National Cancer Institute

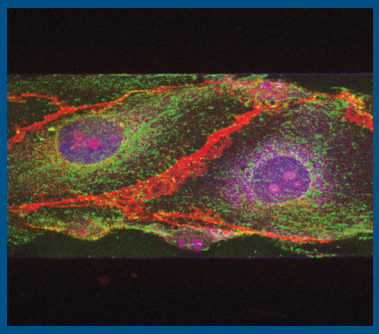
PHYSICAL SCIENCES in ONCOLOGY

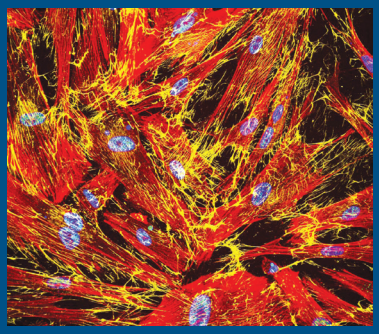
PERSPECTIVES

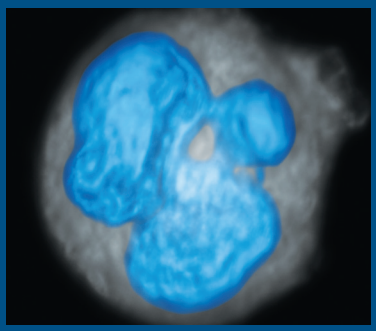
News from the Collaborative Network of Physical Sciences-Oncology Centers | Spring 2011

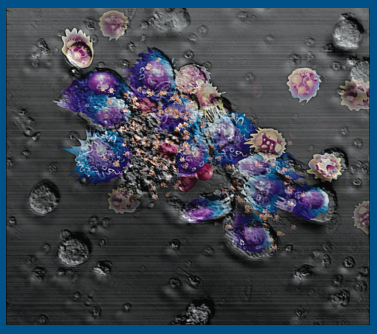
INSIDE

Trans-Network Perspective 2
Center Perspective 4
Investigator Perspective 13
Young Investigator Perspective 15
Program Perspecitve 17
A New Perspective 19

