathways and nuclear deformability. Understanding and targeting the pathways that lead to DNA damage and its repair in migrating cancer cells may ultimately help inform new treatment avenues to prevent the spread of metastatic cancer cells.

A47 Systematic identification of exosome-mediated crosstalk in ovarian cancer

Sheng, Jianting; Yeung,Tsz-Lun; Mok,Samuel C; Wong,Stephen TC

Ovarian cancer, particularly high-grade serous ovarian cancer (HGSC), is the deadliest gynecological malignancy, with a poor clinical outcome and survival rate. The poor patient survival is directly attributable to significant tumor heterogeneity, development of drug resistance, and the lack of effective treatment options for these patients. To date, strategies targeting only tumor cells have proven to be ineffective to eliminate HGSC and prevent drug resistance and metastasis. Therefore, identification of novel molecular targets/pathways altered in ovarian cancer cells, and development of highly effective combination therapies that can shut down these pathways is urgently needed to improve patient survival. Exosomes are small vesicles released by cells capable of transferring internal cellular materials such as non-coding RNAs (ncRNA) to serve as an intercellular communication system. While tumor exosomes have demonstrated angiogenic properties, exosomal lncRNAs and miRNA signatures from cancer associated stromal cells have not been thoroughly investigated, and their functional roles in modulating the malignant phenotypes

of the recipient cancer cells have not been elucidated. Here, we will integrate large-scale experimental omics data with mathematical modeling to systematically uncover comprehensive and complex exosomal-mediated communication between ovarian cancer cells and stromal cells, prioritize the role of ncRNAs in tumor progression and drug resistance, and identify therapeutic agents to disrupt the crosstalk signaling. We use genomic analysis of purified individual stromal and epithelial cell compartments directly from freshly procured clinical specimens without manipulation in culture, ensuring that in vivo gene expression signatures are accurately captured. It allows quantitative analysis of differentially regulated genes in specific cell populations, often missed in traditional whole tumor profiling and not supported by single cell gene sequencing data. This is the first study to apply systems biology approaches to evaluate the role of stromal cell derived exosomal ncRNA from individual stromal cell populations in HGSC. An in-depth understanding of the complex intercellular signaling pathways that regulate tumor growth, metastasis, and drug resistance would help the design of novel therapeutic strategies and reprogramming the tumor microenvironment to inhibit mutual benefits between stromal and tumor cells. The success of this study will identify potential therapeutic targets for ovarian cancer.

A48 Optogenetic control of nuclear body assembly in telomerase-free cancer cells

Huaiying Zhang, Michel Liu , Chanat Aonbangkhen, Robert Dilley, Roger A. Greenberg3,4, David M. Chenoweth, Michael A. Lampson

Telomerase-negative cancer cells rely on an alternative lengthening of telomeres (ALT) pathway that employs homologous recombination to maintain telomere length for immortality. The presence of ALT-associated promyelocytic leukemia (PML) nuclear bodies (APBs) is a unique characteristic of ALT and is used for diagnosis. Inhibiting APB formation leads to telomere shortening, indicating an essential role for APBs in telomere maintenance in ALT. However, there are major gaps in our mechanistic understanding of APBs. Both how they form and how they function in telomere lengthening, which is a crucial part of the ALT cancer phenotype, are unknown. We observed that APBs induced by DNA damage at telomeres exhibit behavior characteristic of liquid phase condensation, leading us to hypothesize that telomere shortening in ALT cells induces nucleation of APB condensates as a mechanism for telomere elongation. The liquid nature of APBs would promote coalescence of APBs to cluster telomeres within APBs, another characteristic of ALT cells. Meanwhile, condensation of APB droplets can concentrate DNA repair factors, providing opportunities for telomeres to use one another as repair templates to elongate within APBs. We developed an optogenetic approach and induced de novo assembly of APB nuclear body. We demonstrated that APB formation follows liquid condensation and the coalesces of APB liquid droplets drives telomere clustering, providing basis for cancer therapy targeting APB phase transition and material properties.

A49 An unexpected relationship between HSF1 foci dynamics and HSF1 function in cancer.

Giorgio Gaglia, Rumana Rashid, Gaurav Joshi, Luke Whitesell, Kris Sarosiek, Susan Lindquist and Sandro Santagata

Heat-Shock Factor 1 (HSF1) accumulates in foci, or nuclear stress bodies, following proteotoxic stress. We use live-cell time-lapse microscopy of human cancer cells to characterize the dynamics of HSF1 foci formation at single-cell resolution, their relationship with HSF1 transcriptional activity and cell fate. We found that the amount and timing of induction of HSF1 foci are finely modulated by the levels of proteotoxic stressors. Surprisingly, we showed that the HSF1 foci induction is anti-correlated with HSF1 cytoprotective transcriptional activity and are positively correlated with cell death. In addition, HSF1 localized within foci showed vastly reduced cellular trafficking ability. We postulate that HSF1 foci are aggregates of inactive HSF1 that are trapped in an immobile state, leading to impairment of the cellular cytoprotective response. Importantly we also observed HSF1 foci in a range of human tumor samples, opening the possibility of selectively targeting cancers with proteotoxic drugs based on their specific likelihood of activating cell death responses.

A50 Mapping chromatin motions using structured illumination reveals loss of genomic cohesion in response to DNA damage

Bonin, Keith; Smelser, Amanda; Salvador Moreno, Naike; Holzwarth, George; Segall, Dave; Vidi, Pierre-Alexandre

DNA is tightly packaged with proteins in the cell nucleus, forming a complex structure called chromatin. Chromatin displays largely stochastic motions best described by sub-diffusive models, and tunable physico-chemical properties of the nucleus may directly regulate or indirectly influence chromatin motions. Chromatin motions are thought to influence most if not all genomic functions including gene expression, DNA replication, and DNA repair. Yet the mechanisms governing chromatin diffusion are poorly understood, in particular in the context of the DNA damage response. Most studies in yeast have shown increased chromatin motions after DNA cleavage, proposed to facilitate homology search during homologous recombination. It is however unclear how much of the yeast knowledge on chromatin dynamics applies to mammalian cells, given the fundamental differences between the two organisms in terms of nuclear organization and DNA repair pathway usage. We developed a novel structured illumination microscopy method to map chromatin motions in mammalian cell nuclei. The method relies on a diffractive optical element that generates a lattice of photoactivated spots in cells expressing histones tagged with photoactivatable GFP. Photoactivated spots correspond to chromatin microdomains that can be tracked with time-lapse imaging. This approach led to the discoveries of (1) a transient decrease in chromatin motions after DNA damage, (2) correlated motions for neighboring chromatin domains, and (3) reduced chromatin cohesion in cells with DNA damage. A unique feature of the method is to provide spatial information on chromatin

motions, used to compare DNA damage sites to undamaged nuclear regions and to understand chromatin dynamics within and across chromosome territories. We anticipate that this approach will yield a better and dynamic understanding of the higher organization of the genome.

A51 Pancreatic Tumor Organoids for Functional Precision Medicine

Raghavan, Srivatsan; Stockslager, Max; Mu, L. Mary; Navia, Andrew; Winter, Peter; Ng, Raymond; Kalekar, Radha; O'Connell, David; Araya, Joshua; Tseng, Moony; Camarda, Nicholas; Keskula, Paula; Gill, Shubhroz; Sicinska, Ewa; Brais, Lauren; Reilly, Emma; Carter, Scott; Clemons, Paul; Schreiber, Stuart; Boehm, Jesse; Aguirre, Andrew; Wolpin, Brian; Shalek, Alex; Manalis, Scott; Hahn, William C.

Pancreatic ductal adenocarcinoma is a deadly disease with few effective targeted therapies, and only 15% of patients with pancreatic cancer carry potentially targetable somatic alterations. As such, there is a critical need for functional approaches to further characterize patient-specific tumor vulnerabilities and to guide clinical decision-making. In collaboration with the Division of Gastrointestinal Oncology at the Dana-Farber Cancer Institute and the Cancer Cell Line Factory at the Broad Institute we have developed a pipeline to perform therapeutic testing on pancreatic tumor resection and biopsy specimens cultured as patient-derived 3D organoids. We have optimized our organoid culture and therapeutic testing processes with the goal of performing drug response testing within a period of 8-12 weeks from biopsy acquisition, a clinically relevant time frame during which most patients will be treated with first- or second-line standard of care therapies. We are now systematically testing a panel of clinically relevant drugs against our cohort of pancreatic organoids to identify the spectrum of sensitivity and resistance, and have also established clinical protocols that allow for the return of patient-specific response data to the treating clinician to potentially guide 3rd-line and beyond clinical trial selection. We observe a range of sensitivities across organoids from different patients, and are beginning to identify patterns in organoid response profiles that may help to functionally classify patients toward specific therapies. In addition, we are applying single cell approaches - clonal organoid expansion, suspended microchannel resonators (SMR) and single-cell RNA sequencing - to characterize tumor heterogeneity and variation in therapeutic response. These studies integrating therapeutic testing in patient-derived culture models and single cell approaches will further our understanding of the mechanisms underlying therapeutic sensitivity and resistance in pancreatic cancer.

A52 3D-printed vascularized murine model as a tool for developing novel immunotherapies

Reynolds, Daniel; Kroll, Katharina; de Lazaro, Irene; Mooney, David; Lewis, Jennifer

Advances in cancer immunotherapy have shown that a complete immune reaction to tumor antigens is required to achieve an effective patient response. However, patient response to immunotherapies, such as checkpoint blockades or therapeutic vaccines, remains variable

and poorly understood. To develop better immunotherapies, there is a need for an in vitro 3D murine melanoma model that is robust, scalable, and can be directly compared to in vivo models. Towards this objective, we use bioprinting to pattern multiple cell types alongside a fugitive ink that, upon removal, yields perfusable channels. These printed features are then encapsulated in an engineered extracellular matrix (ECM) to produce a 3D vascularized murine melanoma model. The construct has high spatial organization and contains a perfusable tumor-associated vasculature, which allows for culture for extended periods of time. Currently, we are focused on developing cell-laden inks with properties that recapitulate the in vivo tumor environment. Once this model is optimized, we will introduce primed T cells and investigate the immune response within this in vitro 3D murine melanoma model. Ultimately, we seek to create a human melanoma model on chip that allows for preclinical treatment testing of novel immunotherapies.

A53 A systems biology driven drug-repositioning strategy identifies digoxin as a potential treatment for Groups 3 and 4 medulloblastoma

Lei Huang; Sarah Garrett Injac;Kemi Cui1; Frank Braun; Qi Lin; Yuchen Du;Huiyuan Zhang;Mari Kogiso;Holly Lindsay; Sibo Zhao; Patricia Baxter;Adesina Adekunle;Tsz-Kwong Man;Hong Zhao;Xiao-Nan Li; Ching C.Lau; Stephen T.C. Wong

Medulloblastoma (MB) is the most common malignant brain tumor of childhood. While outcomes have improved in recent decades, new treatments are still sorely needed both to improve survival and to reduce treatment-related complications. Medulloblastoma is a heterogeneous group of tumors that consists of four subtypes with distinct genomic signatures. Two of these subgroups are defined by a single dysfunctional signaling pathway, WNT and SHH respectively, which has raised the prospect of taking a rational target-based approach to the development of new therapies. Conversely, the other 2 sub-types, Groups 3 and 4, which compose 60-65% of total medulloblastoma cases, are associated with much more complicated genetic, epigenetic and genomic changes and display significant intragroup heterogeneity, limiting their options for 'rational' targeted therapies. We developed a computational systems biology method that incorporates novel algorithms for driver signaling network identification (DSNI) and drug functional network-(DFN)-based drug repositioning to integrate multiple types of genomics profiles for group 3 MB patients (whole genome/exome sequencing, DNA-copy number, DNA-methylation and mRNA expression) with human cancer signaling pathways resources and gene expression profiles of 1,309 drugs in CMAP with drug structure information and effects. By applying the DSNI-DFN method on groups 3 MB data, we identified five members of the cardiac glycoside family as potentially inhibiting the growth of Groups 3 and 4 medulloblastoma, and subsequently confirmed this in vitro. Systemic in vivo treatment of patient-derived orthotopic xenograft (PDOX or orthotopic PDX) models representing Groups 3 and 4 medulloblastoma with digoxin, one of the five cardiac glycosides identified in our in silico analysis, significantly prolonged animal survival and most importantly did so at plasma levels known to be tolerated in humans. Digoxin treatment prolonged survival in a PDOX model of Group 4 MB (ICb-1078MB) to 113 days (n=7) vs. 92 days (n=6) for untreated

controls (log-rank, p=0.001). Digoxin treatment prolonged survival in a PDOX model of Group 3 MB (ICb-2555MB) to 180 days (n=10) vs. 102 days (n=8) for untreated controls (log-rank, p<0.001). In addition, digoxin single agent therapy and radiation showed comparable prolongation of survival (median 180 and 167 days) vs. untreated controls (102 days). Combination therapy with digoxin and radiation together showed a further significant prolongation of survival compared with radiation only (median survival: 219 days vs. 167 days, p=0.04). Transcriptome analysis identified differentially expressed genes both in Groups 3 and 4 PDOX before and after digoxin treatment, indicating the changes in expression of transcription factors, such as LHX9, and mitochondrial function are associated with the mechanism(s) of action of digoxin. Our results demonstrate the power of a systematic drug repositioning method in identifying a highly effective and rapidly translatable new treatment for medulloblastoma. The same method can potentially be used to identify novel driver signaling networks and to accelerate the discovery of new treatments for other human cancers that lack clearly defined druggable targets, through the repositioning of known drugs for new indications.

A54 Linked single-cell biophysical and transcriptional profiles resolve heterogeneity and mechanisms of in vivo resistance in primary human tumors

Winter, Peter; Murakami, Mark; Navia, Andrew; Gupta, Alejandro; Shigemori, Kay; Calistri, Nicholas; Atta, Lyla; Van Scoyk, Alex; Liu, Huiyun; Kimmerling, Robert; Stevens, Mark; Weinstock, David; Shalek, Alex; Manalis, Scott

Technologies that can define drug sensitivity and resistance in primary human tumors and illuminate mechanisms mediating intratumor response heterogeneity are needed to realize personalized cancer therapies. While single-cell RNA-Seq (scRNA-Seq) can reveal molecular circuits that distinguish pre- and post-treatment tumor samples, this information does not necessarily identify the mechanisms that most directly contribute to resistance. To bridge this gap, we developed a platform that couples functional single-cell biophysical profiling and downstream scRNA-Seq from primary human tumor specimens. More specifically, our approach measures single-cell mass and short-term growth using a series of suspended microchannel resonators (SMRs) that trigger the indexed collection of individual cells for RNA-Seq. By serially profiling tumors at distinct phases of treatment, we can track the transcriptional signatures and cell states that mediate cellular fitness and thereby define the evolution of in vivo resistance to targeted therapy. Here we apply this method to longitudinally follow a cohort of patient-derived xenograft models of BCR-ABL-rearranged acute lymphoblastic leukemia (BCR-ABL ALL) during treatment with the BCR-ABL inhibitor ponatinib. Our platform revealed significant inter- and intra-tumor heterogeneity - including differences between spleen- and bone marrow-resident leukemia cells - but highlighted the recurrent emergence of a subset of leukemia cells with smaller mass harboring transcriptomes characteristic of quiescence and late B lymphoid maturation. We hypothesize that these cells constitute a persistent cellular reservoir that ultimately seeds relapse through the acquisition of additional genetic and non-genetic lesions. Our platform is tractable for paucicellular blood and bone marrow specimens, making it ideal for testing

this hypothesis within the context of our upcoming phase I clinical trial of intensified BCR-ABL inhibition in newly diagnosed BCR-ABL ALL.

A55 Synergistic interaction of physics and biology modulate biological barriers in the liver.

