2018 CSBC/PS-ON Podium Presentation Abstracts

Cellular and Extracellular Interactions

Monitoring dynamic heterogeneous responses to microenvironmental signals and therapeutic inhibitors

Laura Heiser, PhD, *Associate Professor, Biomedical Engineering, Oregon Health & Science University*

Cells sense and respond to their environment by activating distinct intracellular signaling pathways, however signal transmission in individual cells is not well understood. To assess the ability of individual cells to respond to the microenvironmental signal IGF, we developed an optimized genetically encoded sensor for IGF-I signaling. We stably expressed this sensor in HeLa cells and used live-cell imaging to monitor dynamic responses to IGF-I in individual cells. Across the population, signaling responses overlapped between different IGF-I doses, with relatively constant responses over time. An information theoretic approach to calculate the channel capacity using variance of the single cell time course data--rather than population data--predicted that cells were capable of discriminating multiple growth factor doses. We validated these predictions by tracking individual cell responses to multiple IGF-I doses. We found that cells can accurately distinguish at least four different IGF-I concentrations, and that the input-output relation varies across the population of individual cells. Furthermore, we monitored responses to the PI3K inhibitor alpelisib and again observed substantial variation in signaling capability across a population of cells, with some cells consistently more or less sensitive than others. These findings indicate that cells can accurately interpret multiple growth factor signals in their extracellular environment, and that there is substantial variation in how cells encode this signal. These observations imply a role for microenvironmental signals to modulate therapeutic response. For example, subpopulations of cells may be subject to signals that lead to pathway activation, whereby a larger drug dose may be required to fully inhibit these cells. This is particularly important in the metastatic setting where cancer cells have disseminated to distinct tissue sites enriched with unique complements of signaling molecules. In ongoing studies, we are performing more in-depth studies of how various pathway inhibitors alter signaling activity at the single cell level, with the goal of identifying optimized co-treatment strategies. In sum, our studies reveal the importance of understanding microenvironmental and therapeutic responses at the single cell level.

Engineered ECM platforms to analyze progression in high grade serous ovarian cancer

Pam Kreeger, PhD, *Associate Professor, Biomedical Engineering, University of Wisconsin*

High-grade serous ovarian cancer (HGSOC) is associated with a poor survival rate of less than 50%. HGSOC originates in the fallopian tube, metastasizes to the ovary, and then disseminates throughout the peritoneum. Given its complex progression and challenges associated with animal models of the disease, there is a recognized need for in vitro models of the HGSOC

microenvironment. The ECM is a key element in biomimicry, and particularly important to consider in the context of cancer, where the composition and architecture of the ECM change drastically with disease progression. Using state-of-the-art mass spectrometry, imaging technologies and thorough immunohistochemical analysis, we are characterizing differences between the ECM of normal and diseased tissues. For example, through histological analysis of pathology samples from human ovaries, we determined that collagen I and III were elevated near cortical inclusion cysts (CICs), structures in the ovarian cortex that have been hypothesized to create a niche environment for HGSOC progression. We further determined that the collagen fibers in this dense region were oriented parallel to the cyst boundary. Using this information from human samples as design parameters, we engineered an in vitro model that recreates the size, shape, and ECM properties of CICs. We found that tumor precursor cells cultured within our model underwent robust invasion that was responsive to stimulation with follicular fluid, and that decreased collagen I concentration or the addition of collagen III to the matrix surrounding FTE cells increased FTE cell invasion. Through these experiments in combination with computational/statistical analysis, we will determine which of the many changes that we identify are causative for disease progression. We expect that the lessons learned from our process can be applied broadly towards the larger goal of developing pathophysiologically-relevant models of all cancer types.

Metabolic status and adaptability of breast cancer stem cells

Gary Luker, MD, *Professor, Radiology and Biomedical Engineering, Molecular Biology and Immunology, University of Michigan*

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.

Fructose Fuels Metabolic and Epigenetic Reprogramming of Liver Metastasis

Xiling Shen, PhD, *Hawkings Family Associate Professor, Biomedical Engineering, Duke University*

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.