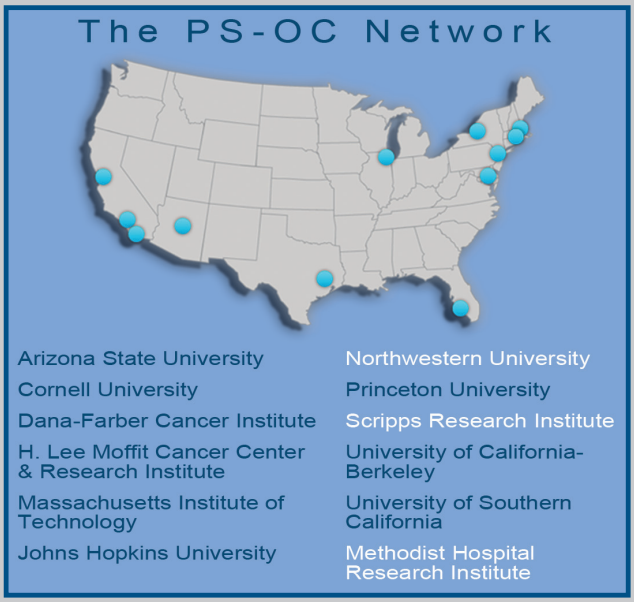








NCI Perspective: Transport Phenomena and Cancer



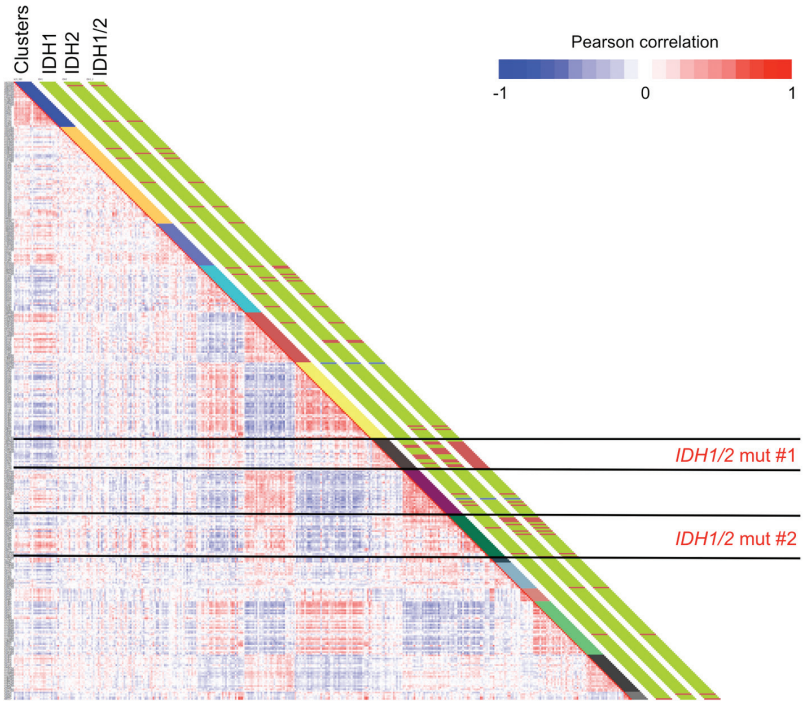
Exploring transport phenomena in cancer encompasses both the study of physical transport mechanisms at the cellular scale and the study of information transfer at the molecular level. Within the Physical Sciences-Oncology Center Program, these distinct scales of transport are examined using physical science perspectives to help elucidate ways to overcome the complex mechanisms of cancer. At the cellular scale, a broader understanding of the physical mechanisms directing transport and bio-distribution of synthetic particles or cells within the microvasculature of a tumor can provide insight into better therapeutic delivery to tumors and the metastatic efficiency of circulating tumor cells (CTCs). Specifically, physical sciences may define limiting factors of particle delivery to tumors for imaging and therapeutic applications and explain why low percentages of CTCs result in formation of secondary tumors. At the molecular level, pursuing experimental approaches that define what information is and how it is decoded and managed in terms of chromosome structure and spatial distribution within the cell may explain questions such as the mechanistic basis for sensing and controlling nucleus size and cell division.

This issue of *PS-OC Perspectives* highlights the use of advanced tools and approaches from the physical sciences that may expand the understanding of these complex transport phenomena in cancer. It also emphasizes the team-based, collaborative approach to science prevalent within each center and across the PS-OC Network and how these connections are leading to new science and new ideas.

Elucidating the Function of Isocitrate Dehydrogenase Mutations in Aberrant Epigenetic Programming

By Ari. M. Melnick

Acute myeloid leukemias (AML) are a heterogeneous group of mostly fatal diseases, for which treatment outcomes have not improved much in the past thirty years. A better understanding of molecular pathogenesis is urgently required to improve diagnostic and therapeutic options for these patients. To more accurately define the molecular basis of AML, scientists from the Cornell University PS-OC undertook a large-scale effort to measure the patterning of epigenetic marks in 750 AML patients enrolled in multicenter clinical trials in Europe and the United States. The studies involved profiling the distribution of DNA methylation, histone modifications and gene expression in purified leukemia cells. Since cell numbers are very limited from these multicenter clinical trial specimens, epigenomic assays had to be adapted for small/selected cell populations. Computational and mathematical modeling approaches had to be created to deconvolute the complex distribution patterns of these data and integrate the various layers of information.



A graphical representation of the correlation values between DNA methylation profiles in 398 AML patients based on hierarchical clustering, where red represents greater correlation and blue lesser correlation between cases.

Although still in progress, this study is already yielding important new information. For example, we have learned that DNA methylation is not randomly distributed in AMLs and in fact occurs in very specific patterns as AML patients group into sixteen different epigenetically defined subtypes. Some of these subtypes are explained by known genetic lesions, while others are defined solely based on their DNA methylation signatures. These novel, epigenetically defined subtypes feature deregulation of biologically distinct pathways and have different clinical outcomes. Even more surprisingly, 45 genes were almost universally aberrantly hypermethylated and repressed (compared to normal hematopoietic cells) in AML patients regardless of their genetic background, suggesting that a common pathway of aberrant epigenetic silencing is required for malignant transformation of bone marrow cells.

One issue that the Cornell group wished to address was the molecular basis of these novel epigenetically defined AML subtypes. To solve this mystery, additional expertise was required, and so the

"This project exemplifies how the PS-OC Network can synergistically unite scientists who otherwise would not interact in order to solve fundamental questions in cancer biology."

Cornell PS-OC teamed up with the Dana Farber PS-OC (Ross Levine and Craig Thompson) and the Northwestern University PS-OC (Jonathan Licht). This collaboration joined mathematical modeling, computational biology, nanoengineered selected cell epigenomics, biochemistry, cell biology, biostatistical and clinical investigation.

Unifying disparate areas of expertise, our trans-network team made the surprising discovery that two of the unexplained novel AML subtypes featured mutations in either isocitrate dehydrogenase 1 (IDH1) or IDH2. IDH1 and IDH2 mutation was strongly associated with global promoter hypermethylation as compared to other AMLs. Mutations in these enzymes were only recently identified in AML and their functional significance was unknown. Normally IDH1 and IDH2 are involved in energy metabolism, catalyzing the interconversion of isocitrate and alpha-ketoglutarate, and regulating the balance between NADP+ and NADPH. It was therefore not at all obvious how dysfunction of IDH1 or IDH2 might influence epigenetic programming. Nonetheless, an important clue was provided by recent work showing that mutant IDH1/2 generates an aberrant neo-metabolite: 2 hydroxyglutarate (2HG), which can partially mimic the structure of alpha-ketoglutarate.