Nizzero, Sara; Tong, Si Qi; Goel, Shreya; Li, Feng; Zhang, Guodong; Li, Zheng; Shen, Haifa; Blanco, Elvin; Ferrari, Mauro

In the past decades much effort has been devoted to the development of selective, efficient, and targeted drugs for the treatment of a variety of cancer. However, despite undoubtable advancements, a major limitation to any type of systemic therapy is the presence of biological barriers that hinder the capability of drugs to reach the target. Among others, the first and most dramatic barrier that systemically administered drugs encounter is the liver, responsible for uptake of ~90-99% of systemically administered nanoparticles and drugs. This dramatic filtering effect constitutes an upstream limit to therapeutic efficacy in the treatment of cancer, especially metastatic disease. In this work, an in depth mechanistic analysis is conduced on liver uptake of microparticles on track to the clinics, with the ultimate goal of identifying the key variables that modulate liver uptake and their synergistic behavior. Uptake by different liver cell populations is studied under static and dynamics in vitro conditions, and compared to in vivo studies of uptake dynamics. A custom liver-on-a-chip model is used to evaluate the effect of shear stress and flow on cell uptake capability, which is then confirmed by small animal studies. As a result, the interaction dynamic of uptake between different liver cell populations under liver physiological conditions is elucidated. This synergy is disrupted with a therapeutic approach aimed at inhibiting liver uptake through saturation. Overall these results open a new paradigm on preconditioning strategies to inhibit the effect of biological barriers in cancer treatment, with a disruptive potential in enhancing therapeutic efficacy.

A56 Quantifying drug combination synergy along potency and efficacy axes

Meyer, Christian; Wooten, David; Paudel, B. Bishal; Bauer, Joshua; Hardeman, Keisha; Westover, David; Lovly, Christine; Harris, Leonard; Tyson, Darren; Quaranta, Vito,

Two goals motivate treating diseases with drug combinations: reduce off-target toxicity by minimizing doses (synergistic potency), and improve outcomes by escalating effect (synergistic efficacy). Surprisingly, established drug synergy frameworks obscure such distinction, failing to harness the full potential of modern chemical libraries. We therefore developed Multidimensional Synergy of Combinations (MuSyC), a formalism based on a generalized, multidimensional Hill-equation with parameters that decouple synergistic potency and efficacy. In mutant-EGFR driven lung cancer, MuSyC provides the insight that combining a mutant-EGFR inhibitor with inhibitors of other kinases may only result in synergistic potency, whereas synergistic efficacy can be achieved by co-targeting epigenetic regulation or microtubule polymerization. In mutant-BRAF melanoma, MuSyC validates a synergistically efficacious combination identified by differential expression analysis. These

findings showcase MuSyC's potential to transform the enterprise of drug-combination screens by precisely guiding translation of combinations towards dose reduction, improved efficacy, or both.

A57 Impact of sex-differences on MRI and genetic correlations in Glioblastoma

Leland S. Hu, Nathan Gaw, Hyunsoo Yoon, Jennifer M. Eschbacher, Leslie C. Baxter, Kris A. Smith, Peter Nakaji, John P. Karis, Paula Whitmire, Andrea Hawkins-Daarud, Kyle Singleton, Pamela Jackson, Susan Massey, Bernard R. Bendok, J. Ross Mitchell, Teresa Wu, Nhan L. Tran, Joshua B. Rubin, Kristin R. Swanson, Jing Li

BACKGROUND: MRI-based modeling can help characterize the intratumoral genetic heterogeneity of Glioblastoma (GBM). Yet, published models to date have neglected the potential impact of sex-differences on the accuracy of MRI-genetic correlations. Specifically, there is growing awareness that female GBM patients can display different genetic/molecular aberrations and phenotypic expression compared to male counterparts. In this exploratory study, we compare MRI signal and key GBM driver alterations across a cohort of male and female GBM patients, using image-guided biopsies and spatially matched multi-parametric MRI. METHODS: We collected 61 image-guided biopsies from 18 primary GBM patients (9/9 male/female). For each biopsy, we analyzed DNA copy number variants (CNV) for 6 core GBM driver genes reported by TCGA: amplifications (++) for EGFR and PDGFRA and deletions (--) for PTEN, CDKN2A, RB1, TP53. We compared regional CNV status with spatially matched MRI texture measurements from co-registered biopsy locations. Advanced MRI features included relative cerebral blood volume (rCBV) on DSC-perfusion, mean diffusivity (MD) and fractional anisotropy (FA) on diffusion tensor imaging. We identified univariate correlations for combined and sex-specific (male, female) subgroups. We also built multivariate predictive decision-tree models for each GBM driver gene and used leave-one-out-cross-validation (LOOCV) to determine area-under-curve (AUC) on ROC analysis to compare accuracies across combined and sex-specific models. RESULTS: We identified multiple univariate correlations between regional CNV status and spatially matched MRI texture features that were specific to either male or female GBM tumors. For instance, EGFR++ specifically correlated with T2W image textures in male biopsies but rCBV textures in female biopsies. In general, sex-specific analyses on decision-tree modeling improved predictive accuracies (AUC) compared to combined (male+female) modeling, particularly for EGFR++ ($p<0.05$), PTEN--($p<0.025$), and TP53--($p<0.025$). CONCLUSION: Sex-differences impact MRI-genetic correlations and warrant further study in larger GBM cohorts.

B01 Metabolic Status and Adaptability of Breast Cancer Stem Cells

Buschhaus, Johanna; Luo, Ming; Burnett, Joseph; Sun Duxin; Wicha, Max; and Luker, Gary

Breast cancer stem cells (BCSCs) represent the subpopulation of malignant cells that cause tumor initiation, metastasis, and recurrence. BCSCs resist therapy with radiation and standard drugs, emphasizing the need to identify new vulnerabilities as therapeutic targets. Here, we investigated the metabolism of BCSCs at the single-cell resolution using

two-photon microscopy with fluorescence lifetime imaging (FLIM) of the endogenously fluorescent molecule NADH. Concurrently, we imaged retention of the fluorescent dye PKH26 or expression of a CRISPR/Cas9-engineered ALDH1A3-mCherry promoter-reporter to identify BCSCs. We previously reported that sorted BCSCs exhibited enhanced oxidative phosphorylation (OXPHOS) relative to bulk tumor cells in 2D culture and rapidly metabolically adapted to the glycolysis inhibitor 2-deoxyglucose (2DG). Since cells rewire signaling and metabolism in 3D environments, we capitalized on two-photon microscopy to quantify metabolism in secondary mammospheres and living animals. In both intact spheres and orthotopic tumor xenografts, BCSCs marked by either fluorescent reporter utilized glycolysis to a greater extent than bulk cancer cells. When treated with 2DG, mammospheres showed increased OXPHOS and a significant decrease in the percentage of ALDH1A3-mCherry+ cells. These data 1) highlight capabilities of FLIM to measure the metabolism of single cancer cells in physiologic environments; and 2) reveal that BCSCs rely on glycolysis, suggesting a potential target for metabolic therapy.

B02 A quantitative proteomic survey reveals signaling alterations and oxidative stress regulation in lung cancer cells driven by stromal fibroblasts.

Martial Boutchueng-Djidjou, Jae-Young Kim, Ki-Cheol Han, Gabriela Wright, Bin Fang, John Koomen, Lily.L. Remsing Rix, Uwe Rix, Eric B. Haura.

Cancer cells and fibroblasts support each other for increased aggressiveness and drug resistance. Cancer cells educate fibroblasts to acquire cancer associated fibroblasts (CAFs) phenotype and CAFs feedback by regulating cancer cells signal rewiring in response to tyrosine kinase inhibitors (TKIs) for their survival and proliferation. Drug sensitivity has been associated to the oxidation state in mutated EGFR driven cancer cell lines treated with EGFR TKI. Furthermore, the importance of ROS scavengers of the Glutathione peroxidase family in drug tolerant persistent cells survival by ferroptosis has been reported. To gain insights on this bi-directional interplay, we applied a 'Cell Type-specific labelling using Amino acid Precursors' (CTAP) to perform quantitative mass spectrometry analysis of lung cancer cells (LCs) and lung fibroblasts (LFs) in co-culture. CTAP allows for simultaneous metabolic labelling of co-cultured cell types leading to differential proteomic analysis. We generated three sets of proteomics data including: shotgun data for whole cell proteomes, activity-based protein profiling (ABPP) using a desthiobiotin-ATP probe for kinases activation, and phospho-tyrosine proteomics data. We selected PC9 lung cancer cells harboring an activating EGFR mutation and the transformed lung fibroblasts (WI-38-VA13). Their mono-cultured cells are used as reference for comparative data analysis. Preliminary results show a downregulation of tyrosine phosphorylation in both LCs and LFs after 24 hours of co-culture suggesting increased activity of cysteine-dependent tyrosine phosphatases as potential mechanism. In parallel, we identified increased level of ROS sensors and scavengers of the Thioredoxin family, the glutathione peroxidases, and AXL receptor. GSEA identified enrichment of proteins with YAP-conserved motifs among the major oncogenic signatures in LCs. Moreover, the ABPP dataset shows a downregulation of MAP4K3 activity in LCs, a component of Hippo signaling pathway. Total AXL, which is

regulated by YAP and is, associated with oxidation, has ten-fold increase in LCs along with upregulation of AXL-Y702. Live cell imaging microscopy shows decreased oxidation level in LCs after co-culture with LFs. Our results suggest a module of co-expressed proteins including the ROS sensors, ROS scavengers, tyrosine kinase receptors such as AXL, as drivers of ROS regulation in LCs by LFs.

B03 Exploring Tumor Architecture from Millimeters to Nanometers

Jessica L. Riesterer, Koei Chin, Kevin Loftis, Melissa Williams, Kevin Stoltz, Joe W. Gray

Tumor architecture is an important descriptor of potential cancer response. Even within the same cancer type there can be subtypes, such as basal versus luminal triple-negative breast cancer. Immune response is impacted by the cellular arrangement with respect to the tumor microenvironment, i.e. stroma, fibroblasts. Likewise, cellular phenotype, and the tumor architecture at a cellular level is a potential descriptor of patient outcome. Being able to view how the tumor is arranged at all scales is clearly important in order to begin predicting therapeutic response. Two methods of viewing quantifiable architectural features are electron microscopy and cyclic immunofluorescence (cycIF), and can be used to image tissues and cells, mouse models, xenografts, and organoids. Scanning electron microscopy (SEM)-based methods can be utilized to view ultrastructure to architecture at high resolution over large areas of cells and tumors. The FEI Maps software allows epon-embedded samples to be imaged over the entire block face at 4-nm resolution and montaged to form a single image. These high-resolution maps provide an overview of the architecture spanning an area of tumor as large as millimeters. When collected at the highest resolution, the map can be 'pan & zoomed' into for viewing the cellular ultrastructural relationship with respect to the architecture. Particularly intriguing regions of interested can be zeroed-in on for higher resolution probing. Two acquisition methods can be utilized to view these regions in three-dimensions at high resolution: focused ion beam (FIB)- and serial block face (SBF)-SEM. Both methods utilize serial physical slicing of the epon-embedded blocks followed by image collection; hundreds of images are collected and aligned in order to render a 3D model of the cell-cell contacts, cell-microenvironment interactions, and ultrastructural features. When we add cycIF, we can begin to understand signaling with respect to architecture and compare measurable features between the two microscopy techniques. cycIF is an iterative process (a cycle) performed on a single slice cut from a block of FFPE tissue. Each cycle involves four sequential steps: (1) staining with fluorophore-conjugated antibodies against different protein antigens; we currently use antibodies conjugated to Alexa 488, 555 and 647; (2) staining with Hoechst 33342 to mark nuclei; (3) four-channel imaging at low and high magnification (10X, 20X and 40X objectives); (4) fluorophore oxidation using hydrogen peroxide, high pH and UV light followed by a wash step. A pre-bleaching cycle is performed prior to incubation with primary antibodies to reduce the level of auto-fluorescence and minimize non-specific background staining by secondary antibodies. Pre-bleaching involves incubation with secondary antibodies alone followed by fluorophore oxidation. The current approach has been optimized for samples prepared in the standard manner for pathologic diagnosis of

cancer (4-5 μm thick FFPE slices mounted on a glass slide). Immunostaining is performed for 6 cycles using a core set of validated antibodies indicated inelected to interrogate aspects of differentiation, proliferation, architecture, immune infiltrate, vasculature, fibroblasts and other stromal cells related to tumor architecture.

B04 Single cell analysis of therapy resistance in cancer

Eduardo Torre, Ben Emert, Arjun Raj

Therapies targeting mutated proteins hold much promise in the treatment of cancer, but the emergence of resistance to these therapies presents a major barrier to cures. Recent work, including some results from our lab in melanoma, shows that non-genetic cellular plasticity may provide a mechanism of resistance to these therapies. Furthermore, the addition of the drug itself converts this transient plasticity into a new, stably resistant cell state via cellular reprogramming. We describe a genome-wide method of identifying high-memory rare-cell expression programs that are therapy resistant, a new method for tracing cellular heterogeneity back in time, and discuss some recent efforts to map the molecular underpinnings of rare-cell formation.

B05 3D Tumor Biofabrication

W. Gregory Sawyer, Padraic P Levings, Colin J. Anderson, Steven C. Ghivizanni, C. Parker Gibbs

Cancer is a complex and heterogeneous disease involving more than just cancer cells. A diverse cast of components in the surrounding microenvironment interact with cancer cells and actively facilitate malignant progression in a three-dimensional (3D) structure. For decades, drug development relied on the simplistic two-dimensional (2D) cancer model derived from cell lines that grow submerged in a monolayer format. Although this configuration was amenable to high throughput screening, the loss of dynamic interaction between cancer cells and the host microenvironment changed the cell morphology and signaling networks, resulting a suboptimal model that was not reflective of native tumors. The demands of biofabrication have driven a tremendous amount of research effort in 3D tissue culture technology and, more recently, in 3D printing. The need to use 3D tissue culture techniques more broadly in all of cell biology is well-recognized, but the transition to 3D has been impeded by the convenience, effectiveness, and ubiquity of 2D culture materials, assays, and protocols, as well as the lack of 3D counterparts of these tools. The recent discovery of Liquid Like Solids (LLS) and 3D biofabrication provides the technical support needed for 3D culture. Cancer is a complex and heterogeneous disease involving more than just cancer cells. A diverse cast of components in the surrounding microenvironment interact with cancer cells and actively facilitate malignant progression in a three-dimensional (3D) structure. For decades, drug development relied on the simplistic two-dimensional (2D) cancer model derived from cell lines that grow submerged in a monolayer format. Although this configuration was amenable to high throughput screening, the loss of dynamic interaction between cancer cells and the host microenvironment changed the cell morphology and signaling networks, resulting a suboptimal model that was

not reflective of native tumors. The demands of biofabrication have driven a tremendous amount of research effort in 3D tissue culture technology and, more recently, in 3D printing. The need to use 3D tissue culture techniques more broadly in all of cell biology is well-recognized, but the transition to 3D has been impeded by the convenience, effectiveness, and ubiquity of 2D culture materials, assays, and protocols, as well as the lack of 3D counterparts of these tools. The recent discovery of Liquid Like Solids (LLS) and 3D biofabrication provides the technical support needed for 3D culture. The development of 3D culture systems that can accurately and reproducibly model tumor biology in vitro, while providing the flexibility and ease of use of conventional cell culture may provide a major advance to cancer research. Engineered 3D printed microtissues of cancer cells, fibroblasts, and endothelial cells in co-culture offers the potential to recapitulate the functional and molecular heterogeneity found in patient tumors. In 3D printed tumoroids we have observed differential zones of extracellular matrix (ECM) production, cell proliferation, apoptosis and necrosis that likely arise from adaptive responses of tumor cells to hypoxic and nutrition/metabolite stress gradients. Relative to conventional monolayer culture, it is widely accepted that cells in 3D aggregates more closely exhibit the biology of cells in the context of their native tissues, and their response to stimulation.