Computational Modeling of Cancer for Precision Medicine

Pan-cancer analysis of time-to-distant metastasis in the context of node-positive and node-negative disease

Andrew Gentles, PhD, *Assistant Professor, Biomedical Informatics Research, Stanford University*

Significant evidence exists that in some cancers, systemic permissiveness for metastasis of malignant cells from primary tumors to distant sites is mediated by interactions occurring in lymph nodes. We conducted a pan-cancer analysis of the association between gene expression levels and time-to-distant metastasis (DMFS), comparing these to associations with overall survival (OS). Expression of MHC genes (particularly class II) showed favorable associations for

time-to-metastasis as well as overall survival, with higher expression portending longer survival. However, a significant number of genes showed stronger association with DMFS than with OS, despite the fact that these would be expected to be strongly correlated given the clinical fact that metastasis is a strong driver of death from cancer. By performing these analyses separately in node-positive and node-negative disease, we further identified genes that were differentially related to DMFS. We further explored the influence of specific cell types on DMFS by applying the CIBERSORT algorithm to deconvolve bulk expression profiles. We found that specific immune populations were associated positively or negatively with time to metastasis, based on a previously validated signature matrix of 22 cell types. Notably, modulation of EMT-related genes, and changes in expression of immunosuppressive checkpoint pathways reflected earlier metastasis. Leveraging RNA-seq data generated on flow-sorted populations in head and neck squamous carcinomas, and melanoma, we also identified specific gene expression programs in fibroblasts that influenced DMFS. Overall, our results provide a map of the relationship between gene expression and cancer metastasis, in the context of lymph node involvement.

Joining Forces: Combining Machine Learning and Mechanistic Models to Predict Tumor Cell Density for Glioblastoma Patients

Kristin Swanson, PhD, *Professor, Neurosurgery, Mayo Clinic*

Glioblastoma is the most aggressive primary brain cancer, with poor survival that can be largely attributed to intra-tumoral heterogeneity. While these tumors are primarily monitored via contrast-enhanced (CE) T1-weighted and T2-weighted magnetic resonance (MR) images, these standard clinical images are known to be non-specific in their correlation with tumor cell density. This lack of relationship makes it difficult to define the specific regions of interest to target for surgery and radiation. Previous efforts have shown some promise in better interpreting these images utilizing either machine learning (ML) or mechanistic modeling independently. But methods to harness the strengths of both methods are sorely needed to make clinically actionable progress. Here we present a novel, first-of-its-kind, hybrid model which brings together a graph-based semi-supervised machine learning approach with a mechanistic partial differential equation model of glioblastoma growth, known as the Proliferation-Invasion (PI) model, to generate predictive tumor cell density maps with high accuracy. Our ML approach bridges cell density as quantified from image-localized biopsies with texture analysis of multiparametric MR images. To incorporate the mechanistic model, the PI model is first used to generate an independent prediction of cell density which is then introduced into the ML algorithm through a Laplacian matrix, which ensures regions with similar predictions from the PI model will have similar predictions in the final model. We have applied our proposed ML-PI model framework to 18 patients with a total of 82 image localized biopsies. Each patient's tumor was imaged with multi-parametric MR images, including T1-weighted, CE T1-weighted, T2-weighted, dynamic contrast-enhanced (DCE) imaging, diffusion weighted imaging (DWI), and diffusion tensor imaging (DTI). In this cohort, our hybrid model was able to achieve higher accuracy in cell density prediction than either of the independent models (ML or PI) alone, with a mean accuracy prediction error of 0.084 vs 0.227 for PI alone and 0.220 for ML alone. We hope that with more verification, this tool can be used to not only

guide spatially localized therapies such as surgery and radiation, but also help broadly in the interpretation of images for glioblastoma patients.

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

Alexander (Sandy) Anderson, PhD, *Chair, Integrated Mathematical Oncology Department H. Lee Moffitt Cancer Center*

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.