Through an in-depth genetic analysis of the large AML patient cohort studied by the Cornell group, Dr. Levine observed that IDH1/2 mutations are mutually exclusive with loss of function mutations in another gene called TET2. TET2 is an alpha ketoglutarate-dependent enzyme that mediates cytosine hydroxymethylation, which is associated with subsequent DNA demethylation. Because of its structure 2HG was postulated as a possible inhibitor of alpha ketoglutarate-dependent enzymes. Dr. Thompson found that mutant IDH1 or IDH2 did indeed block TET2-mediated DNA hydroxymethylation. Most significantly, either mutant IDH1/2 or TET2 loss of function induced global DNA hypermethylation in hematopoietic cells. Strikingly, AML patients with DNA methylation profiles similar to IDH1/2 mutants but with wild type IDH1/2 loci, turned out to harbor TET2 loss of function mutations; there was accordingly extensive overlap between the aberrant DNA methylation signatures of TET2 or IDH mutant patients. Finally Drs. Levine and Licht showed that transduction of mutant IDH alleles or depletion of TET2 induced differentiation blockade and stem cell expansion in normal hematopoietic cells, which is consistent with the leukemia cell phenotype.

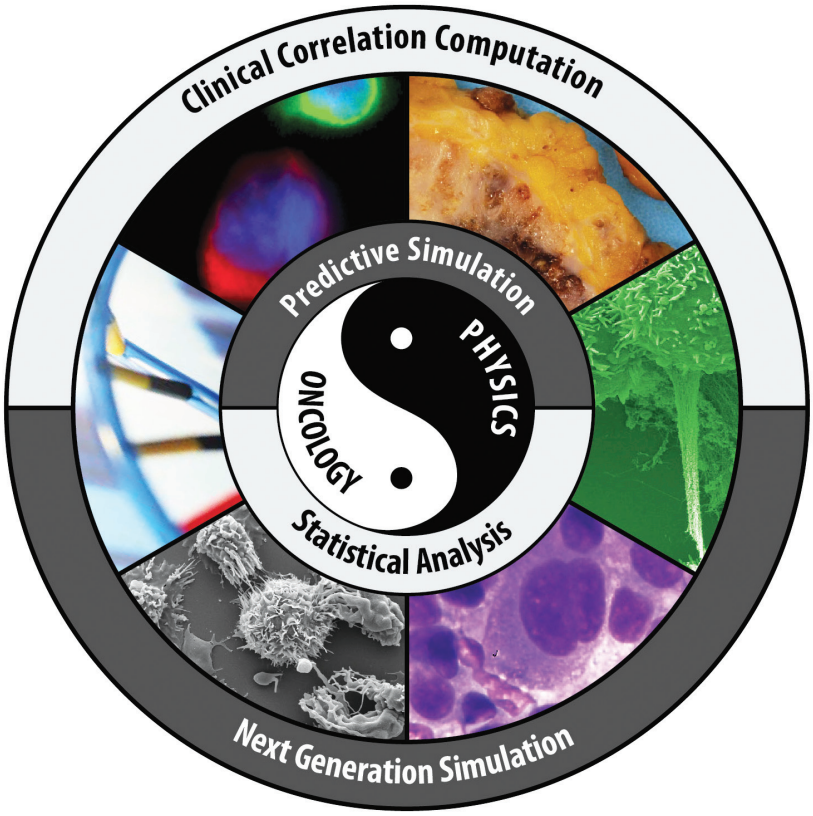
The data suggest that 2HG produced by mutant IDH1/2 inhibits TET2, leading to reduced hydroxymethylation and increased cytosine methylation, thus directly linking for the first time defects in energy metabolism enzymes with aberrant epigenetic programming. These results were recently published in *Cancer Cell* and underline the power of integrative epigenomics and genetic studies to define new paradigms in cancer biology. The results are promising from a therapeutic standpoint, since they suggest that development of small molecule inhibitors to selectively block mutant IDH1/IDH2 might repair the defect in DNA methylation and overcome the leukemic phenotype. This project exemplifies how the PS-OC Network can synergistically unite scientists who otherwise would not interact in order to solve fundamental questions in cancer biology.