B06 Stratifying mitochondrial heterogeneity of pancreatic tumors by single-cell impedance cytometry

Nathan S. Swami , David F. Kashatus, Gustavo Rohde and Todd Bauer

Tumor development is governed by interactions between oncogene and tumor suppressor pathways, as well as by a host of environmental influences from the surrounding stroma. Combinations of oncogenic mutations and the heterogeneous genetic and phenotypic background of the human population lead to heterogeneity in disease presentation, progression and treatment response. Mitochondria, which are key regulators of cellular metabolism and life-death decisions, undergo constant cycles of fusion and fission to allow the cell to quickly adapt to environmental conditions and to promote overall cellular health. Since the mitochondrial phenotype in tumor cells arises through a combination of inputs from activated oncogenes, loss of tumor suppressors, and interactions with stromal cells and the tumor microenvironment, we seek to quantify its heterogeneity to assess its functional impact on oncogenic signaling and tumor-stroma interactions of relevance to pancreatic tumors. The mitochondrial phenotype in cells represents a particularly intricate set of subcellular features, such as its branch size, number, shape, surface area, connectedness, etc. Current approaches to quantify the mitochondrial phenotype based on imaging and image processing are time-consuming and cumbersome, while flow cytometry methods are unable to isolate cells based on this phenotype, due to their inability to spatially resolve mitochondrial features and rapidly recognize the underlying phenotypes. In this context, electrical impedance and polarizability acquired on single-cells flowing through a microfluidic channel, possesses real (magnitude) and imaginary (phase) components that can serve as a powerful and label-free metric of the mitochondrial phenotype, since respective frequency regions correspond to particular subcellular

phenotypes determined based on their subcellular electrophysiology. Specifically, we show that single-cell impedance spectra are sensitive to genetic manipulation of mitochondrial morphology in multiple diverse cell lines and the altered subcellular cytoplasmic electrophysiology enables microfluidic isolation of cells with distinct mitochondrial phenotypes. Furthermore, cytometry of mitochondrial phenotypes within single-cells can be measured at throughput levels of 50-100 cells/s for analyzing heterogeneity from 10000-100000 tumor cells obtained from liquid biopsies. In ongoing work, we seek to mathematically correlate mitochondrial spatial organization to physically measured impedance characteristics and employ patient-derived xenografts to develop statistical models for mitochondria-based prediction of patient outcomes and theragnosis.

B07 Identifying key signaling network in AXL-driven chemotherapy resistance

Bae, Song Yi; Meyer, Aaron S.

Despite the development of multiple effective therapeutics, drug resistance is a major barrier to lung cancer treatment. A common mechanism of chemoresistance is expansion of mesenchymal-like cells within tumors leading to poor drug-response and metastasis. AXL, a receptor tyrosine kinase aberrantly activated in many tumors, plays a critical role in driving mesenchymal state. AXL signaling simultaneously contributes to the survival of cells, promotes resistance of those cells to therapy, and directs tumor cell migration resulting in potently increased metastatic capacity. However, the details of how the receptor's activation lead to consequent changes in drug response are still unclear. Here, we propose to study global downstream signaling changes during chemotherapy resistance mediated by AXL in a mesenchymal cell state using a panel of phosphorylation site mutants and phosphoproteome analysis. We hypothesize that a conserved subset of downstream signaling is required for AXL-driven chemoresistance. First, we detected the activation of AXL by chemotherapeutic agents in H1299, an AXL-expressing non-small cell lung cancer (NSCLC) cell line. Among the drugs, paclitaxel significantly increased AXL activation. In addition, the selection of wild-type (WT) AXL-expressing cells by paclitaxel was observed in H1299 AXL knockout cells and HCC827, another NSCLC cell line expressing minimal endogenous AXL. These findings confirmed the association of AXL in paclitaxel-driven drug resistance. Then, 17 AXL mutant vectors in which each intracellular tyrosine residue is substituted with phenylalanine were assessed for their resistance promoting capacity. According to the results so far, 8 of 17 AXL mutants were identified to lose the ability to enrich AXL-expressing cell population in HCC827 cells after paclitaxel treatment. Key molecules distinguished from the study to direct chemoresistance will be valuable in predicting drug response of cancer cells in mesenchymal cell state and improve our ability to identify effective treatment strategies.

B08 Memory sequencing reveals heritable single cell gene expression programs associated with distinct cellular behaviors

Sydney Shaffer, Benjamin L. Emert, Ann E. Sizemore, Rohit Gupte, Eduardo Torre, Danielle S. Bassett, Arjun Raj

Non-genetic factors can cause individual cells to fluctuate substantially in gene expression levels over time. Yet it remains unclear whether these fluctuations can persist for much longer than the time for a single cell division. Current methods for measuring gene expression in single cells mostly rely on single time point measurements, making the time of a fluctuation of gene expression or cellular memory difficult to measure. Here, we report a method combining Luria and Delbruck's fluctuation analysis with population-based RNA sequencing (MemorySeq) for identifying genes transcriptome-wide whose fluctuations persist for several cell divisions. MemorySeq revealed multiple gene modules that express together in rare cells within otherwise homogeneous clonal populations. Further, we found that these rare cell subpopulations are associated with biologically distinct behaviors in multiple different cancer cell lines, for example, the ability to proliferate in the face of anti-cancer therapeutics. The identification of non-genetic, multigenerational fluctuations has the potential to reveal new forms of biological memory at the level of single cells and suggests that non-genetic heritability of cellular state may be a quantitative property.

B09 Evaluating Ovarian Cancer 3D Nodule Mechanical Properties using Brillouin Confocal Microscopy

Conrad, Christina; Gray, Kelsey M.; Stroka, Kimberly M.; Rizvi, Imran; Scarcelli, Giuliano

The mechanical properties of nodules have been linked to epithelial-mesenchymal transition and upregulation of survival pathways in ovarian cancer. However, current mechanical characterization methods are limited in various experimental designs due to the requirement of contact or perturbation to the sample. Our lab has developed Brillouin Confocal Microscopy, an optical non-contact method to map the mechanical properties of biological materials with high 3D resolution without need for labeling. Here, we use Brillouin microscopy to evaluate mechanical properties of in vitro 3D ovarian cancer tumor nodules. We demonstrated three-dimensional mapping of mechanical properties of tumor nodules with a spatial resolution of 1μm transverse and 2μm axial. We validated this technique by correlating the Brillouin-derived 'Brillouin Shift' to the gold-standard elastic modulus measured using both Atomic Force Microscopy (AFM) and a Micro-Scale Mechanical Tester (MicroSquisher). To vary the mechanical state of the nodules we increased osmotic pressure and found nodules to present increased Brillouin shift and elastic modulus. This is expected because the increase in extracellular sucrose concentration causes efflux of water from the cells causing stiffening of the whole nodule. We also treated the tumor nodules with carboplatin, a chemotherapy commonly used to manage ovarian cancer drug, and measured a significant decrease in Brillouin shift ($p<0.0005$ via unpaired t-test assuming equal variances). Brillouin Confocal Microscopy has been shown to be a promising method to evaluate mechanical properties of 3D cancer

nodules. Moving forward, we plan to utilize this technology for evaluating mechanical effects of nodules in response to phenomena relevant to ovarian cancer disease pathology.

B10 Robust Method for the Alignment of Serial Whole Slide Histology

Gatenbee, Chandler; Tessi, Mark Robertson; Anderson, Alexander A.R.

The importance of the tumor ecology is often acknowledged, but has been difficult to describe quantitatively, due to limited number of stains that can be applied to tissue, and/or large amounts of training data required for cell type prediction. Multiplexing with mass cytometry is a promising new technology that gets around this limitation, but it is highly expensive, requires new samples, and is often applied only to small sections of the tumor. Using machine learning, graph theory, and computer vision, we have developed a method to align series of whole slide tissue slices stained for different markers, even when the original ordering of the slices is unknown. While the accuracy of this method is not at the level of multiplexing, the benefits are that it requires only a computer and little processing power, is free, can be used to align tissues of any size, and can be applied to existing datasets for which the original samples may no longer be available. The proposed method has successively been applied to large datasets of dual-stained immunohistochemistry images from colorectal cancer and DCIS, as well as large collections of serial slices (47 slides) stained for H+E. Once tissue slices have been aligned using our method, existing quantitative methods from ecology can be used to both describe the individual tumor ecology (cell abundances, spatial interactions, regional variation, etc...), and also compare tumor ecologies across groups, such as primary/metastases, responders/non-responders, tumor grade, tumor subtype, etc... . Given that the ecology imposes Darwinian selection pressures within the tumor, we believe that application of this method and subsequent ecological analysis will provide insights into how cellular interactions regulate tumorigenesis, and how those interactions affect outcome.

B11 Engineered extracellular matrix polymer scaffolds for precisely defined cellular microenvironments

Muniz, Ayse; Brooks, Michael; Neale, Dylan; Sze, Angela; Wicha, Max; Lahann, Joerg

The cellular microenvironment, comprised of a complex milieu of biomacromolecules and disparate cell types, plays an extensive role in tissue behavior. Study of the microenvironment has a broad range of applications, from understanding disease pathogenesis to designing structures for regenerative medicine applications. Current strategies for studying or controlling interactions between cells and their microenvironment rely on combining cells and biological compounds on two-dimensional surfaces or engineered three-dimensional (3D) constructs. Generally, these methods are insufficient at recapitulating native tissue microenvironments, do not allow for precise control over cell morphology, alignment, or the study of complex cell-cell or cell-extracellular matrix (ECM) interactions. Therefore, a need exists for more sophisticated 3D engineered structures that both maintain the natural microenvironment found in vivo

while being easy to fabricate, scalable, and robust enough to manipulate. Our lab has recently pioneered a novel micromanufacturing process, termed '3D jet writing,' that produces user-defined biocompatible 3D polymer structures with high precision, and can be used in tissue engineering applications. Highly porous polymer scaffolds made using this method support an engineered ECM (eECM) through the deposition of highly fibrillar, native-like ECM components. Importantly, control over ECM composition, topography, and scaffold geometry are critical parameters that are orthogonally controlled using this platform. Here, we characterize the platform and utilize single-cell transcriptomic analysis to establish its biorelevancy for engineering complex tissue that more faithfully recapitulates the breast tumor microenvironment relative to other standard 2D and 3D approaches. Our results underscore the importance of culture condition as a driver of tumor heterogeneity, which is well recognized as an emerging driver of drug development and patient diagnosis and treatment.

B12 Engineered extracellular matrix scaffolds induce directionally persistent cell migration

Neale, Dylan; Muniz, Ayse; Vargas, Diego; Wawryszyn, Mirella; Buschhaus, Johanna; Owen, Sarah; Solorio, Luis; Nagrath, Sunitha; Luker, Gary; Lahann, Joerg

Aligned tissue architecture is ubiquitous in human physiology and observed across various tissue microenvironments, including the tumor microenvironment. Notably, the extracellular matrix (ECM) surrounding tumors takes on a pro-fibrotic characteristic in which there is an increased deposition of collagen-I (COL-I) that can present in a highly aligned orientation. These aligned COL-I tracks have been correlated with poor prognosis and are presumed to facilitate metastatic tumor cells. Although the role of COL-I has been extensively explored in this context, other evidence suggests that fibronectin and ECM bound hyaluronic acid are, among other ECM components, associated with tumorigenic tissue. Hence, there is growing interest in utilizing three-dimensional (3D) cell culture systems to understand the pathobiology associated with these tumorigenic tissue signatures, as they likely play a role in metastasis. This interest is, in part, due to the growing consensus that two-dimensional (2D) cell culture substrates do not recapitulate critical biophysical components of the tumor tissue niche. Here, we demonstrate an advancement in creating aligned, 3D fibronectin constructs through a shear-driven fibril deposition process. Using these aligned engineered extracellular matrices (aEECM), we found that aligned fibronectin significantly influences the directional orientation of fibroblasts, their polarity and promotes directionally persistent cell motility. We further demonstrate the ability to incorporate other critical ECM components such as hyaluronic acid and COL-I; thus, setting the stage for future studies to investigate the role of tumorigenic tissue signatures on tumor cell migration and phenotype. This in vitro technology can therefore be leveraged to study the reciprocal interactions between tissue architecture and composition at the invasive front of tumors.

B13 Stratifying mitochondrial heterogeneity of pancreatic tumors by single-cell impedance cytometry

Nathan S. Swami, David F. Kashatus, Gustavo Rohde and Todd Bauer

Tumor development is governed by interactions between oncogene and tumor suppressor pathways, as well as by a host of environmental influences from the surrounding stroma. Combinations of oncogenic mutations and the heterogeneous genetic and phenotypic background of the human population lead to heterogeneity in disease presentation, progression and treatment response. Mitochondria, undergo constant cycles of fusion and fission to allow the cell to regulate and adapt to the interactions arising from tumor development. Hence, we seek to quantify heterogeneity in mitochondrial phenotypes to assess its functional impact on oncogenic signaling and tumor-stroma interactions of relevance to pancreatic tumors. However, due to the intricate mitochondrial phenotypes such as its branch size, number, shape, surface area and connectedness, imaging-based approaches are cumbersome and flow cytometry is unable to spatially resolve the underlying phenotypes. Cytometry approaches based on electrical impedance that are acquired on single-cells flowing through a microfluidic channel, possesses real (magnitude) and imaginary (phase) components that can serve as a powerful and label-free metric of the mitochondrial phenotype, since the subcellular electrophysiology measured at set frequency regions can correspond to particular subcellular phenotypes. Specifically, we show that single-cell impedance spectra are sensitive to genetic manipulation of mitochondrial morphology in multiple diverse cell lines, including those from tumor biopsies, and the altered subcellular cytoplasmic electrophysiology enables microfluidic isolation of cells with distinct mitochondrial phenotypes. Furthermore, cytometry of mitochondrial phenotypes within single-cells can be measured at throughput levels of 50-100 cells/s for analyzing heterogeneity from 104-105 tumor cells obtained from liquid biopsies. In ongoing work, we seek to develop a mathematical model correlating single-cell impedance spectra to mitochondrial spatial organization using a library of mitochondrial phenotypes and employ clinically-relevant patient-derived xenografts (PDXs), alongside features of PDXs (KRAS mutation status, patient survival, etc.) as labeled data, to develop statistical models for mitochondria-based prediction of patient outcomes and theragnosis. We envision that this technological platform will advance the ability to quantify differences in the relative proportion of cells with particular mitochondria phenotypes across patient categories to ultimately aid in the prediction of patient survival in prognostic studies and enable patient-screening for precision medicine.

B14 Biophysical roles of platelets in the pre-metastatic niche on transport of circulating tumor cells

Kenji Yokoi, Arturas Ziemys, Lidong Qin, Milos Kojic, Megumi Kai, Yan Ting Liu and Mauro Ferrari

Understanding the mechanisms of metastases has been a challenge. Classically, two hypotheses attempt to explain the metastatic process: “mechanical and hemodynamic

hypothesis" and the "seed-and-soil hypothesis". Recent studies reported that distant microenvironments are primed and ready for the arrival of circulating cancer cells (CTCs), creating a "pre-metastatic niche" for future metastatic growth. Various clinical evidences indicate a relationship among platelets (PLTs), its activation and metastasis. Whereas the effects of circulating PLTs on CTCs (seed) and the effect of PLTs accumulating at already metastasized tumor cells (seed) are known, the roles of PLTs in the initiation and development of the pre-metastatic niche (soil) and the effects of these PLTs on mechanical and hemodynamic transport of CTCs have not been reported. Here, we identified 4T1 murine breast cancer cells spontaneously metastasized to lungs and liver, but not to other major organs in Balb/C mice after implantation into the primary site. Significant increases in PLT accumulation and activation were found only in lungs and liver after the implantation, before any detectable metastasis, suggesting that PLT accumulation and activation occur in these organs before initiation of spontaneous metastasis. Next, we used Intravital Microscopy to image and quantify spatial and temporal changes in PLT transport in liver capillaries of normal mice, 4T1 primary tumor-bearing mice, and the primary tumor-bearing mice pre-treated with Eptifibatide, an inhibitor of PLT activation. We found that PLT velocity was more heterogeneous and slower in the livers of the primary tumor-bearing mice as compared to those in normal mice. Treatment with Eptifibatide reversed these modifications of PLTs transport, indicating anti-PLT reagent can modulate blood flow dynamics in liver of the primary breast tumor bearing mice. To determine the biophysical effects of PLTs on cancer cells transport, we used our microfluidics chip, which consist of microarrays of cell trapping barriers with gaps decreasing. Cancer cells with less frictional properties with gap surfaces and less stiffness can be transported a further distance within the chip. The chips were pre-coated with or without PLTs, and perfused with 4T1 cells. PLT coating led to a higher proportion of trapped 4T1 cancer cells at barriers with larger gap sizes compared to non-coated condition. Furthermore, coating the gaps with activated PLTs reduced transport of 4T1 cells more than non-activated PLTs. Our PLT and CTC transport modeling analyses provide mechanistic insights into physical forces governing biological phenomena. The feedback coming from the models to in vivo and in vitro experiments allows the de-convolution of the biological complexity and the refinement of critical physical properties that can be exploited in designing anti-metastatic therapies using anti-platelet reagents.