Modeling malignant myelopoiesis to increase efficacy of targeted leukemia therapy

John Lowengrub, PhD, *Chancellor's Professor, University of California, Irvine*

Chronic myeloid leukemia (CML) is a blood cancer in which there is dysregulation of maturing myeloid cells (granulocytes) driven by a chromosomal translocation that creates the fusion gene, BCR-ABL1. Although there has been much progress in the treatment of CML using

tyrosine kinase inhibitors (TKI), there are still unmet clinical needs. For example, there is still a small cohort of patients who, for reasons that are still unknown, do not respond to initial TKI treatment. Further, a significant proportion of patients who appear to have a complete molecular remission while on TKIs experience a relapse of CML when TKI treatment is discontinued. Mathematical modeling of CML hematopoiesis can provide insight into these processes. Current mathematical models of CML are highly simplified (e.g., linear) and have been used to estimate treatment-related model parameters, but fail to describe the mechanisms that underlie primary resistance and treatment-free remission. Here, we explore how more physiologically accurate, data-driven mathematical models of CML hematopoiesis that incorporate feedback control and lineage branching can provide such insight. Although it is recognized that feedback plays a role in CML hematopoiesis, the interactions are poorly understood. In many cases, it is not known which cell types are providing and receiving the feedback, what signals are used, and what aspects of proliferative cell behavior they influence (i.e. cell cycle speed, self-renewal probability, or progeny fate choice). We developed an automated method for model selection that integrates data gleaned from experiments and single cell RNA sequencing to select plausible classes of feedback models. We first apply this approach to normal hematopoiesis and identify models that have desired system properties, e.g., stable equilibria, and make predictions about system behavior upon perturbation. New experiments are shown to validate model predictions, determine model parameters and suggest model refinements. When extended to incorporate CML hematopoiesis, our initial assessment shows that feedback/branching models are more robust, have a better fit to alternative patterns of patient response than simple linear models, and suggest new treatment strategies.

Genetic and Proteomic Interactions

Metabolome-wide features of therapeutic responses in head and neck cancer

Melissa Kemp, PhD, *Associate Professor, Biomedical Engineering, Georgia Institute of Technology*

NAD(P)H metabolism is integrally connected with the mechanisms of action of quinone-based chemotherapeutics (through futile redox cycling) and radiation therapy (through antioxidant response and nicotinamide demand for DNA repair) and is altered in many refractory tumors. This makes NAD(P)H metabolism an ideal target for enhancing sensitivity and improving patient outcomes. Characterizing a repertoire of HPV- head and neck squamous cell carcinomas with multi-omics and leveraging TCGA datasets, we are establishing a framework for developing personalized, genome-wide metabolic models to determine the cumulative NADPH producing enzymes that may be used as prognostic indicators of therapeutic outcomes, specifically to the pre-clinical NQO1 cycling drug beta-lapachone. We envision this systems approach will facilitate identification of molecular targets and nutrient supplementation that - when implemented in combination with beta-lapachone - will enhance therapeutic potency within HNSCC tumors that exhibit heterogeneity of NQO1 expression.

A structure-based physical interactome for the human proteome: Biophysical mechanisms for cellular dysregulation and a complementary resource for high-throughput experimental PPI methods

Diana Murray, PhD, *Program Director, Systems Biology, Columbia University*

This work presents a novel, holistic perspective of the human physical interactome. The Protein Data Bank (PDB) is a repository of experimentally determined protein structures and has become a rich resource for protein complexes. PDB structures provide the strongest experimental support for direct, biophysical protein-protein interactions (PPIs) from a wide range of species. The Predicting Protein-Protein Interactions (PrePPI) algorithm leverages these structures to predict more than one million functional protein-protein interactions (PPIs) for 85% of the human proteome. Almost 300K of these PPIs are predicted to be direct and constitute a physical human interactome. The PrePPI database provides atomic coordinates for each predicted binary protein complex. Queries on the physical interactome recapitulate known and identify novel PPIs that underlie information flow in signaling pathways. For instance, the PrePPI-predicted path, PIK3R-ITSN-PKC-AKT, identifies a physical model for the functional interactions among PI3K, ITSN and AKT, the mechanisms of which have been elusive. An emerging role for ITSN in cancer is an area of intense research. Further, each protein consists of multiple modular protein domains and the PrePPI algorithm identifies the domains that mediate the PPIs: the PI3K-SH3 interacts with the ITSN-SH3; the ITSN-PH interacts with the PKC-C2; and PKC-kinase interacts with AKT-kinase. This PrePPI path supports a signaling mechanism at the plasma membrane surface of cells which involves the lipids PI(4,5)P2, an important source of second messengers, and PI(3,4,5)P3, a potent mitogen. Compellingly, the ITSN-PH/PCK-C2 PPI occurs through an interface that allows both domains to bind their lipid partners. Because of the reduction in dimensionality from solution (3D) to membrane (2D), membrane-mediated PPIs can occur with binding affinities two to three orders of magnitude lower than currently detectable by most experimental assays. Along with the many models for solution (3D) PPIs, the PrePPI interactome constitutes a powerful complementary resource to high-throughput Y2H, AP-MS, and other PPI databases. Finally, the proteins in the PrePPI physical interactome can be annotated with cancer-driving mutations and as drug targets, thereby providing specific experimentally testable models for cellular dysregulation, drug MoA, mechanisms of drug resistance, and additional targets for combination therapies.