Dr. Ari M. Melnick is an Associate Professor at Weill Cornell Medical College where he directs the Epigenomics Core Facility and leads a

The Physics and Mathematics of Metastasis Across Time and Space: The Scripps Research Institute PS-OC

By Peter Kuhn

The Scripps Research Institute PS-OC formed through a unique collaboration between the Scripps Research Institute, Oregon Health Sciences University (OHSU), University of Southern California, Scripps Clinic, University of California-San Diego Moor's Cancer Center and Billings Clinic. Our Center investigates heterogeneity of fluid phase biopsies in cancer to provide a new fundamental understanding of the physical processes of cancer metastasis in patients with epithelial cancers across the dimension of space in the human organism and over the dimension of time during the course of disease.



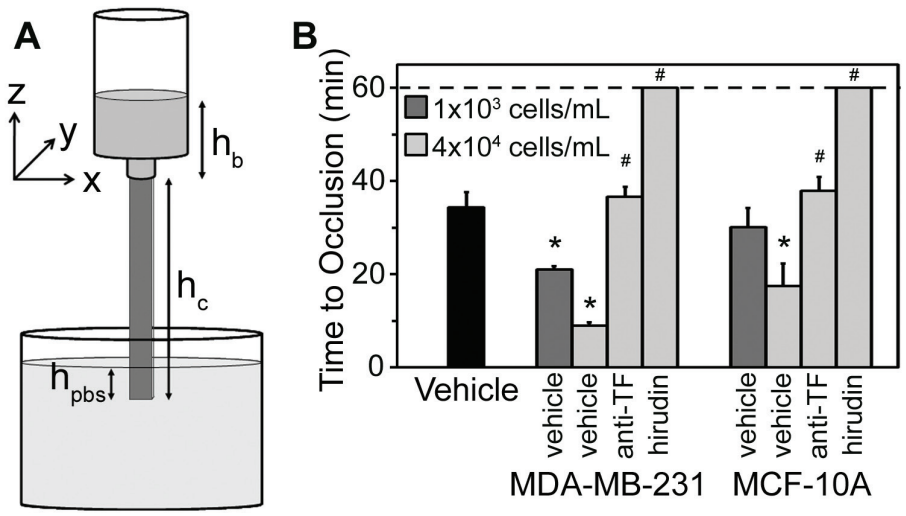
At the Scripps PS-OC, first-principles analysis drives the design of measurements, which will provide orthogonal data sets on cancer patients' primary and fluid biopsies. The resulting model will be a comprehensive new view of the metastatic process.

THREE PROJECTS

Project 1: Project 1, a team led by Owen McCarty, OHSU, is measuring the biophysical properties of cancer cells. Although significant progress has been made in deciphering the molecular and genetic features of epithelial cancers, much is still unknown about the behavior and effects of cancer cells in the fluid phase during transit through the circulation. Thrombosis is a common clinical manifestation of cancer and circulating tumor cells (CTCs) may play a pathogenetic role in this process. The presence of coagulation-associated molecules on cancer cells has been described, but the mechanisms by which CTCs augment or alter coagulation remains unclear. We have utilized suspensions of a metastatic adenocarcinoma cell line, MDA-MB-231, and a non-metastatic breast epithelial cell line, MCF-10A, as models of circulating tumor cells to determine the thromobogenic activity of these blood-foreign cells. In human plasma, both metastatic MDA-MB-231 cells and non-metastatic MCF-10A cells significantly enhanced clotting kinetics. The effect of MDA-MB-231 and MCF-10A cells on clotting times was cell number-dependent and inhibited by a neutralizing antibody to tissue factor (TF) as well as inhibitors of activated factor X and thrombin. Using fluorescence microscopy, we found that both MDA-MB-231 and MCF-10A cells supported the binding of fluorescently-labeled thrombin. Furthermore, in a model of thrombus for-

"Our Center investigates heterogeneity of fluid phase biopsies in cancer to provide a new fundamental understanding of the physical processes of cancer metastasis...across the dimensions of time and space."

mation under pressure-driven flow, MDA-MB-231 and MCF-10A cells significantly decreased the time to occlusion. Our findings indicate that the presence of breast epithelial cells in blood can stimulate coagulation in a TF-dependent manner, suggesting that tumor cells that enter the circulation may promote the formation of occlusive thrombi under shear flow conditions.