B15 Revealing mechanisms of cancer-related protein complexes through atomic-resolution cryo-electron microscopy

Moritz, Michelle; Wang, Feng; Brilot, Axel F., Agard, David A.

A traditional route to understanding the molecular mechanisms of protein complexes at atomic-resolution that lends insight to drug design has involved x-ray crystallography. However, many complexes of interest are so flexible and/or dynamic that the formation of high-quality crystals is prohibited. Recent advances in cryo-electron microscopy (cryo-EM) technology have made it a feasible alternative method for determining high-resolution structures of protein complexes of ~150KDa or larger. In practicality, another bottleneck

has been the need for recombinant expression, large-scale purification and reconstitution of complexes of interest. To circumvent this difficulty, we are developing affinity-EM grids capable of rapidly and efficiently isolating a dual-tagged protein complex of interest out of mammalian cell extracts. The strategy is to absorb the complexes to magnetic beads, elute in very small (microliter scale) volumes, and then to capture the complexes on derivatized affinity-EM grids for immediate analysis by cryo-EM. The goal is to provide a rapid and efficient method to evaluate many different protein complexes of interest that are identified through cancer proteomics. As an example, our progress on a cryo-EM structure of a ~390KDa protein complex that is required for microtubule nucleation (the γ-Tubulin Small Complex), in association with a regulatory kinase that has been implicated in certain cancers (casein kinase-1 δ), will be described.

B16 Unjamming and cell shape changes in breast cancer

Kim, Jae Hun; Fredberg, Jeffrey

Cancer cells most often invade neighboring tissues as collective groups, e.g. protruding sheets, clusters or strands, retaining epithelial markers. This finding suggests that instead of an epithelial-to-mesenchymal transition (EMT), some alternative migratory program might be activated. Here we propose that such a program might be provided by the unjamming transition, a process in which a cellular collective undergoes a transition from a solid-like phase to a liquid-like phase. This transition is marked not by EMT markers but by characteristic changes of cell shape. Across vastly diverse epithelial systems, there exists a fixed relationship between cell shape (aspect ratio, AR), its variation (SD), and cell jamming; as a cell layer becomes more and more unjammed, cell shape becomes progressively elongated and more variable To test whether malignant cells conform this same jamming relationship, we used engineered breast-carcinoma cell lines with diverse levels of transforming potential. We tested confluent layers of MCF10A cells expressing breast cancer associated traits, 10A.ErbB2 cells that are highly proliferative, 10A.14-3-3ζ cells that lack E-cadherin and 10A.vector cells. We also tested H-Ras mutated MCF10A variants with increasing malignant potential, MCF10AT cells that are pre-malignant and MCF10ca1a cells that are fully-malignant. Remarkably, all carcinoma cell lines we tested fall on the same relationship that we had established in various epithelial cell types. Moreover, these cell lines aligned roughly with invasive potential along the jamming relationship; a more aggressive cell line was more unjammed. But MCF10ca1a was an exception, being a more aggressive cell line and being more jammed. To test the theoretical prediction in which weaker cell-cell adhesion leads to greater jamming, we examined cell shapes of MCF10A cells in which adhesion proteins in tight junctions, adherent junctions and desmosomal complexes were knocked down one at a time by small interfering RNA (siRNA). Strikingly, 15 siRNAs out of 16 siRNAs caused cells to become less elongated, but all cases fell on the jamming relationship. This finding comprises a striking confirmation that loss of adhesion promotes jamming, but not unjamming. Taken together, this result suggests that an unjamming transition provides a cancer cell collective a gateway to motility.

B17 HiSIF, a computational model of Hi-C data, identifies cell-type-specific chromatin interactions

Yufan Zhou, Xiaolong Cheng, Sandun Jayarathna, Yini Yang, Tian Li, Diana L. Gerrard, Ryan Laseter, Seth E. Frietze, and Victor X. Jin

Most of current computational methods on Hi-C analysis were designed to identify Mb size topological associated domains (TADs). However, these domain-based analyses often fail to unveil the underlying functional and mechanistic relationship of higher order chromatin structure and specific gene regulation. In spite of an improved in situ Hi-C protocol which can achieve a fine resolution (1-5Kb), an extremely higher sequencing depths requirement makes it impractical for many studies. In this study, we developed a novel statistical model and software tool, HiSIF (Hi-C Significant Interacting Fragments) for the Hi-C data analysis. HiSIF uses a Poisson mixture model with a power-law decay background to classify the highly significant interacting fragment (SIF) among random vs proximate ligations. We further use a Landau fit function to identify cell type-specific SIFs among multiple cell lines or biological samples. We evaluated HiSIF on data publicly available and compared its performance to some existing programs. We further tested the model in our newly generated Hi-C data in breast cancer cells, MCF7, tamoxifen resistant MCF7 (MCF7-TamR), T47D and tamoxifen resistant T47D (T47D-TamR). Interestingly, we identified 8,309 SIFs associated with promoter-distal loops (PDLs) in MCF7 cells with 48% of them lost during acquired endocrine resistance, and 4,135 PDLs in MCF7-TamR cells with 23% of them gained during acquired endocrine resistance. However, we identified 4,579 PDLs in T47D cells with 76% of them lost during acquired endocrine resistance, and 4,554 PDLs in T47D-TamR cells with 82% of them gained during acquired endocrine resistance. Clearly, T47D-TamR cells have higher gained looping events than MCF7-TamR cells, suggesting that the aggressive breast cancer cell may experience higher rewired chromatin interactions during acquired endocrine resistance.

B18 Integration of chromatin accessibility and gene expression data across multiple mouse models helps to characterize CD8 T cell dysfunction in cancer and chronic infection

Yuri Pritykin, Lauren Fairchild, Christina Leslie

Loss of CD8 T cell function results in lack of effective immune response to cancer, and only a subset of patients respond to T-cell stimulatory therapies. Recent studies in mouse models of cancer and chronic viral infection applied genomic assays to profile various states of impaired T cell function and demonstrated that T cell dysfunction is epigenetically imprinted. However, comprehensive characterization and classification of T cell dysfunction across models based on their epigenetic and transcriptional profiles is lacking. We reanalyzed a large collection of recently published chromatin accessibility (ATAC-seq) and gene expression (RNA-seq) data sets. Batch effect correction using generalized linear modeling allowed us to map into the same space profiles of chromatin accessibility peaks in gene promoters and enhancers from different studies. We observed that epigenetic profiles

of dysfunctional tumor-infiltrating T cells and exhausted T cells in chronic viral infection were surprisingly extremely similar. Furthermore, a recently characterized discrete distinction between epigenetic profiles of early (day 7-8) and late (day 28-30) dysfunction in the tumor was recapitulated in the model of chronic infection. Overall we observed across mouse models that T cells committed to becoming dysfunctional early after an immune challenge, rather than first mounting and then loosing an effector response. Genes with massive differential accessibility of their promoter and enhancer peaks during development of dysfunction observed consistently across models, including transcription factors (TF) well studied in immunity such as Lef1, Satb1, Ikzf2, Nfatc2, Runx2, are good candidates for further validation of their role. We associated absolute levels of chromatin accessibility in peaks of each sample with TF binding using regularized negative binomial regression, and estimated the effect of each TF using cross-validation. This allowed us to map chromatin accessibility profiles into the TF activity space of much lower dimensionality. Interestingly, this mapping largely preserved the hierarchy of relative similarities between samples. We identified key TFs associated with open or closed chromatin in different cell states. For example, binding of well known effector factors Eomes and Batf was associated, not surprisingly, with closed chromatin in naive cells and highly open chromatin in effector cells. Strikingly, the strongest association with closing chromatin in dysfunction, consistently across mouse models, was observed for Tcf7/Lef1 binding, further suggesting Lef1 role in establishing the terminal CD8 T cell dysfunctional state. This analysis provides a better systematic understanding of cell-intrinsic mechanisms driving different functional states of CD8 T cells, and the developed computational methods are broadly applicable in other experimental setups where diverse cell states are profiled by high-throughput genomic assays.

B19 Universality properties of Random Matrix Theory to study single cell multiomics

Aparicio, Luis; Bordyuh, Mykola; Rabadan, Raul

One of the main problems in developing therapies for cancer patients is tumor heterogeneity. Single-cell biology in its different analysis layers (omics), is a powerful tool to study this heterogeneity but it comes with its own problems like the big amount of noise generated both from technical and biological levels. Random Matrix Theory (RTM) is a natural framework to address the noise problem because it is mathematically developed to deal with systems with a large number of components (such as genes, bio-molecules or cells) interacting according to unknown laws. RMT is a field with many applications in different branches of mathematics and physics and one of its outstanding properties is the so called universality, which consists in the insensitivity of certain statistical properties to variations of the probability distribution used to generate the random matrix. Universality makes RMT a suitable framework to mathematically describe gene-cell expression data coming from single-cell multi-omics approaches. Therefore, RMT is natural tool to study clinically interesting questions in biological systems like the heterogeneity and evolution of

tumors. In this talk we will present the methodological approach based on RMT and its applications to tumors in the context of Glioblastomas.

B20 Non-genetic bet hedging as a survival strategy in cancer cell populations

Harris, L. A., Frick, P. L., Paudel, B. B., Hayford, C. E., Tyson, D. R., and Quaranta, V.

Targeted therapies have been developed to treat cancers 'addicted' to specific driving oncogenes, such as EGFR-mutant non-small cell lung cancer (NSCLC), BRAF-mutant melanoma, and HER2-postive breast cancer. While remarkably successful in the short term, tumor recurrence in the long term is essentially universal. Mechanisms of tumor recurrence are usually thought of in terms of genetic mutations, either pre-existing or acquired during the course of therapy. However, recent research points to a role for non-genetic processes in the response of cancer cells to targeted agents. Many bacterial species are known to employ a 'bet hedging' strategy to survive antibiotic treatments, whereby cells diversify across multiple phenotypes, each with differential fitness to different challenges. Here, we report evidence that cancer cell populations also employ bet hedging as a strategy to survive anticancer drug treatments. Our working hypothesis is that cancer cells diversify across phenotypes in the absence of drug to survive initial drug onslaught and that surviving sub-populations act as reservoirs susceptible to genetic mutation from which tumor recurrence can emerge. This view is supported by experimentation and mathematical modeling that reveal a high degree of clonal heterogeneity within drug-naïve, isoclonal oncogene-addicted cancer cell populations and variation in drug response with increasing culture passage number.

B21 Integrative genomics approaches to study SETDB1 dysregulatory network in multiple myeloma

Junwen Wang, Panwen Wang, Leif Bergsagel, Keith Stewart

Multiple myeloma (MM) is the second most commonly diagnosed blood cancer. Discovering key genes for MM pathogenesis and understanding the underlying molecular mechanisms is essential for novel treatment strategies. The SETDB1 gene, a gene in the well-known MM causing region in chromosome 1q21, encodes a histone methyltransferase that trimethylates histone H3 on lysine 9 (H3K9me3). It is reported to cause multiple cancers, but its role in MM has rarely been studied. We analyzed over 653 samples at the whole genome, whole exome and transcriptome levels sequenced through the MMRF CoMMpass project. By analyzing the multi-OMICs data, we found that increased SETDB1 expression was highly associated with poor patient survival, and germline variants in the gene's enhancer/promoter region were associated with MM risk. We will present a novel computational method to predict SETDB1's upstream regulators and to identify functional noncoding variants, by studying how a variant affects the co-expression relationship between SETDB1 and its binding TF. The method will be improved upon the widely used eQTL method, and is generalizable to study other genes.

B22 CELL GROWTH RATE DICTATES THE ONSET OF GLASS TO FLUID-LIKE TRANSITION AND LONG TIME SUPER-DIFFUSION IN AN EVOLVING CELL COLONY

Malmi Kakkada, Abdul; Li, Xin; Samanta, Himadri S.; Sinha, Sumit; Thirumalai, Dave

Collective migration dominates many phenomena, from cell movement in living systems to abiotic self-propelling particles. Focusing on the early stages of tumor evolution, we enunciate the principles involved in cell dynamics and highlight their implications in understanding similar behavior in seemingly unrelated soft glassy materials and possibly chemokine-induced migration of CD8+T cells. We performed simulations of tumor invasion using a minimal three-dimensional model, accounting for cell elasticity and adhesive cell-cell interactions, as well as cell birth and death, to establish that cell-growth-rate-dependent tumor expansion results in the emergence of distinct topological niches. Cells at the periphery move with higher velocity perpendicular to the tumor boundary, while the motion of interior cells is slower and isotropic. The mean-square displacement $\Delta(t)$ of cells exhibits glassy behavior at times comparable to the cell cycle time, while exhibiting superdiffusive behavior, $\Delta(t) \approx t^{\alpha}$ ($\alpha > 1$), at longer times. We derive the value of $\alpha \approx 1.33$ using a field theoretic approach based on stochastic quantization. In the process, we establish the universality of superdiffusion in a class of seemingly unrelated nonequilibrium systems. Superdiffusion at long times arises only if there is an imbalance between cell birth and death rates. Our findings for the collective migration, which also suggest that tumor evolution occurs in a polarized manner, are in quantitative agreement with in vitro experiments. Although set in the context of tumor invasion, the findings should also hold in describing the collective motion in growing cells and in active systems, where creation and annihilation of particles play a role.

B23 Scalable parameterization of large-scale mechanistic models enables drug response prediction

Froehlich, Fabian; Kessler, Thomas; Weindl, Daniel; Shadrin, Alexey; Schmiester, Leonard; Hache, Hendrik; Muradyan, Artur; Schütte, Moritz; Lim, Ji-Hyun; Heinig, Matthias; Theis, Fabian; Lehrach, Hans; Wierling, Christoph; Lange, Bodo; Hasenauer, Jan

Mechanistic models are essential to deepen the understanding of complex diseases, such as cancer, at the molecular level. Nowadays, comprehensive molecular and phenotypic characterizations are possible in high-throughput experiments. Yet, the integration of such datasets with the vast prior knowledge on signaling pathways is limited by the availability of mathematical models and scalable computational methods. Here, we present a computational framework for the parameterization of large-scale mechanistic models and its application to the prediction of drug response of cancer cell lines from exome and transcriptome sequencing data. This framework is over 104 times faster than state-of-the-art methods and therefore renders previously infeasible mechanistic modeling problems feasible. With this framework, we parameterized a model describing major cancer-associated signaling pathways (>1200 species and >2600 reactions) using drug response data. The parameterized model can accurately predict effect of drug combinations

from single drug data. This is the first integration of massive, high-throughput datasets using large-scale mechanistic models. We anticipate this to be the starting point for development of more comprehensive models allowing a deeper mechanistic insight.