Systematic identification of the actionable kinase dependencies of chemotherapy-resistant triple- negative breast cancer

Jean-Philippe Coppé, PhD, *Research Scientist, University of California, San Francisco*

Triple-negative breast cancer (TNBC) accounts for approximately 15% of all breast cancer cases, with over 35,000 newly diagnosed women per year in the USA. TNBC patients are at high risk of recurrence, and neo-adjuvant standard chemotherapy leads to Pathological Complete

Response in only about 30% of patients. No targeted therapy has yet been conclusively established to improve outcome. The management of TNBC will significantly improve once mechanisms responsible for TNBC resistance to chemotherapies will be identified. Here, we applied a new functional proteomic strategy to reveal which (dys)regulated phospho-signaling circuits are the effective dependencies of chemotherapy-resistant TNBC cells. The high throughput kinase activity-mapping (HT-KAM) assay is our new screening technology to assess the catalytic activity of many kinases in parallel. HT-KAM relies on collections of biological peptide probes that are computationally derived from PhosphoAtlas (Olow and Chen et al, 2016 Cancer Research), and are physically used as combinatorial sensors to measure the activity of kinase enzymes in biological extracts. The HT-KAM system provides access to a vast, untapped resource of meaningful measurements, whether readouts are interpreted irrespective of which enzymes phosphorylate which probes, or analyzed to convert global phospho-signatures into functional profiles of kinase activities. Kinome maps reveal how signaling networks are re-wired by drug interventions in the context of different cellular backgrounds or exogenously mutated proteins/pathways, and provide insight into potentially targetable pathways. We previously successfully showed that the HT-KAM platform identifies new, actionable kinases responsible for intrinsic or acquired targeted-therapy resistance in BRAFV600E colorectal cancer cells and melanoma tumors from patients (Coppé et al, in revision; Ruiz-Saenz A et al, in preparation). To study TNBC, we used a 615-peptide sensor library that supports the mapping of >100 kinases and represents >900 functional kinase-substrate interactions relevant to tumor biology, and matches 100's of druggable components of signaling circuits. We characterized the phospho-catalytic signatures of 10 TNBC cell lines (BT549; HCC1143; HCC1395; HCC1937; HCC38; HCC70; HS578T; MDA231; MDA436; MDA468), either untreated or treated with chemotherapeutic drugs (5-FU, Carboplatin, Doxorubicin) at IC50 concentrations. We also included a panel of 8 luminal BC cells (BT483, CAMA1, HCC1428, MCF10A, MCF10F, MDA134, MDA175, ZR75.1). Based on our advanced preliminary data, we anticipate showing a comprehensive map of the oncogenic kinome of TNBCs, and how chemotherapies re-wire their signaling networks. The most hyperactive and conserved pathways establish a priority map of strategic kinase-hot-spots to explore as therapeutic candidates. We find that chemotherapies differentially induce kinases such as SRC, AKT, IKK, CHEK, AURK, SGK, which can be inhibited to improve therapeutic response in TNBC.

Systems approach for mapping functional cancer genome atlases in vivo

Sidi Chen, PhD, *Assistant Professor, Genetics and Systems Biology, Yale University*