*Cultured breast epithelial cells promote TF-dependent occlusive thrombus formation in flowing blood, ex vivo. Human sodium citrate-anticoagulated whole blood was mixed with vehicle, MDA-MB-231 or MCF-10A cells ($4x10^4$ or $1x10^3$/mL), for five minutes at room temperature. In selected experiments, blood was treated with a neutralizing antibody to TF (anti-TF, 20 µg/mL) or the thrombin inhibitor, hirudin (20 µg/mL), in the presence of MDA-MD-231 or MCF-10A cells. (A) Treated blood was recalcified with $CaCl_2/MgCl_2$, added to a reservoir to a set height (hb), and allowed to drain through collagen-coated capillaries into a PBS bath as shown. (B) The time to thrombotic occlusion was recorded. Data are mean±SEM; n=3-6. *P<0.05 versus vehicle treatment in the absence of cells. #P<0.05 versus vehicle treatment of corresponding cell type at $4x10^4$ cells/mL*

Project 2: This project, led by Kelly Bethel, focuses on the level of functionally relevant structural properties of cell populations and grouping behaviors of cancer cells with other cells in their microenvironment by taking a comprehensive sampling across the time and space heterogeneity of the tumor via solid and fluid biopsies.

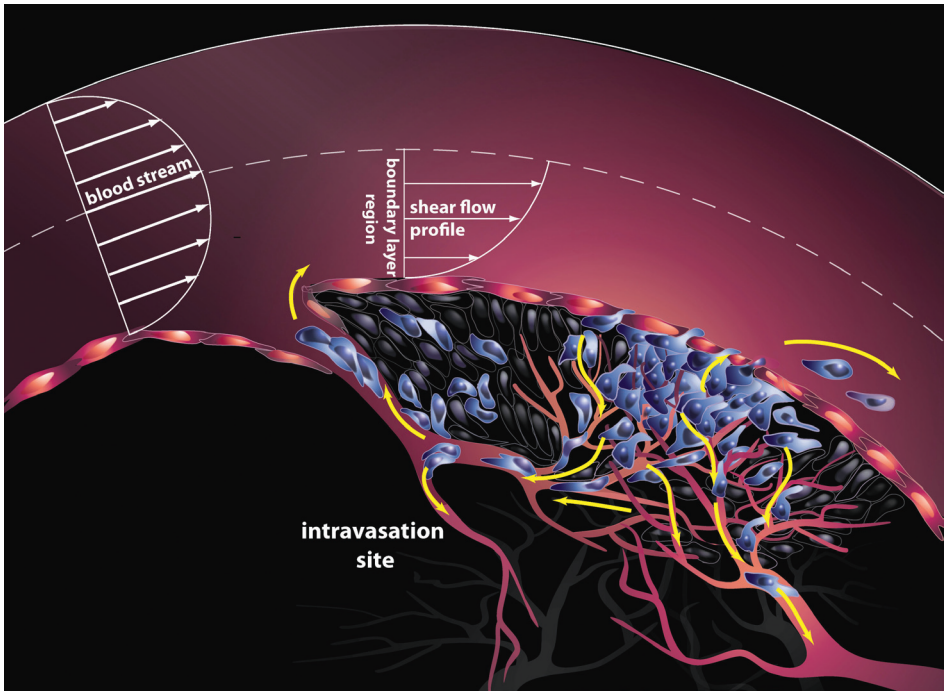
Project 2 is pursuing two objectives. First, it seeks to create a tumor topology by exploring tumor cell heterogeneity across geography and assessing tumor cell heterogeneity in individual patients with lung and colon cancer, across space and time to produce a tumor topology: primary tumor mapping, locoregional tumor mapping and evolution over time. Second, it also seeks to delineate travel strategies in the third tumor microenvironment, the bloodstream, by characterizing interactions there. This aspect of the project will use empirical measurements to understand the variables involved in how the bloodstream and blood proteins may function as a transient 'tumor stroma' for metastasizing cells, affecting how they conduct their migration through the circulation.

During our research, clusters and emboli have been found in a majority of patients. Circulating tumor cell association enumeration revealed that ~50% of CTCs reside in clusters in both breast and prostate patients, while less than 20% appear in healthy donor (HD) patients. High resolution imaging on a Zeiss LSM710 confocal microscope was performed in order to confirm cell association between cell populations and non-stained entities. These images confirm association of CTCs with each other,

"Our Education and Outreach Unit sponsors monthly meetings featuring guest speakers and has also organized two pathology boot camps to help illuminate the medical and clinical aspects of pathology."

white blood cells, and platelet-like structures.

Project 3: A third team, led by Paul Newton, is developing quantitative/computational models describing tumor cell dynamics and dissemination in the microcirculation through establishing the fundamental physical parameters of CTCs in the bloodstream. During the past year, Newton's team developed stochastic models of intravasation and circulating CTCs as well as a metastatic progression model using a Markov chain/network framework. The model can be used for predictive metastatic development as the disease progresses, providing results in the form of probabilistic distributions (pdfs) that can be tailored to individual patients using initial state vectors. What drives the model dynamics is a "transition matrix" which allows our research team to experiment with different scenarios and predict or design outcomes.