B24 GenePattern Notebook: an integrative analytical environment for cancer research

Michael Reich, Thorin Tabor, Peter Carr, Edwin Juarez, David Eby, Ted Liefeld, Helga Thorvaldsdóttir, Barbara Hill, Pablo Tamayo, Jill P Mesirov

As the availability of genetic and genomic data and analysis tools from large-scale cancer initiatives continues to increase, the need has become more urgent for a software environment that supports the entire 'idea to dissemination' cycle of an integrative cancer genomics analysis. Such a system would need to provide access to a large number of analysis tools without the need for programming, be sufficiently flexible to accommodate the practices of non-programming biologists as well as experienced bioinformaticians, and would provide a way for researchers to encapsulate their work into a single 'executable document' including not only the analytical workflow but also the associated descriptive text, graphics, and supporting research. To address these needs, we have developed GenePattern Notebook, based on the GenePattern environment for integrative genomics and the Jupyter Notebook system. GenePattern Notebook unites the phases of in silico research - experiment design, analysis, and publication - into a single interface. GenePattern Notebook presents a familiar lab notebook format that allows researchers to build a record of their work by creating 'cells' containing text, graphics, and executable analyses. Researchers add, delete, and modify cells as the research evolves, supporting the initial research phases of prototyping and collaborative analysis. When an analysis is ready for publication, the same document that was used in the design and analysis phases becomes a research narrative that interleaves text, graphics, data, and executable analyses. The online notebook format allows researchers to explain the analytical and scientific considerations of each step in any level of detail, promoting reproducibility and adoption. Notebooks can also be shared between researchers for collaborative development. GenePattern Notebook features are designed to help nonprogramming users create and adapt notebooks. We have developed additional cell types allowing users to choose analyses, specify inputs, navigate results, send result files to new analyses, and create richly formatted text, all without the need for programming. A free online GenePattern Notebook workspace is available at http://www.genepattern-notebook.org, where researchers can develop, share, and publish notebook documents. We have provided a collection of template notebooks that walk users through various genomic and machine learning analyses, and are collaborating with cancer research laboratories to create integrative cancer genomics notebooks.

B25 Glioblastoma invasiveness may predict response to therapies with high or low blood brain barrier penetrability

Massey, Susan Christine; Urcuyo, Javier; Hawkins-Daarud, Andrea ; Jackson, Pamela R.; Tuma, Ann C.; Marin, Bianca Maria; Gupta, Shiv; Burns, Terence; Giannini, Caterina; Tran, Nhan; Hu, Leland; Sarkaria, Jann; Swanson, Kristin R.

Introduction: Glioblastoma (GBM) is a very aggressive primary brain cancer, noted for its diffuse infiltration into surrounding normal-appearing brain. This particular nature makes GBM notoriously difficult to treat, as these diffusely invading cells cannot be resected surgically, are difficult to target with radiation therapy, and thus must be targeted with chemotherapy. However, this too presents a challenge, as these invading GBM cells reside beyond the dense tumor regions where angiogenesis causes disruption of the blood brain barrier (BBB) and allows drugs to more readily enter the central portion of the tumor. Thus, it is critical to determine predictors of drug distribution in individual patients' tumors and surrounding brain tissue to ensure the invading GBM cells are exposed to the therapy. Objective: Determine predictors of drug distribution and effect from non-invasive imaging using minimal mathematical approaches. Methods: Following experiments treating murine orthotopic patient-derived xenografts (PDXs) of GBM with various anti-tumor therapies, we compiled data from both the xenografts and the original patients from which the PDX lines were derived. This data included bioluminescence imaging (BLI), lamin-stained histological sections, and magnetic resonance imaging (MRI) from the PDXs, as well as patient MRIs and clinical data. Using the time series BLI data from PDXs, we developed and parameterized minimal differential equation models of PDX tumor growth for individual PDX lines, adjusted for lead-time bias using a nonlinear mixed effects approach. This gave us an overall growth rate for each of the PDX lines across multiple subjects. Next, we compared these growth and invasion characteristics with therapeutic response to various agents. Results: Individual PDX lines have different growth kinetics, and recapitulate the kinetics observed in the original patients from which the lines are derived. Further, these growth kinetics appear to be correlated with differential drug response, with more diffusely infiltrating tumors responding better to drugs with higher CSF to plasma ratios. Conclusion: While further work is needed to verify our results across more PDX lines, our results suggest that noninvasive imaging-based characterization of tumor invasiveness may be able to aid in matching patients to the best therapy for their individual tumors.

B26 Cell Fate Relationships Mapped by p-Creode Trajectory Analysis of Single-cell Data

Charles Herring, Amrita Banerjee, Eliot McKinley, Alan Simmons, Qi Liu, Robert Coffey, Ken Lau

Modern single-cell technologies allow multiplexed sampling of cellular states within a tissue. However, computational tools that can infer developmental cell-state transitions reproducibly from such single-cell data are lacking. Here, introduced is p-Creode, an unsupervised algorithm that produces multi-branching graphs from single-cell data,

compares graphs with differing topologies, and infers a statistically robust hierarchy of cell-state transitions that define developmental trajectories. p-Creode is applied to mass cytometry, multiplex immunofluorescence, and single-cell RNA-seq data. As a test case, we validate cell-state-transition trajectories predicted by p-Creode for intestinal tuft cells, a rare, chemosensory cell type. We clarify that tuft cells are specified outside of the Atoh1-dependent secretory lineage in the small intestine. However, p-Creode also predicts, and we confirm, that tuft cells arise from an alternative, Atoh1-driven developmental program in the colon. These studies introduce p-Creode as a reliable method for analyzing large datasets that depict branching transition trajectories.

B27 Intermittent hypoxia and tumor-immune interactions: A multiscale approach to understanding spatiotemporal heterogeneity

Robertson-Tessi, Mark; Gillies, Robert; Gatenby, Robert; Anderson, Alexander

Heterogeneity is widely observed between and within tumors and the potential clinical significance of these variations is increasingly recognized. The tumor microenvironment selects for phenotypes that are best adapted to survive and grow. However, this environment is temporally and spatially heterogeneous, resulting in local fluctuations of nutrients, growth factors and immune cells. Tumor cells adapt to changing conditions through a variety of mechanisms with different timescales, which have significant implications for the application of therapies. Using a hybrid multiscale mathematical model of tumor growth in vascularized tissue, we investigate the selection pressures exerted by variations in the tumor microenvironment. Key components of the model include: cellular metabolism; an adaptive immune system; and a dynamic vasculature that exhibits intermittent hypoxia. Tumor cells can evolve along several phenotypic axes, which have associated cost-benefit functions. The model was calibrated using in vitro and murine experiments. Results show that tumors develop heterogeneous spatiotemporal structures that we call 'metaphenotypes', which collectively have an evolutionary advantage in that region of the tumor. These emergent metaphenotypes are dependent on timescales of microenvironmental change: intermittent hypoxia selects for plastic, rapidly responding tumor strategies; prolonged nutrient deprivation selects for aggressive, hard-wired, glycolytic cells; and excess immune pressure leads to formation of PD-L1 palisades on the tumor edge. We show how therapy timing and dose can change the evolutionary course of the tumor and affect outcomes. By categorizing each therapy response as a function of initial tumor metaphenotype, we implement drug sequences that promote a synergistic response.

B28 The Same But Different: Identifying Distinct Imaging Ecologies in Male and Female Glioblastomas

Bencomo, Tomas; Hawkins-Daarud, Andrea; Singleton, Kyle; Swanson, Kristin R.

Glioblastoma (GBM) is a primary brain cancer known for its aggressive nature. Inter- and intra-tumoral heterogeneity is generally credited for the slow to non-existent progress in improving outcomes. Sex differences have been identified in the prevalence and prognosis

of various genetic modifications, including IDH1 mutation, EGFR mutation and MGMT methylation. Such observations beg the question of whether there are inherent biological differences in male and female GBMs. We hypothesize that imaging patterns may be different between the sexes. That is, the relationship between the different MRI features of the tumor across the different multiparametric MRIs may be distinct for females and males. In this initial study, we investigated multiparametric pretreatment imaging of 101 patients, 42 females and 59 males, including T1-weighted, T1-weighted contrast enhanced with gadolinium (T1Gd), T2-weighted, and Fluid Attenuated Inversion Recovery (FLAIR) sequences. All images were first coregistered to other images within the same date for the same patient, then run through an inhomogeneity correction algorithm (N4) and finally normalized such that the intensity in the CSF regions had a mean of zero. Pairwise voxel correlations were then assessed for each patient's imaging for mean, standard deviation, and range of the intensities from different MRI sequences as determined on 8x8 windows. When looking across the entire brain, our preliminary results, show that the pairwise correlations between the mean imaging intensities between T1/T1Gd, T1/T2, T1/FLAIR, and T1Gd/T2 pairs all showed similar patterns between the male and female groups. However, the correlations of the mean intensity between the T2 and FLAIR sequences demonstrated different patterns with the male images tending to have more negative correlations and the female images having more positive correlations. As T2 and FLAIR images both emphasize the non-specific edematous swelling in the brain, the difference in correlation patterns of the T2 and FLAIR intensities between the sexes is of great interest. It is suggestive that the hyperintensity seen on these images may reflect a difference in immune response. Future studies will focus exclusively on the tumor area and will also make use of image-localized biopsies to identify the relative presence of key immune constituents.

B29 Exploiting space and trade-offs in drug scheduling using adaptive therapy

Jill Gallaher, Pedro M. Enriquez-Navas, Kimberly A. Luddy, Robert A. Gatenby, and Alexander R. A. Anderson

Over the past decade, there has been an explosion of new, targeted therapies for cancer. However, for advanced disease, having a vast arsenal of treatment options does not always lead to sustained outcomes. Targeted treatments are too specific for heterogeneous tumors and need to be used in combination to target all cells to avoid recurrence. Cytotoxic treatments can attack a wide variety of proliferating cells but are more taxing to the patient's health. Despite the growing acknowledgement that heterogeneity is driving treatment failure, it is not often recognized that a successful treatment must be designed with the evolutionary response of the tumor in mind. We investigate the role of spatial heterogeneity in the efficacy of adaptive therapy, an evolutionary-based treatment strategy that aims to balance cell kill with toxicity, by controlling the resistant population through competition with the sensitive population. Adaptive therapy aims to keep a constant tumor volume by adjusting the dose such that a shrinking tumor will receive a lower dose while a growing tumor will receive a higher dose. Using an off-lattice agent-based model, we

simulate the outcomes of different population mixes exposed to two general treatment strategies with an anti-proliferative drug: a continuous application given at the maximum tolerated dose or an adaptive strategy that incorporates dose-modulation and treatment vacations to sustain control of the tumor's sensitive and resistant cell populations. We assume that there is a trade-off between proliferation and drug sensitivity, so that the slower growing resistant cells get trapped in the interior of the tumor during growth and can hide from the drug during treatment. The more homogeneous, sensitive tumors are cured with continuous treatment, but even a few resistant cells will cause eventual recurrence. We find that we can maintain a steady tumor size with adaptive therapy, as long as there are sufficient sensitive cells to suppress resistant cell growth. We explore two different scheduling parameters for the adaptive therapy strategy: one that emphasizes more dose modulation, and another that mostly relies on treatment vacations for maintenance. We find that they can both control the same tumor types, but with dose modulation, the average dose rate is significantly lower. Further, we find that cell migration and phenotypic drift disrupts the efficacy of adaptive therapy in general, but this can be partly preserved through a more vacation-oriented schedule. We also show how adaptive therapy can control multiple metastases with similar or dissimilar compositions.

B30 Updated motor clutch model for cell traction recapitulates dynamics observed in single-molecule FRET-based force sensors

Sarah Anderson, Steven Tan, Alex Dunn, David Odde

The motor clutch model has been widely used to model the force transmission system in cells. The model consists of a series of molecular clutches, modeled as Hookean springs, pulled by a moving actin filament. The actin causes force to build on the clutch bonds until a force-dependent unbinding event occurs[1]. Under this framework, single bonds experience a gradual and monotonic increase in force until an instantaneous return to a zero-force state upon unbinding (Figure 1a).

The Dunn lab recently developed integrin-binding FRET-based molecular tension sensors that can measure forces experienced by a single integrin *in vitro*. The majority of the sensors exist in a nearly constant non-zero force state that persists for several seconds, a result that could not be explained by the original motor clutch model. We therefore sought to determine changes to the existing model that would more accurately explain the dynamics observed by the FRET sensors. Three potential models were developed that could explain the observed results.

The first method that achieved constant non-zero clutch forces involved using a Kelvin-Voigt Model to impose viscous relaxation on the clutches after unbinding. The binding rate was then assumed to be dependent on the distance between the clutch and actin filament, so immediately after unbinding, the probability of rebinding was high. This led to an unbinding-rebinding behavior that appears as clutches holding at approximately constant forces.

The second method involved a more detailed modeling of the dynamics of adhesion proteins talin and vinculin. As talin unfolds in response to force, revealing vinculin binding sites, the number of connections between an integrin and actin increases[2]. This was modeled as integrin springs connected in series to 2 to 12 clutch springs in parallel. When many clutches are attached to each integrin, as the clutches bind and unbind, the force on the integrin stays relatively constant.

The final method implemented a dynamic actin network, where each motor pulls on an actin filament, which can bind to a single clutch. The actin filaments are connected by dynamic crosslinks, which can bind and unbind. In this case, the dynamics of the crosslinks dominate, allowing clutches to reach a force equilibrium (Figure 1b).

Though the original motor clutch model was unable to explain results found by the Dunn lab, changes to the base model have been able to capture single molecule dynamics and force distributions. Future work will focus on further experimental testing of these three scenarios.

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B31 Parameter analysis of the cell migration simulator 1.0 with an efficient computational method

Jay C. Hou, Liam Tyler, Brian T. Castle, Victor H. Barocas, Daniel F. Keefe, David J. Odde

Cell migration simulator 1.0 version (CMS1.0) has been proposed in Bangasser et al. [1] to predict the migration of neuronal growth conces and glioma cells on hydrogel substrates with various stiffness. CMS1.0 has been verified successfully by comparing the stiffness sensitivity of cell traction and F-actin retrograde flow rate between embryonic chick forebrain neurons and U251 glioma cells with assumed different amount of active motors (e.g. myosin II) and clutches (e.g. integrins and associated adaptor proteins) [1]. Furthermore, CMS1.0 has predicted the shifting of optimal stiffness of random motility, cell aspect ratio, F-actin flow, and traction energy for U251 cells with simultaneous drug inhibition of myosin motors and integrin-mediated adhesions [1]. CMS1.0 is implemented in Matlab (MathWorks) and has been distributed on the lab website (http://oddelab.umn.edu). With the Gillespie stochastic process in the simulator, the number of steps per second increases proportionally to the number of clutches, and hence in order to capture the behavior of cell with high motor and clutch number (e.g. U251 cells with 10000 motors and 7500 clutches), it takes days (5+ days) to complete the simulation of 1 parameter set for 10 cells in 6 hour simulation, which largely reduces the practicability of the model. Also, due to the long simulation time, the parameter analysis of the model has not been investigated thoroughly. In this study, a C++ version of CMS1.0 has been

implemented with more than 30 times higher efficiency and the parameter analysis of the model has been studied to better understand the model prediction.

The C++ code is written following the algorithm of the Matlab code, while the number of For-loops is minimized and they only loop over engaged clutches instead of all clutches. The efficiency of the C++ code varies according to the parameters, and overall it is about 30 times faster than the Matlab code. For the previous example, it takes 5 hours instead of 5+ days using Matlab code, and for the cell with lower motor and clutch number (e.g. neuron cells with 1000 motors and 750 clutches), it takes 10 mins instead of 9 hours using Matlab code. The parameter analysis has been studied, including the number of clutches (), the number of motors (), the polymerization rate (), and the module birth rate (). The rest parameters values are prescribed according to Bangasser et al. [1] with lower motor and clutch numbers. When increases, the F-actin flow rate decreases with higher optimal stiffness, and eventually goes to a stalled system, which is consistent with Bangasser et al. [2]. Interestingly, when decreases, the optimal stiffness of motility increases, and ultimately decreases in magnitude (Fig. 1(a)), showing that the higher motor/clutch ratio can promote migration. In the opposite, when increases, the F-actin flow rate increases with higher optimal stiffness, and eventually goes to free-flowing system, which is also consistent with Bangasser et al. [2]. Similarly, when increases with a higher motor/clutch ratio, the optimal stiffness of migration increases (Fig. 1(b)). When increases 10-fold, the random motility coefficient increases about 20-fold for all stiffness (Fig. 1(c)), showing that the polymerization of actin filament is crucial for the migration. In the opposite, when increases, more modules are created and migration is reduced to a stalled condition (Fig. 1(d)). With the efficient C++ code implemented, a complete parameter analysis of CMS1.0 can be studied, and the physical and experimental interpretations can be further investigated.