International consortia such as TCGA mapped a comprehensive catalog of molecular alterations in the cancer genome. However, for many novel molecular alterations in patients, it is still unclear which of them, or their combinations, are necessary, sufficient, additive, antagonistic or synergistic in causing cancer and subsequent tumor progression. With genome editing, it is now feasible to systematically and quantitatively assess the contribution of each gene and various combinations to cancer evolution directly in vivo. This leads to the concept of mapping the functional cancer genome atlas (FCGA) for each cancer type. I will discuss the initial work towards mapping of most frequently mutated genes in cancer genomes in two highly lethal

cancer types. Our initial effort to map a provisional FCGA started from hepatocellular carcinoma (HCC). We developed a direct in vivo AAV-CRISPR screen approach, which generated highly complex, yet genetically defined, autochthonous liver tumors, for which we devised a novel readout strategy by directly sequencing variants at predicted sgRNA cut sites using molecular inversion probe sequencing (MIPS). The combination of AAV-CRISPR autochthonous mutagenesis and MIPS readout illuminated the mutational landscape of tumors, demonstrating quantitative maps of a large collection of variants. This screen revealed a functional map of drivers in liver tumorigenesis in fully immunocompetent mice, identifying novel functional tumor suppressors such as Setd2, B2m, Kansl1, Arid2, Kdm5c, Zc3h13 and Cic. We extended this approach for mapping of FCGA for glioblastoma (GBM), a disease with single digit five-year survival. We performed an AAV mediated autochthonous CRISPR screen in GBM. Stereotaxic delivery of an AAV library targeting genes commonly mutated in human cancers into the brains of conditional Cas9 mice resulted in tumors that recapitulate human GBM. Capture sequencing revealed diverse mutational profiles across tumors. Notably, across all genes represented in the mTSG library, the experimental mutational frequencies observed in these AAV-CRISPR pooled mutagenized mouse tumors correlated with the clinical mutational frequencies in human patients tumors in two independent patient cohorts. Co-mutation analysis identified co-occurring driver combinations such as Mll2, B2m-Nf1, Mll3-Nf1 and Zc3h13-Rb1, which were subsequently validated using AAV minipools. This study provides a functional landscape of gliomagenesis suppressors in vivo. The concept of FCGA and the AAV-CRISPR-MIPS approach can be applied to virtually the whole genome in any cancer types, or be focused down to the level of personalized mutations in each patient's cancer. Such studies can be performed in combination with many pre-clinical or co-clinical settings, providing new and powerful avenues for therapeutic discovery.

Non-coding Genome

Micromechanics and structure of metaphase chromosomes and the cell nucleus

John Marko, PhD, *Professor, Molecular Biosciences, Physics and Astronomy, Northwestern University*

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).

Nuclear rupture at sites of high curvature compromises retention of DNA repair factors

Dennis Discher, PhD, *Professor, Chemical and Biomolecular Engineering, Bioengineering, and Mechanical Engineering and Applied Mechanics, University of Pennsylvania*

The nucleus links physically to cytoskeleton, adhesions, and extracellular matrix - all of which sustain forces, but any relationships to DNA damage are obscure. Here, nuclear rupture and cytoplasmic mis-localization of multiple DNA repair factors results from high nuclear curvature imposed by an external probe or else by cell adhesion to either aligned collagen fibers or, most simply, stiff matrix. Mis-localization is enhanced by lamin-A depletion, requires hours for nuclear re-entry, and correlates with increases in pan-nucleoplasmic foci of the DNA damage marker, phospho-H2AX. Excess DNA damage is rescued in ruptured nuclei by co-overexpression of multiple DNA repair factors as well as by soft matrix or inhibition of actomyosin tension. Increased contractility has the opposite effect, and stiff tumors with low lamin-A indeed exhibit increased nuclear curvature, more frequent nuclear rupture, and excess DNA damage, suggestive of curvature stress in vivo. At least one clinical study of breast cancer, which also tends to have low lamin-A, shows mis-localization of multiple DNA repair factors and high DNA damage in biopsies from patients with worst prognosis. Ongoing studies of genomic variation include use of a novel fluorescence reporter for aneuploidy, which already shows increased aneuploidy after partial knockdown of DNA repair factors.