Stochastic models for intravasation and CTC circulation. (A) Growth from primary pushes cell mass through vessel wall lining. (B) Corner vortices pinch off cell at base and contribute to shear/normal forces. (C) Shear and normal forces on cell surface in boundary layer act on protruding cellular mass. (D) Stochastic fluctuations in these forces sporadically exceed threshold for cell mass to stay intact. (E) Cell or cells on front line get torn from tumor and enter bloodstream.

EDUCATION and OUTREACH

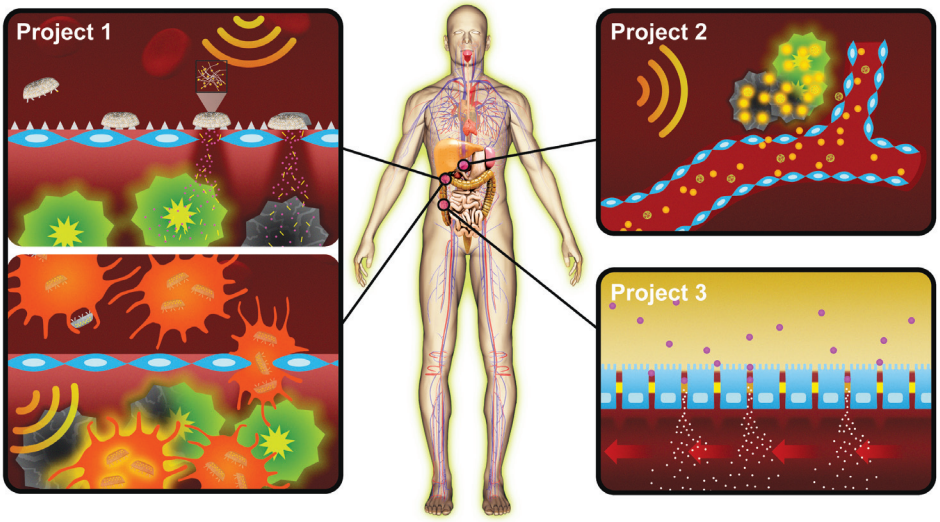
Our Education and Outreach Program was developed to achieve two goals: 1) to improve understanding of cancer's complexity; and 2) to raise awareness that this understanding can be achieved through the application of physical sciences-based approaches to cancer research. To realize these goals, our Education and Outreach Unit sponsors monthly meetings featuring guest speakers and has also organized two pathology boot camps to help illuminate the medical and clinical aspects of pathology. The Unit has also helped recruit students and faculty from the physical sciences to work on the hypotheses defined in the Center's research projects by giving these researchers the opportunity for daily interactions with medical oncologists and pathologists. This collaborative environment provides a unique opportunity for generating deep insights into some of the most pressing questions in the field of cancer research.

Peter Kuhn is an Associate Professor of Cell Biology at the Scripps Research Institute and the Principal Investigator for the Scripps Research Institute PS-OC.

The Center for Transport Oncophysics: The Methodist Hospital Research Institute PS-OC

By Damian Walsh

The Center for Transport Oncophysics at Houston (CTO) brings together research teams from Methodist Hospital Research Institute, University of Texas M. D. Anderson Cancer Center, University of Texas at Austin, University of Texas Health Science Center, Rice University, Harvard Medical School and Massachusetts General Hospital. The CTO articulates into three tightly integrated projects under the unifying theme of the quest for a deeper understanding of the multi-scale differentials in transport properties that accompany and help define the evolution of malignancies. These differentials manifest themselves with the emergence or the modulation of pathology-associated biophysical and biological barriers to transport. The operational corollary to the unifying basic science theme of the CTO is the quest for innovative, physical sciences-based methodologies that afford the exploitation of these differentials for improved diagnostics and treatment of cancer.



The CTO's three projects collectively seek to understand both the physics of mass transport within a cancer lesion and mass exchanges between cancer and surrounding host biology. The Center focuses on two specific cancer types: colorectal cancer and liver metastasis. Investigators are integrating mathematics, innovative engineered transport probes and state-of-the-art imaging to elucidate the transport physics of various physical and biological barriers related to tumorigenesis and drug delivery.

THREE PROJECTS

Project 1: Project 1 is led by Mauro Ferrari (Methodist Hospital Research Institute), Isaiah Fidler and Renata Pasqualini (University of Texas M. D. Anderson Cancer Center) and is developing a broader understanding of the physical barriers and biological factors involved in the progression of liver metastasis in orthotopic models of colorectal cancer (CRC). This project is also investigating the design of novel biocompatible delivery carriers able to overcome or exploit these barriers with favorable pharmacokinetics and tissue distribution profiles for highly efficient delivery of novel therapeutic and imaging agents. One of the therapy methods to be investigated includes thermal radiofrequency (RF) ablation.