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B32 Investigating Ras biology on lipid membranes: a DOE/NCI Collaboration

Dwight Nissley and Fred Streitz

Despite recent successes in the treatment of cancer, and the promise of emerging therapeutic approaches, such as immunotherapy, there are a significant number of patients for whom there are few therapeutic options. One such class of cancers are those which contain mutations in RAS, an oncogene that is a driver in up to 30% of all human cancers. The NCI RAS Initiative was established to investigate and target RAS using emerging experimental and drug-targeting technologies. The Initiative is focusing on direct targeting of the RAS protein as well as better understanding RAS biology in the

context of cellular membranes where RAS interacts with proximal effectors, such as RAF to propagate oncogenic signaling. Recently the NCI and DOE formed a partnership (Joint Design of Advanced Computational Solutions for Cancer-JDACS4C) that includes a pilot that has developed a multiscale simulation platform to study RAS/RAF/membrane dynamics, elucidating interactions and mechanisms that cannot be fully accessed with current experimental technologies. The JDACS4C RAS Pilot leverages extensive biophysical, biochemical, structural and molecule dynamic studies on RAS and RAF to inform and parameterize a multiscale simulation which in turn generate hypotheses that can be tested experimentally. In order to simulate biologically relevant length and time scales the Pilot developed a novel massively parallel multiscale simulation framework where machine learning connects a macroscale continuum model to an ensemble of microscale molecular dynamics simulations. This approach makes it possible to survey large membrane patches containing hundreds of RAS molecules and then dive into the molecular details of specific foci where the RAS proteins and associated lipids can be analyzed more extensively. This coupling of molecular detail to the cellular-scale makes it possible to analyze RAS-membrane interactions at unprecedented scale and simulate RAS biology with sufficient instances to be statistically relevant and capture the breadth of RAS-membrane interactions.

B33 NCI - Department of Energy Collaborations : Extending Frontiers of Predictive Oncology and Computing

Greenspan, Emily; Gryshuk, Amy; Lauzon, Carolyn; Stahlberg, Eric

In 2016, the National Cancer Institute and the US Department of Energy established an inter-agency collaboration to explore the frontiers in cancer research through the utilization of unprecedented levels of computing planned for exascale. Supported by initiatives in precision medicine (PMI) and the National Strategic Computing Initiative (NSCI), in Exascale Computing, and the Cancer Moonshot, the collaboration aims to accelerate the missions of both agencies, advancing cancer research with new capabilities and insights while helping prepare the computing infrastructure needed to support future big data challenges. Through innovative development and application of modeling, simulation, deep learning, uncertainty quantification, cutting-edge data and computational science to cancer challenges, the collaboration is developing computational approaches to deepen understanding of cancer biology, help identify potential new treatments, and better understand factors involved with cancer outcomes at the individual patient. Current collaborative efforts include the Joint Design of Advanced Computing Solutions for Cancer (JDACS4C), the CANcer Distributed Learning Environment (CANDLE) - a deep-learning exascale computing project as part of JDACS4C, and Accelerating Therapeutics for Opportunities in Medicine (ATOM) - a public-private consortium aimed at dramatically improving cancer drug discovery. Collaborations involve scientists at the NCI, at four DOE national laboratories (Argonne, Oak Ridge, Lawrence Livermore, and Los Alamos), and

Frederick National Laboratory for Cancer Research. The presentation will provide insights into the current collaborative efforts described below as well as insights into DOE resources available to the cancer research community. JDACS4C Pilot 1 - Developing advanced data-driven models to predict tumor response to drug treatments, including incorporating of uncertainty quantification to improve experimental design, as well as developing hybrid data-driven+mechanistic models. JDACS4C Pilot 2 - Developing new multi-scale molecular simulation capabilities using molecular, functional and structural information to better understand and predict behaviors of RAS biology. JDACS4C Pilot 3 - Developing new approaches for data extraction from pathology reports and other sources to integrate data for to gain insight into patient trajectories, potential anomalies and other factors impacting patient outcomes. CANDLE - Generalizable and scalable deep-learning and uncertainty quantification capabilities for cancer research ATOM - Utilizing diverse molecular data, simulation and data-driven models to improve rate of successful cancer drug discovery.

B34 Causal interactions from proteomic profiles: molecular data meets pathway knowledge

Ozgun Babur, Augustin Luna, Anil Korkut, Funda Durupinar, Metin Can Siper, Ugur Dogrusoz, Joseph E. Aslan, Chris Sander, Emek Demir

Measurement of changes in protein levels and in post-translational modifications, such as phosphorylation, can be highly informative about the phenotypic consequences of genetic differences or about the dynamics of cellular processes. Typically, such proteomic profiles are interpreted using simple enrichment or clustering methods that do not attempt to explain the dynamics that shaped the data. Here, we present a computational method to generate causal explanations for proteomic profiles using prior mechanistic knowledge in the literature, as recorded in cellular pathway maps. We detect knowledge fragments that-when considered collectively-provide evidence for a causal link between two measurements. This approach, in essence, mimics the literature search of a biologist for relationships that explain their data. Automating this task enables asking multiple interesting questions that would be too time-consuming to do through manual analysis. Since this process systematically considers hundreds of thousands curated mechanisms, it also is more comprehensive, unbiased, and more consistent in terms of the generated hypotheses. We tested this framework on an EGF stimulation dataset, detected EGFR activation with its signaling downstream to MAPKs, and a feedback inhibition on EGFR itself. We analyzed platelet activation and detected a plausible link from MAPK signaling to apoptotic signaling, which we validated by perturbing MAPK proteins. We analyzed ovarian cancer and breast cancer mass spectrometry datasets from two CPTAC projects, focusing on general and subtype specific signaling, as well as detecting regulators of well-known cancer proteins. We analyzed RPPA datasets from 32 TCGA projects and found that AKT signaling is the most frequently varied signaling among patients in the same study. Causal pathway analysis, that combines molecular profiles with prior biological knowledge captured in

computational form, is a powerful discovery tool for a range of cellular profiling data types and biological questions. The method is freely available at http://causalpath.org.

B35 Quantitative multidimensional characterization of protein-protein interactions in head and neck cancer

Danielle Swaney, Margaret Soucheray, Jisoo Park, Neil Bhola, Toni Brand, Kuymin Kim, Fan Zheng, Minkyu Kim, Silvio Gutkind, Jennifer Grandis, Trey Ideker, Nevan Krogan

DNA sequencing of cancer genomes has produced a wealth of information regarding the mutational landscape of cancer, however, translating these alterations into functional and clinical outcomes is complicated by the significant heterogeneity among cancers of the same type, and even within a single tumor. Previous work has demonstrated that placing these alterations in the context of protein interaction networks can transform heterogeneous genetic mutations into stratified classes of cancer subtypes with distinct survival rates. Here we have used a quantitative affinity purification mass spectrometry (APMS) approach to characterize the dynamic nature of protein-protein interactions for genes altered in head and neck squamous cell carcinoma. APMS experiments have been performed for approximately 32 genes across a panel of 3 different cell lines. Additionally, we have analyzed the effect on protein-protein interactions for several recurrent point mutations on genes, such as TP53, PIK3CA, NFE2L2, and HRAS. From this analysis we find that while some canonical PPIs are maintained across cell lines, the majority of PPIs are context dependent and found exclusively in a single cell type and do not correlate with protein expression. Several of these novel cell type specific interactions have subsequently been validated by coIP western, and functional studies to target these interactions and determine their impact on cell viability are currently in progress. PIK3CA is the most commonly altered oncogene in head and neck cancer, and consequently, we have characterized the protein interaction landscape for 15 different PIK3CA mutants found in head and neck tumors. This approach allowed us to cluster protein interaction regulation by point mutation, to reveal disparate mutations similar interaction profiles. For example, a subset of these mutants that show a strong interaction preference with the receptor tyrosine kinase ERBB3 (HER3), for which targeted therapies are now in clinical trials. Overall, these results demonstrate the highly dynamic and context dependent nature of protein-protein interactions and illustrate the utility of multidimensional experiments across cell types, mutational status, drug perturbation, etc. to reveal novel PPIs that can enhance our understanding of disease biology and serve as candidates for potential therapeutic intervention.

B36 Molecular analysis towards the optimization of in vitro cancer models

de Lazaro, Irene; Kroll, Katharina; Reynolds, Daniel; Zhang, David; Adu-Berchie, Kwasi; Lewis, Jennifer & Mooney, David

To date, cancer research relies heavily on animal models that do not fully recapitulate human biology. In vitro cultures of human cancer cells help avoid inter-species differences.

However, cell growth in 2-dimensional (2D) monolayers fails to capture the complexity of the in vivo microenvironment and its 3D architecture, among other important features known to strongly influence cell invasion, migration and resistance to chemotherapy. Altogether, these differences are likely at least partially to blame for the poor pre-clinical to clinical correlation faced by many cancer studies. In this project, we aim to build ex vivo 3D melanoma models that better resemble their in vivo counterparts. Initial studies focus on the mouse melanoma cell line B16F10 to allow direct in vivo to in vitro comparison and validation that the in vitro model captures key features of the in vivo tumors. We have performed cellular, gene and protein expression analyses to compare the molecular signatures of different 3D melanoma in vitro models, generated by cell aggregation or 3D printing techniques and with varying cellular composition, to that of their in vivo counterparts grown in syngeneic mice. The results are being utilized to fine-tune the design of the 3D in vitro models to ensure maximum recapitulation of in vivo biology.

B37 Multiscale mapping of the functional architecture of cancer systems

Zheng, Fan; Yu, Michael; Ono, Keiichiro; Flagg, Mitchell; Kim, Minkyu; Swanney, Danielle; Demchak, Barry; Kreisberg, Jason; Krogan, Nevan; Ideker, Trey

The modular, interdependent multi-gene systems which govern cancer remain poorly understood. It is well-established that any given cancer gene operates in multiple distinct cellular contexts. However, it remains unclear how to represent these diverse roles in a single comprehensive model with predictive capabilities and robust multi-omic data integration. To that end, we integrate many physical and functional networks from a wide range of molecular studies, assembling a comprehensive multiscale map of human cancer's functional systems. This map organizes protein complexes, signaling pathways, and inter-pathway crosstalk in a single hierarchy of cellular subsystems. The model's many uncharacterized modules offer intriguing hypotheses with robust data support; in examining these modules, we predict novel functional roles for hallmark cancer genes, established biomarkers of unclear function, and wholly uncharacterized genes. Applied to somatic mutation profiles in The Cancer Genome Atlas (TCGA) patients, this map reveals patterns of subsystem alteration, identifying systems targeted by mutations at multiple scales above the individual gene. This map facilitates interpretation of omics data in terms of affected subsystems; we integrate new protein-protein interactions identified with AP-MS to elucidate the selective rewiring of these modules in multiple breast cancer cell lines. This map also enables integration of mutation and clinical data in TCGA patients across many cancer types, bridging genotype and phenotype to connect patient survival, treatment resistance, and tumor histology to subsystem alteration. To facilitate model interpretation, we created HiView, a web-based hierarchy browsing tool, which allows users to visualize and navigate hierarchies, contextualizing each module by displaying the underlying data and relevant entries in gold-standard databases.

B38 Regulation of a Chemoresistant Small Cell Lung Cancer Phenotype with Immunosuppressive Properties

Maddox, Sarah; Wooten, David; Wandishin, Clayton; Kochen, Michael; Pino, James; Tyson, Darren; Lopez, Carlos F.; Quaranta, Vito

Small cell lung cancer (SCLC) is an aggressive lung tumor type that metastasizes early and relapses rapidly after chemoradiation therapy. It is now clear that SCLC tumors exhibit phenotypic heterogeneity that occurs spontaneously, is not correlated to driving mutations, and promotes formation of a microenvironment robust to perturbations via a network of feedbacks amongst cancer cell phenotypes. Defining origins, and molecular and functional properties of SCLC phenotypes is therefore a promising route to new treatment strategies. Applying consensus clustering, and network analyses including WGCNA, ARACNe, and MAGINE to transcriptomics data of 50 SCLC cell lines and 81 primary human tumors, we identified a network of ~33 transcription factors that controls expression of SCLC co-expressed gene modules. Boolean simulations show that, at equilibrium, the dynamics of the SCLC TF network specify at least 4 distinguishable phenotypic SCLC states. Of these, three phenotypes align with previously identified subtypes, namely, the neuroendocrine tumor-propagating cell (NE; TPC), the supporting mesenchymal-like (ML; non-NE), and thea recently described Myc-high NeuroD-high variant NE phenotype. The fourth one represents a new NE/TPC variant that displays broad chemoresistance to SCLC standard-of-care therapeutics. This variant is characterized by high levels of expression of CGRP (CALCA), which has been shown to have immunomodulatory properties. We are currently testing the role of this NE/TPC variant in tumor relapse and inhibition of immune response to SCLC tumors, and will report at the meeting.

B39 Typing tumors using pathways selected by somatic evolution

Sheng Wang; Jianzhu Ma; Wei Zhang; John Paul Shen; Justin Huang; Jian Peng; Trey Ideker

Many recent efforts to analyze cancer genomes involve aggregation of mutations within reference maps of molecular pathways and protein networks. Here, we find these pathway studies are impeded by molecular interactions that are functionally irrelevant to cancer or the patient's tumor type, as these interactions diminish the contrast of driver pathways relative to individual frequently mutated genes. This problem can be addressed by creating stringent tumor-specific networks of biophysical protein interactions, identified by signatures of epistatic selection during tumor evolution. Using such an evolutionarily selected pathway (ESP) map, we analyze the major cancer genome atlases to derive a hierarchical classification of tumor subtypes linked to characteristic mutated pathways. These pathways are clinically prognostic and predictive, including the TP53-AXIN-ARHGEF17 combination in liver and CYLC2-STK11-STK11IP in lung cancer, which we validate in independent cohorts. This ESP framework substantially improves the definition of cancer pathways and subtypes from tumor genome data.

B40 Pathway activity profiling differentiates metaplastic breast cancer histological subtypes

McQuerry, Jasmine A; Jenkins, David F; Johnson, W. Evan; Yost, Susan; Yuan, Yuan; Bild, Andrea H

Purpose: Analysis of dysregulated signaling pathways in rare cancers has proven challenging due to limited access to patient materials and requirement of intact, non-degraded RNA for next-generation sequencing. We customized an oncogenic pathway gene expression panel compatible with degraded RNA from fixed formalin, paraffin embedded (FFPE) patient biopsies and profiled patients with metaplastic breast cancer (MpBC), a rare breast cancer subtype. Patients and Methods: Activity of various biological pathways was profiled from FFPE samples from nineteen patients with MpBC and 8 patients with invasive ductal carcinoma with triple negative breast cancer (TNBC) phenotype using a custom gene expression-based assay of 345 genes. Results: Patients with metaplastic breast cancer of mesenchymal (chondroid and/or osteoid) histology demonstrated increased SNAI1 pathway activity compared to patients with other metaplastic subtypes or with invasive ductal TNBCs. Additionally, cytoskeletal and keratinization genes were downregulated in MpBC samples compared to TNBCs, and epithelial-to-mesenchymal transition genes were upregulated in MpBC. Expression of stemness markers correlated with patient recurrence-free and overall survival. Conclusion: This study demonstrates that MpBC exhibits increased SNAI1, EMT markers, and dysregulation of the cytoskeleton compared to TNBC, an aggressive breast cancer subtype also known to have increased levels of EMT and stemness compared to estrogen receptor positive breast tumors. Gene expression patterns identified by this custom pathway assay suggest that, although often histologically triple negative, patients with MpBC have distinct pathway activation compared to patients with invasive ductal TNBC. Incorporation of targeted therapies may lead to improved outcome for MpBC patients, especially in those patients with a histological mesenchymal component.