Squish and squeeze - the role of the nucleus and lamins in breast cancer metastasis

Jan Lammerding, PhD, *Associate Professor, Biomedical Engineering, Cornell University*

During cancer cell invasion and metastasis, tumor cells migrate through interstitial spaces and transendothelial openings substantially smaller than the diameter of the cell. The cell nucleus is the largest and stiffest organelle, making nuclear deformation a rate-limiting factor in the passage of cells through such confined environments. Since the nuclear envelope proteins lamins A and C are a major determinant of nuclear stiffness and their expression is altered in many cancers, we examined their levels in breast cancer and their role in nuclear mechanics, cell invasion, and disease progression. Analysis of patient-derived breast tumor tissues and human and mouse breast cancer cell lines revealed significantly lower lamin A/C levels in highly aggressive breast cancer cells than in less metastatic cells. Low levels of lamin A/C correlated with increased nuclear deformability. Increasing expression of lamin A in breast cancer cells

with normally low levels of lamin A/C significantly impaired their invasive properties, while depletion of lamin A/C increased invasive potential through micron-scale microfluidic constrictions and dense collagen matrices. The role of lamins in mediating aggressive cancer phenotypes was not limited to invasion, as cell proliferation was increased with reduced lamin A/C levels both in in vitro cell models and in patient tumors. Increasing lamin A expression in metastatic cells also altered expression of several cell adhesion and extracellular matrix proteins, as revealed by stable isotope labeling with amino acids in cell culture (SILAC) analysis, suggesting additional pathways by which lamins impact cancer cell invasion. Importantly, analysis of breast tumor tissue microarrays showed that low levels of lamin A/C correlated with reduced disease-free survival. Furthermore, reduced lamin A/C levels in patient-derived tumors were associated with increased Akt phosphorylation, and inhibition of the PI3K/Akt pathway increased lamin A/C levels in vitro, providing a possible explanation for the altered lamin A/C levels. Taken together, our studies indicate that downregulation of lamin A/C could promote both cancer cell invasion and outgrowth in breast cancer. Insights gained from these studies could improve prognostic approaches; ultimately, targeting regulator pathways associated with altered lamin expression may offer novel therapeutic avenues to control metastatic disease in breast cancer.

Enhancer Reprogramming Promotes Breast Cancer Cell Lineage Plasticity to Achieve Endocrine Resistance

Jason Liu, PhD, *Assistant Professor, University of Texas Health Science Center - San Antonio*

While patients with ERα-positive luminal breast cancer receive drug/endocrine therapies for a period of 5 years, more than 30% of these patients eventually develop drug/hormone resistance and recurrence, which is a persistent challenge in breast cancer treatment. However, the underlying molecular mechanisms, especially the dysregulations of gene expression that promote cancer phenotypic progression, are unclear. Enhancers are very important distal DNA regulatory elements that control temporal- or spatial-specific gene expression patterns in development and diseases. Estrogen and ERα are critical for breast cancer development through binding predominantly at distal enhancers. Increasing evidence suggests that many other oncogenic transcription factors (TFs) are involved in functional regulation of ERα-bound enhancers. However, it is not well known about the functional interaction mechanisms between these oncogenic TFs and ERα and how these interactions are altered and result into gene dysregulations during cancer progression. Our omics data showed that multiple oncogenic TFs collaboratively interact with ERα to determine the activation of ERα-bound enhancers. These TFs including ERα interact with enhancers through both cis-binding (DNA motif dependent binding) and trans-binding (protein-protein tethering binding). Remarkably, we observed significant changes in the interaction among the TFs, as well as in the cistromes of these TFs during hormone resistance progression. Moreover, these changes are associated with genome-wide enhancer gain/loss reprogramming that promotes cell lineage plasticity characterized with the loss of luminal lineage markers and gain of basal lineage markers. Our preliminary data also suggest that, among the ERα-interacting TFs, several critical oncogenic

TFs, which have been shown to be involved in cancer progression, might mediate the gain and loss of ERα enhancers. It's previously known that the expression level and functional activities of oncogenic TFs can be regulated by microenvironmental signals. Therefore, our data support that the combinations/interactions between ERα and oncogenic TFs are altered in response to cancer microenvironmental signals, and these alterations architecturally reprogram ERα-bound enhancers, resulting into transcriptional program transitions that promote lineage plasticity and cancer invasive progression.

Translational Applications of Physical Oncology and Systems Biology

Quantifying drug combination synergy along potency and efficacy axes

Vito Quaranta, MD, *Professor, Biochemistry & Pharmacology, Vanderbilt University*

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.

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

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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.

Linked single-cell biophysical and transcriptional profiles resolve heterogeneity in PDX models of B-cell acute lymphoblastic leukemia and suggest mechanisms of in vivo resistance

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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.

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

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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 conducted 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.