Project 2: Project 2, under the leadership of Steven Curley (University of Texas M. D. Anderson Cancer Center) and Lon Wilson (Rice University), is exploring issues related to the progression kinetics of a primary hepatic tumor (hepatocellular carcinoma or HCC). It will develop a broader understanding of the biophysical barriers involved in RF-based thermal therapy and MRI/CT imaging of HCC by employing gold nanoparticles (AuNPs) and fullerene particles (nano-C60). This will include the transport of the nanoparticles towards the lesion; the sufficient and specific accumulation of the nanoparticles within

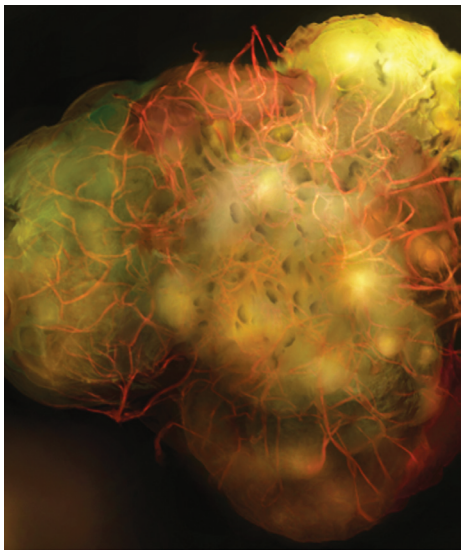
"The CTO articulates into three tightly integrated projects seeking a deeper understanding of the multi-scale differentials in transport properties that accompany and help define the evolution of malignancies."

the tumor cells; the heat generation upon RF activation and the heat transfer to the surrounding tissue. This goal will be achieved through an integrated process where *in vitro* testing and *in vivo* studies are combined with predictive *in silico* mathematical models.

Project 3: Project 3 is led by Nicholas Peppas (University of Texas-Austin) and is addressing various physical barriers that a chemotherapeutical agent, administered orally, meets before being systemically absorbed and reaching the target tumor tissue. These barriers include, for example, changes in pH across the gastrointestinal (GI) tract; enzymatic degradation; and epithelial transport through different mechanisms. To study and tackle these barriers, investigators will use engineered polymeric carriers. The engineered delivery vehicle capable of being loaded with the target compound and subsequently releasing it, with attention focused on the efficiency of each physical process involved, must first be designed and tested for facing each of the physical barrier's components. The pH increase that occurs when passing from the stomach to the upper small intestine is the trigger to initiate release so this carrier must be able to selectively release the compound in response to a pH shift used to simulate this physiological event. The kinetics of release from the carrier must be appropriate given the projected residence time for the carrier particles in the region of the digestive tract most suitable for absorption of the therapeutic agent.

TWO RESEARCH CORES

Core 1: Core 1, led by Paolo Decuzzi (Methodist Hospital Research Institute) and Vittorio Cristini (University of Texas Health Science Center) provides mathematical tools to model and predict the behavior of small molecules and nano-sized particulate systems in terms of (i) transport dynamics within the authentic patient-specific vasculature accounting for the specific/non-specific adhesive interactions with the vascular endothelium and for the permeability of the vessel walls to both plasma and blood-borne agents; (ii) extravasation from the vascular compartment to the extravascular matrix through active (transcytosis) and passive (intravascular gaps) mechanisms; (iii) transport across the extravascular matrix and distribution within the tumor microenvironment; (iv) control of the tumor growth and angiogenic response; and (v) heat generation through remote RF activation with modeling of thermal cell damage and apoptosis.



Tumor morphology as simulated in 3-D by a multiscale model of cancer coupling molecular information with the cell/tissue-scale physics of mass/momentum/energy transport; this picture was made by Dr. F. Jin and Dr. H. Frieboes under the direction of Prof. V. Cristini, with the help of medical illustrator E. Keller.

Core 2: Core 2 is led by Andy Yun (Harvard Medical School and Massachusetts General Hospital) and Dr. Rebecca Richard-Kortum (Rice University) and concerns Advanced Intravital Microscopy. This Core provides the three projects with a number of unique advanced optical systems; collaborates with the project investigators in the design, execution, and analysis of animal experiments; and develops new in-

"Our Education and Outreach Unit administers a monthly workshop series, 'Tools for Nanomedicine,' that gives students hands-on experience in areas such as live confocal imaging, flow cytometry, and more."

strumentation and methodology as needs arise. The Core has established protocols for *in vivo* tracking of nanoparticles in various organs. Further, it has developed low-cost, battery-powered, multi-modal imaging devices which can be used to track the transport of optically active agents throughout 3-D tissue. It has also devised simple strategies to improve spatial resolution and to enable sub-surface imaging. Using GRIN lenses and leveraging the increase in sampling that comes from moving the probe in tissue, team members will image with diffraction-limited resolution.