B41 Associating post-translational modifications with chromatin accessibility in medulloblastoma

Clarence K Mah, Miriam Adam, Pamela Milani, Brook T Wassie, Tobias Ehrenberger, Tenley C. Archer, Scott L. Pomeroy, Ernest Fraenkel, Jill P. Mesirov, Lukas Chavez

Medulloblastoma is one of the most common pediatric brain tumors. Previous studies have shown that medulloblastoma is extremely heterogenous and comprises at least four major consensus subgroups: wingless (WNT), sonic hedgehog (SHH), Group 3, and Group 4. To identify functional pathways associated with subtypes of medulloblastoma, we quantitatively profiled global proteomes and phospho-proteomes of 45 medulloblastoma samples (Archer et al., Cancer Cell, in press). We identified distinct pathways associated with two subsets of MB SHH tumors, and found post-translational modifications of MYC that are associated with poor outcomes in Group 3 tumors. To further analyze the regulatory mechanisms that cause subgroup specific post-translational modifications, we used

ATAC-sequencing and mapped the open chromatin in 22 out of the 45 previously analyzed medulloblastoma samples. By a supervised comparison between medulloblastoma samples with distinct post-translational modifications, we identified a large amount of subtype specific non-coding regulatory elements ('enhancers'). Through an integrated analysis of open chromatin, transcription factor binding sites and gene expression, we have identified candidate transcription factors and core transcriptional circuitries potentially responsible for global differences in translation and proteostasis. We now aim to functionally test the relevance of these core transcriptional circuitries for regulating post translational modifications, tumor development, and for identifying subtype specific drug targets.

B42 Regulation of cancer cell metabolism: Focusing on pyruvate kinase type M2 site-specific phosphorylation and its pivotal role in malignant transformation

Apostolidi, Maria; Vanaja, Kiran; Park, JinSeok; Wyler, Amy; Boggon, Titus; Levchenko, Andre; Rinehart, Jesse

Pyruvate Kinase is a key metabolic enzyme that controls the supply of energy into the cell. Among its different isoforms of liver and muscle, the muscle type 2 (PKM2) is found upregulated in several types of cancer and has been tied directly to altered regulatory processes. PKM2 phosphorylation sites that are reported so far lack of mechanistic insights and are yet to be explored. In the present work we investigate PKM2 differential phosphorylation in a normal versus tumor cell environment. In silico analysis coupled with mass spectrometry revealed a panel of different phosphorylation sites which potentially impact PKM2 function. Phosphorylation in position S37 was verified as the most abundant across different human carcinoma cells lines of tissue varieties and grades. This PKM2 phospho-form has been recently proposed to regulate and support tumor growth in lymphomas and glioblastomas, however it's exact mechanism of action in relation to cancer types and states remains poorly characterized. Using a tumor cell model consisting of high grade estrogen receptor positive adenocarcinomas with high metastatic potential and poor prognosis to therapy response, we aim to connect PKM2-pS37 phospho-form to phenotype alteration and metabolism reprogramming. Our preliminary results indicate multiple regulatory pathways that potentially impact PKM2 phosphorylation in our metastatic tumor model in response to altered metabolic cues. We propose, for the first time, an elegant regulatory mechanism which uses PKM2 phosphorylation as a molecular switch that supports tumor growth, invasion, and metastasis. Moreover, we utilize the unique orthogonal translation system developed in our laboratory to produce recombinant PKM2 proteins with genetically incorporated phosphoserine in position S37 for critical structural and biochemical analysis. These studies will result to an integrated structural and functional characterization of PKM2 site-specific phosphorylation and will contribute to our understanding of cancer initiation, promotion, and progression. Taken together all our efforts may reveal phospho-PKM2 tumor related pathways that can be therapeutically exploited.

B43 Can 'Oncogene Induced Senescence' explain nevus growth?

Ruiz, Rolando; Chen, Chi-Fen; Krasieva, Tatiana; Ganesan, Anand; Lander, Arthur

Mutational activation of the Braf proto-oncogene in melanocytes (pigment producing cells of the skin) triggers proliferation for a certain amount of time, but eventually melanocytes arrest and generate nevi (common 'moles'). Their arrest has been attributed to 'oncogene induced senescence' (OIS), a phenomenon that has mainly been studied in vitro, but it is unlikely that nevus arrest is due to true senescence because nevi readily regrow after a partial excision and return to about their original size. Understanding the mechanisms that control nevus size could be important in understanding how melanocyte-derived cancers (melanoma) escape growth control. To investigate this process, we use a mouse model in which the formation of nevi can be induced by conditionally activating Braf in melanocytes. Distributions of final nevus sizes in this model suggest that all cells in a nevus stop growing at about the same time, which is very different from the predictions of models such as OIS, in which growth arrest is a random, cell-autonomous event. Mathematical modeling shows that one mechanism that can produce such coordinated growth behavior is collective negative feedback control of cell lineage progression. Multiphoton imaging of nevi in the inducible mouse model suggests that there are indeed two distinct melanocyte cell types, which could represent lineage stages. We have used single cell RNA sequencing to identify gene expression signatures associated with these two cell types, and are exploring models to explain how interactions between them could account for the control of nevus size.

B44 Rewiring of regulatory networks in breast cancer by transcription factor isoforms

Juan Fuxman Bass, Martha L Bulyk, and Marc Vidal

One of the ultimate goals of cancer systems biology is to generate predictive and dynamic models of tumorigenesis by identifying and quantifying all perturbed functional interactions in a cancerous cellular system. Our central hypothesis is that, among the combined effects of multiple types of functional perturbations, those emerging from cancer-specific gene expression of alternative isoforms are crucial for tumorigenesis. Genome alterations such as amplification, deletion, translocations and mutations, are often considered primary events of cancer progression. However, cancer-specific isoforms resulting from alternative splicing, alternative sites of transcriptional initiation, and/or alternative transcriptional termination sites, have also been shown to have functional impact on tumorigenesis. In particular, changes in gene regulatory networks (GRNs) by transcription factor (TF) isoforms have been shown to play a major role in tumorigenesis and metastasis in multiple types of cancer. While a few examples of functional characterization of driver cancer-specific TF isoforms have been reported, what remains unclear is the extent to which differences in TF isoforms between normal and cancer tissue affect global GRNs and how such regulatory network rewiring leads to altered gene expression programs in cancer. Indeed, hundreds of differential TF isoforms have been identified between normal and cancer samples, but the vast majority remain uncharacterized at the functional level. In this project, we propose an

initial step toward this long-term goal, which consists of characterizing and modeling the effect of large numbers of breast cancer-specific TF isoforms in the context of cancer interactome networks. We aim to combine network modeling and high-throughput systematic experimental strategies at the level of molecular protein-protein and protein-DNA interactions to predict cancer drivers and suppressors. The resulting hypotheses will be tested experimentally using various large-scale functional assays in breast cancer as a model system. As part of the experimental testing, we will establish state-of-the-art genome editing methodologies for testing the effects of isoform-specific perturbations on GRNs in mammalian cells. Altogether, this project will constitute an important step towards the long-term goal of contextualizing and functionalizing large numbers of TF isoforms implicated in breast cancer. Further, the lessons learned from the data analysis and integration will lead to the identification of novel cancer drivers and suppressors, the generation of mechanistic models of GRN rewiring in cancer and provide a framework for the design of novel therapeutics.

B45 Rule-based Modeling of the Notch Signaling Pathway in Small Cell Lung Cancer

Melaine Sebastian

Small cell lung cancer (SCLC) is an aggressive neuroendocrine tumor, comprising 15% of all lung cancer cases (American Cancer Society, 2015). It is the most lethal form of lung cancer (5-10% 1-yr survival) due to its rapid growth, early metastasis, and recalcitrance to repeated treatment (Gazdar et al, 2017). Previous work (George et al., 2015) has identified the transmembrane receptor gene family NOTCH as having inactivating mutations in ~25% of SCLC human tumor samples, more than any other gene besides the obligate TP53 and RB1 mutations. Other work (George et al., 2015; Hassan et al., 2014; Wael et al., 2014) has implicated Notch1 in suppression of motility and cell proliferation and in induction of MET (mesenchymal-to-epithelial transition). Thus, loss of Notch1 function may be responsible for driving EMT (epithelial-to-mesenchymal)-like phenotypes, such as loss of apical-to-basal polarity that is observed in some SCLC tumor cells (Ito et al., 2015).
The Notch receptor family consists of four transmembrane contact-dependent receptors, Notch1 through 4, and canonical ligands DLL1, DLL2, DLL3, Jag1, and Jag2 (Borggrefe & Oswald, 2009). After ligand binding, Notch is cleaved twice, first extracellularly and then intracellularly. Upon the second cleavage event, the intracellular region of Notch migrates freely through the cytosol and into the nucleus, where it complexes with RBPJ and MAML1 to activate transcription of its target genes. Target genes of Notch include transcription factors such as Hes1 and Hes5, which in turn repress genes such as ASCL1, an oncogene involved in neuroendocrine differentiation (Kageyama et al., 2015). A signaling axis consisting of NOTCH, ASCL1, RB1, and TP53 has been shown to drive a pathway by which secondary SCLC can arise from non-small cell lung cancer (Meder et al., 2016). Additionally, our lab and others (Lim et al, 2017) have demonstrated that the heterogeneous cell types that comprise SCLC tumors can be delineated, in part, by their Notch-expressing status. Furthermore, Notch-expressing cells have been shown to be chemoresistant and to provide

trophic support to their non-Notch expressing neighbors (Lim et al., 2017). Altogether, the Notch signaling pathway has emerged as an integral player in the pathology of SCLC. To further our understanding of the Notch signaling pathway in SCLC, we have constructed a rule-based kinetic model using PySB, a Python-based modeling and simulation framework (Lopez et al., 2013). The model extends a previously published core pathway model of Notch signaling (Agrawal 2009) by including molecular species of specific interest in SCLC, such as ASCL1, MYC, and additional Notch receptor isoforms (Notch2, Notch3, Notch4). We perform a dynamical systems analysis within the context of ordinary differential equations (ODEs) to identify stable steady states of the system and perform stochastic simulations (Gillespie, 1976) to observe spontaneous transitions between states and quantify state-transition probabilities. Future work will involve using the model to glean insight into how the Notch pathway drives heterogeneity in SCLC and to identify druggable molecular targets to improve SCLC treatment.

B46 Regional strategies for expanding the evolving continuum of Physical Sciences - Oncology Network (PS-ON) research advocacy experiences

Samson, Susan; Zahir, Nastaran; Judge, Sheila M.; Cornew, Stuart; Riter, Bob; Francoeur, Jeri; Meyn, Anne; Cynkin, Laurie; Northey, Jason J.; Weaver, Valerie M.; Baas, Carole

Background: The National Cancer Institute (NCI) Physical Sciences - Oncology Network (PS-ON), initiated in 2009, is an interdisciplinary hub currently consisting of eighteen regions across the nation to support the emergence of new scientific frontiers, principles, and opportunities within physical sciences and oncology. Based on the belief that the increasing momentum for cross disciplinary connectivity between biologists, physicists, mathematicians, chemists, biomedical engineers, oncologists would be enriched and enhanced by vigorous and diverse public and/or advocacy support, the PS-ON leadership, at program inception, incorporated the advocate voice in setting a national research agenda. Methods: While the regional advocacy programs operate independently and utilize multi-level, multi-method strategies to expand the evolving umbrella of research advocacy experiences, they are connected through an administrative structure that communicates NCI program priorities to enhance capacity in the approaches utilized across the eighteen PS-ON regions. Impact: As integral team members, advocates bring real-time diverse patient experiences, diverse professional expertise, and concerns into pioneering, innovative research practices. PS-ON regional engagement/communication strategies include: 1) integrating advocate perspectives to shape basic science research agendas, 2) developing conceptual models/roadmaps to holistic engagement focusing on organizational foundations and best practice strategies, 3) applying guiding frameworks and toolkits for setting the terms of principled engagement/shared governance/bidirectional collaboration, 4) implementing education, outreach, and professional development programs for early stage investigators, students, and patient communities, and 5) translating, communicating and disseminating laboratory innovations into society. Discussion: To better understand

and fully address the complexities of intersecting physical sciences and oncology advocacy engagement, we explore the unique culture and guidelines set by selected participating institutions. Meeting key challenges regarding programmatic scope and policy impact requires a shift to a new, rapidly evolving paradigm. In parallel to incentives and policy measures created through federal and professional organizations, we offer recommendations for strengthening regional programs and encouraging equitable partnerships for advocates at earlier stages of research to help propel convergent science innovation.

B47 The Power of Patient Advocacy in Effective Cancer Prevention and Treatment

Dedmon, Ashley

Patient advocacy is critical in cancer research, it focuses on the total patient. A patient advocate navigates patients through barriers, educates them on the cancer process, understands the perspective of the researcher and doctor, and can counsel patients specific to their needs. As a caregiver of two parents with cancer diagnoses and a hereditary gene that predisposes me to cancer, a consistent source of reliable information and comfort was necessary for my family to make decisions that protected our physical and emotional well-being. I am what you would call a previvor. A previvor is an individual who is predisposed to cancer, but the onset of disease has not occurred. Therefore, the approach to patient advocacy is a twofold intervention that addresses disease prevention at the beginning of the process and survivorship at the end of the process. This process helps facilitate in narrowing the margin for the onset of disease to occur, previviorship and/or to catch and treat the disease early and survivorship. Since my involvement as a patient advocate, doctors have increased hereditary cancer screenings and identified women who are predisposed or high-risk of developing cancer. Women who are high risk have identified their family history has reduced and monitored their cancer through lifestyle changes, surveillance, preventative therapy, and procedures. In addition, women who have been diagnosed have identified their family history, received education on their diagnoses, rights and any equipment to make informed decisions about treatment options. Patient advocacy comes in many forms and utilizes many approaches to the mental health, emotional and social well-being for patients and their families. Being a patient advocate is multifaceted with the primary focus of providing central support to individuals who struggle with the reality of being diagnosed with cancer and the effects of cancer treatment. The goal for patient advocacy for cancer patients is to provide continuous education regarding their diagnosis and treatment options. Women who are high risk for breast cancer, another goal is to increase preventative measures, promote early detection and monitor breast health.

B48 Moffitt-PSOC Training Program and Scientific Community Outreach

Jeri Francoeur, Robert Gateby, Heiko Enderling, Alexander Anderson

The Moffitt-Physical Sciences Oncology Center has been instrumental in reaching out to the local community, state and nationally by creating a scientific training and outreach program. The aims of this training and outreach program are to train experimental, clinical and quantitative scientists cross-consortium to create a seamless interface between biological experimentation, quantitative mechanistic modeling and clinical trials. In addition, to educate a new generation of scientists at the interface of clinical oncology and quantitative mechanistic modeling. And, finally, to expand Moffitt's reach to the scientific and general public communities by sharing model frameworks and experimental data and education about quantitative sciences in the personalized treatment area.

B49 PBCF - The Trusted Bioresource Core for the PS-ON and CSBC Investigators

Ghosh, Sudakshina

The Physical Sciences-Oncology Network Bioresource Core Facility (PBCF) at ATCC is a central resource for all investigators of the Physical Sciences-Oncology Network (PS-ON) and Cancer System Biology Consortium (CSBC). The PBCF serves as a centralized distributor and repository that provides the PS-ON and CSBC with standardized stocks of well-authenticated sets of non-malignant and cancerous cell lines, their derivatives, cell culture reagents, in addition to the related standard operating procedures. Investigators outside of the PS-ON and CSBC, who agree to actively participate in data sharing and integration efforts are welcome to join. Biomaterials distributed by the PBCF include: • Cancer and normal cell lines • Genomic DNA • Total RNA • Protein lysates • Media kits • Relevant procedures (SOPs) Biomaterials provided by PBCF are freely available to PS-ON & CSBC investigators for the cost of shipping and handling only. For more information on PBCF, please visit http://pson.cancer.gov/bioresources

B50 Finding tumor subpopulations in heterogeneous single cell data using machine learning algorithms and random matrix theory

Mykola Bordyuh; Luis Aparicio; Raul Rabadan

Intratumoral heterogeneity is among the greatest challenges in precision cancer therapy. Recent developments in high-throughput single-cell RNA sequencing (scRNA-seq) provide a unique platform to study diverse cellular populations of tumors, regulatory mechanisms of cell development and potentially inform the selection of targeted combination therapies. Despite breakneck pace of development of single-cell technologies, computational data analysis of single cell data has many challenges (sparsity and noisiness of data to name a few). We emphasize the common challenges that single cell data provides and how these challenges vary between different platforms. Furthermore, we introduce a theoretical framework of Random Matrix Theory (RMT) that allows to deal with many single cell data analysis challenges in a universal and simple way across different platforms and introduce a novel machine learning algorithm that synergistically with random matrix theory allows to detect small cell subpopulations.

B51 Personalized adaptive therapies for metastatic melanoma: A Phase i approach

Eunjung Kim, Inna Smalley, Zeynep Eroglu, Vernon Sondak, Robert Gatenby, Keiran Smalley, Alexander Anderson

Despite the impressive responses of to targeted therapies, most metastatic melanoma patients ultimately fail therapy. A major drive of treatment failure is intratumor heterogeneity. Adaptive therapy is an evolution-based strategy that exploits heterogeneity via the cost of resistance e.g. molecular synthesis needed to survive treatment. For resistant cells, benefits exceed costs during therapy, however, in the absence of therapy, sensitive cell are more fit due to the cost of resistance. Therefore treatment holidays allow sensitive cells to outcompete their resistant counterparts, potentially extending response times whilst reducing treatment time. The recent adaptive therapy clinical trial in metastatic prostate cancer, using abiraterone, has proven to be more effective than standard of care. We suggest that adaptive therapy can also be useful for melanoma, this requires mathematical models in order to explain the response and resistance in individual patients. Here, we apply the previously developed Phase i trial framework (virtual clinical trial) with a goal of i) Integrating the heterogeneity of actual patient responses and preclinical studies, ii) Predicting and optimizing treatment schedules for individual cancer patients. Our framework consists of mathematical model development, virtual patient generation, and treatment optimization. To describe treatment response dynamics and to investigate the underlying mechanisms of resistance, we develop a compartment model. We first tested mathematical model driven adaptive therapy on Xenograft melanoma mouse models. The model parameters were estimated under no-treatment, continuous, intermittent schedules. The calibrated model predicted an effective fixed intermittent schedule (8 day on/4 day off), which was confirmed by our subsequent Xenograft experiments. We then predicted mouse specific adaptive treatment schedules, based on model calibration of mouse tumor volume changes. The mouse specific adaptive schedules were far more effective than continuous (~ 50% of continuous therapy). The resulting treatment on-off schedules were quite diverse across the animals. Next, we estimated model parameters, such as growth rate and death rate due to treatment, from patient plasma LDH (Lactic Acid Dehydrogenase) that is known to correlate with patient overall tumor burden. We minimized the difference between predicted LDH and actual individual patient LDH. Model calibration results in a suite of parameter sets that fit the data equally well, defining a virtual cohort of patients. Using this virtual cohort, we predict responses to various intermittent therapy schedules and an adaptive therapy.

B52 Improving treatment of colorectal cancer by using tumor evolutionary dynamics.

Enriquez-Navas, Pedro M; Abrahams, Dominique; Gillies, Robert J; Gatenby, Robert A.

Colorectal cancer (CRC) is the third most common cancer diagnosed in USA with 97,000 new cases expected in 2018. A common site of metastatic CRC is to peritoneal cavity, where

treatment options are limited and long term survival is rare. In these cases, usual therapy combines aggressive debulking of visible tumor nodules followed by the peritoneal administration of heated intraperitoneal chemotherapy (HIPEC) using mitomycin C (MC). This procedure has been used for decades with very limited scientific justification. Even though most patients have not previously been exposed to MC, resistance typically develops rapidly and only 20% of patients show long-term survival. Importantly, however, we view HIPEC as an extraordinary clinical 'laboratory' that allows investigation of short cancer cell adaptive response to chemotherapy. Several mechanisms of resistance to MC have been described, including the overexpression of multidrug resistant systems (MDRs), and the protective effect of the stromal cells (e.g. tumor infiltrated fibroblasts). Also, it has been shown that hypoxia increases the sensitivity of cells to MC. Notably, neither of these therapies has been used to improve HIPEC outcome. In prior work, we have demonstrated that integrating evolutionary principles into cancer chemotherapy can improve outcomes. To apply this approach to HIPEC it is necessary to understand the cellular mechanisms of resistance and their evolutionary costs. To improve and understand CRC dynamics during and after HIPEC treatment, we are using murine preclinical models (CT-26 cells injected in BALB/C mice) treated with MC in combination with Verapamil or Glutamine to exploit the cost-of-resistance or modify tumor microenvironment (transient hypoxia), respectively. Tumor volumetric and IHC data are being used to understand the evolutionary dynamics of these tumors after different combinations of treatments, which are compared to continuous dosage of MC. Additionally, we are collaborating with Moffitt GI department to obtain pre- and post- HIPEC tumor samples to understand resistance dynamics during HIPEC treatment.

B53 Localization of erlotonib relative to MRI-based tumor extent in PDX glioblastoma model: Towards a mathematical model for the interface between MRI and drug distribution

Jackson, Pamela R.; Ranjbar, Sara; Randall, Elizabeth; Regan, Michael; Abdelmoula, Walid M.; Lopez, Begona G.C.; Massey, Susan C.; He, Lihong; Macura, Slobodan; Hu, Leland; Agar, Jeffrey N.; Sarkaria, Jann; Agar, Nathalie; Swanson, Kristin R.

Clinical neuro-oncology relies on the hyperintensity of gadolinium (Gd) contrast agent on magnetic resonance imaging (MRI) in tumor regions to confirm that the blood-brain barrier (BBB) is locally compromised. While the extent of Gd hyperintensity may indicate that systemically-administered drug is being distributed to the tumor regions, little is known about how a drug is distributed and how it may relate to the Gd hyperintensity. Additionally, glioblastomas (GBMs) are diffusely invading neoplasms with a significant fraction of the overall tumor cells spread peripheral to the Gd abnormality, which raises uncertainty as to how or if the rest of the diffuse tumor is affected by drug. Given the gap in understanding drug delivery to the brain, we propose a quantitative approach to model drug delivery in GBM based on MRI and matrix-assisted laser desorption/ionization mass spectroscopy imaging (MALDI). T2-weighted (T2) and T1-weighted with Gd contrast (T1Gd) MRI images were acquired for an animal with the GBM12 orthotopic GBM patient

derived xenograft (PDX) line dosed with 100mg/kg erlotinib. A T1Gd region of interest (ROI) captured the Gd-associated hyperintensity. MALDI was performed and aligned with MRI images. A Drug ROI was created to represent the increased intensity of erlotinib on MALDI images. Since the Drug ROI encompassed the T1Gd ROI, we subtracted the two ROIs to create a 'Drug No T1Gd' ROI, which represented the drug region beyond the edge of the T1Gd ROI. A 'Brain ROI' was created to represent the region of the brain outside of the drug's spread. The MALDI intensities for the three ROIs were all significantly different ($p<0.05$), with the T1Gd ROI having the highest mean, followed by the Drug No T1Gd and the Brain ROIs. By developing a quantitative understanding of drug distribution, we can make more robust predictions regarding treatment efficacy in the clinical setting.

B54 Towards clinical multimodal NLO imaging system for dermatology

Fast, Alexander; Osseiran, Sam; Evans, Conor

In this work we present advances toward in vivo multimodal clinical imaging system. Integration of coherent raman imaging with confocal reflection allows for simultaneous morphological and chemical contrast in skin tissue imaging. We target chemical fingerprints of pheo and eumelanin pigments to furthering the understanding of melanoma pathogenesis in a non destructive fashion that allows for longitudinal studies.

B55 Discrimination of Response to CAR T-Cell Therapy Using a Novel Response Metric Incorporating Tumor Growth Kinetics in Recurrent GBM Patients

De Leon, Gustavo; Johnston, Sandra K; Singleton, Kyle W; Rickertsen, Cassandra R; Bayless, Spencer; Yee, Sara; Khalifa, Ali S; Clark-Swanson, Kamala R; Porter, Alyx; Bendok, Bernard; Rodriguez, Analiz; Badie, Behnam; Sahoo, Prativa; Mrugala, Maciej M; Rockne, Russell C; Brown, Christine; Swanson, Kristin R

Glioblastoma (GBM) is a primary malignant brain tumor known for its heterogeneity and dismal median survival of 12-14 months for newly-diagnosed GBM patients and 5-7 months for recurrent GBM patients. Immunotherapy has shown promise in the treatment of GBM but there is a major limitation in translating imageable radiological changes. The current standard guideline, iRANO, suggests a six-month waiting period due to an inflammatory response commonly induced by immunotherapy capable of mimicking tumor progression in MRI. We have developed a personalized response metric, Days Gained (DG), based on a predictive model for tumor growth kinetics capable of distinguishing between responders from non-responders. DG measures the degree to which treatment deflects the tumor off of each patient's predicted imageable (untreated) growth curve. We investigated the application of DG in six recurrent GBM patients from the City of Hope Phase I Trial of CAR T-Cell (CART) therapy genetically engineered to target IL13R α 2. Using VTK, volumetric tumor segmentations on T1Gd and T2/FLAIR MRIs were computed and used to simulate tumor growth dynamics. To quantify the DG metric, patients were required to have two pre-CART and one post-CART MRI to assess tumor growth velocity prior to and during treatment. Iterative Kaplan-Meier analysis was performed to identify DG thresholds

discriminatory of patient survival following four cycles of CART infusions. In our preliminary results, patients with a tumor response lower than 59.9 DG on T2/FLAIR MRI was predictive of survival. However, DG was not able to discriminate survival using T1Gd. In previous work for non-immunotherapies, higher DG scores correlated with increased survival. In the case of immunotherapy, however, we anticipated the opposite due to the inflammatory response associated with immune-based therapy. Our personalized DG response metric demonstrates promise in distinguishing patients who would benefit from CART immunotherapy earlier than other response criteria.

B56 Molecular determinants involved in TCR proximal signaling

Natarajan, A., Chenghao, G., Zhou, Y., Nadarajah, V., Felsovalyi, K., Wang, W., Cardozo, T., Bracken, C., Zhu, C., Krogsgaard, M.

T cell recognition of antigen and resulting proximal signaling are key steps in the initiation of the adaptive immune response. Identification of the specific extracellular contacts between the T cell receptor (TCR) and CD3 subunits upon recognition of peptide-major histocompatibility complexes (pMHC) gives more precise guidance for immunotherapeutic strategies that modulate T-cell immunity by targeting signaling through the TCR-CD3 complex. Previous studies that targeted the antigen binding site for enhancing T-cell responses to tumor antigens often lead to off-target effects and toxicity. Recently, we used nuclear magnetic resonance (NMR) spectroscopy, mutational analysis and computational docking to derive a 3D structure of the extracellular TCR-CD3 assembly. Further, biomolecular force probe (BFP) measurements allowed us to determine how 2D affinity and force-modulated TCR-pMHC kinetics depend on TCR-CD3 interaction sites and affect transduction of extracellular pMHC-TCR ligation into T cell function. This places us in a unique position to translate our findings towards improved immunotherapy strategies. Our hypothesis is that by modulating TCR-CD3 interactions in specific ways, immune-mediated cytotoxicity can be increased without losing specificity for the cancer antigen. To test our hypothesis, we mutated specific TCR-residues that potentially interact with CD3 to increase the affinity of the TCR-CD3 interaction, resulting in better CD3 tetramer binding, cytokine response as well as altered 2D affinity of CD3 binding to purified TCRs. In the future, mutagenesis by in vitro combinatorial retroviral TCR display will be used to further to optimize the TCR-CD3 interaction and resulting mutants will be analyzed for enhanced T-cell effector function and tumor rejection in pre-clinical relevant mouse tumor models to develop more effective T cell therapies for human patients.

B57 Evolution of immune cell subtypes and phenotypes during MK-3475 (aPD1) treatment in patients with advances gastrointestinal cancers

Pierre Wallet, Lance Pflieger, Neena Leggett, Andrea Bild, and Jeffrey Chang

Immune cell function is regulated by a variety co-stimulatory and co-inhibitory pathways. Programmed cell death protein 1 (PD1) and its ligand programmed death-ligand 1 (PD-L1) are part of a co-inhibitory pathway that plays a major role in tumor immune escape by

inhibiting T lymphocyte proliferation, survival and effector functions. Additionally, PD1/PDL-1 promotes differentiation of CD4+ T cells into regulatory T cells and stimulates tumor cell resistance to cytotoxic lymphocytes. In many cancers, including gastrointestinal, PD-L1 expression has been correlated with poor prognosis. Recent cancer immunotherapies using immune checkpoint blockade attempt to reverse these co-inhibitory pathways and aid the immune response. Here, we present results from a phase I/IIA clinical trial where patients with advanced gastrointestinal malignancies were treated with mFOLFOX6 (chemotherapy) first followed by MK3475 (anti-PD1) immunotherapy. We collected PBMCs at time points corresponding to treatment cycles prior to treatment, after chemotherapy and after chemotherapy/anti PD1 combined treatment. We utilized single-cell RNA sequencing to generate a classification scheme for immune cells subtypes for each patient sample. Subtype clusters were confirmed by orthogonal FACS analysis and followed over the course of treatment. Based on RECIST1.1 classification criteria, we found that responder patients start the trial with a higher rate of lymphocytes CD3+ than non-responder. Responder patients have more CD8+ T cells and less NK cells present in the PBMCs compare to non-responder after the anti-PD1 treatment. Moreover, T cells in responder patient have unique phenotypes such as activated interferon signaling pathways. Finally, analysis at late time points showed an increase in the number of lymphocytes in responder patients that persisted after treatment. Taken together, these results highlight that PBMCs immune populations may be utilized as an early indicator of patient response to chemotherapy/immunotherapy PD1 treatment in advanced GI malignancies.

B58 UniChip enables long-term recirculating unidirectional perfusion with gravity-driven flow for Body-on-a-Chip systems

Ying I. Wang, Danielle LaValley, Paula Miller, Michael L. Shuler

Body-on-a-chip systems are promising “human surrogates” that may be used to simulate human response to drugs in preclinical drug development. We have previously developed a pumpless platform that combines gravity-driven fluid flow and a rocking motion to create reciprocating flow between reservoirs for recirculation. Such platform allows design of self-contained and highly integrated systems that are relatively easy and cost-effective to construct and maintain. To integrate vasculature and other shear stress-sensitive tissues into pumpless body-on-a-chips, we propose “UniChip” fluid network design, which converts reciprocating flow input into unidirectional perfusion in the channel(s) of interest by utilizing supporting channels and passive fluid control. The design enables for the first time unidirectional organ perfusion with recirculation on the pumpless platform and provides an effective backflow-proof mechanism. We demonstrate that measured performance of the UniChip aligns closely with predictions from a computational model. We then compare unidirectional flow with bidirectional flow and demonstrate that for shear stress-sensitive vascular endothelial cells, unidirectional perfusion by UniChip devices results in cell elongation and alignment to the direction of flow, continuous network of VE-cadherin at the cell borders, realignment of F-actin and suppressed cell proliferation. The resulting system offers a robust platform that is simple to operate and cost effective. We have developed and

are currently testing two prototype multi-channel, multi-organ UniChip systems, a colon-liver two-organ chip to model colorectal cancer cancer liver metastasis, and a tumor-liver-bone marrow three-organ chip to study chemotherapeutic toxicity with relevant drug metabolism and hematological side effects.