EDUCATION AND OUTREACH

The Education and Outreach Unit within the Center for Transport Oncophysics seeks to develop trainees capable of tackling cancer-related problems while using approaches from the physical sciences and engineering. Toward this end, many exciting new programs have been implemented. Most significantly, the Unit administers a monthly workshop series, "Tools for Nanomedicine," that gives students hands-on experience in areas such as live confocal imaging, flow cytometry, and sample preparation for transmission electron microscopes. Videos of these workshops have been archived on the CTO website for easy reference. By partnering with the Cornell PS-OC, the Unit has also made a Cornell course in nanobiotechnology available to students via videoconference. Additionally, the CTO's Nanomedicine Scholarly Concentration in the University of Texas Health Science Center Medical School currently has five medical students working on didactic laboratory projects Finally, the Unit also helped organize the CTO's annual workshop, which drew over 90 participants from the Houston area and featured presentations from CTO investigators as well as guest speakers Robert Austin and Jan Liphart from the Princeton and University of California-Berkeley PS-OCs, respectively.

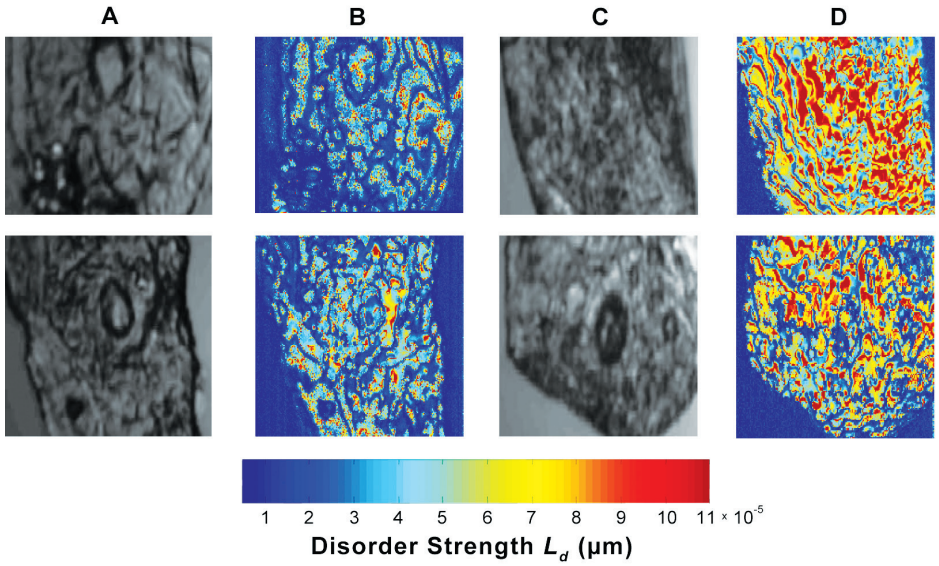
Damian Walsh is the Manager of the Department of Nanomedicine at the Methodist Hospital Research Institute.

The CTO's fall workshop attracted more than 90 participants from the Houston region and featured presentations from Center PI Mauro Ferrari as well as investigators from two other PS-OCs, Robert Austin (Princeton) and Jan Liphardt (University of California-Berkeley).

The Coding, Decoding, Transfer and Translation of Information in Cancer: The Northwestern University PS-OC

By Benette Phillips

Led by Jonathan Widom, PhD, and Jonathan Licht, MD, the Northwestern University PS-OC investigates the molecular basis of information flow in normal cells and in cancer. Each of the Center's five projects—which are collaborative efforts spanning Northwestern University, the California Institute of Technology, the University of Chicago, and the Weizmann Institute—merge molecular and cell biology with the physical sciences to achieve a quantitative and predictive understanding of the regulation of gene expression. Four projects examine selected properties of DNA and its higher-order compacted forms to better understand how DNA structure, composition, and epigenetic modification become dysregulated in cancer while the fifth project investigates how protein degradation is regulated.



The above figure, taken from a study led by Vadim Backman of Project 3, contrasts representative buccal epithelial cells from cancer-free COPD patients and patients harboring lung cancer. (A) Bright-field microscopy image from a COPD patient. (B) Partial-wave spectroscopy (PWS) microscopy pseudocolor heatmap of disorder strength of nanoscale intracellular architecture from a COPD patient. (C) Bright-field image from a lung cancer patient. (D) PWS pseudocolor heatmap of disorder strength from a lung cancer patient. The bright-field images from COPD and lung cancer patients were indistinguishable. However, the disorder strength of the same cells obtained from PWS analysis of the cancer patient was markedly increased when compared to that of the COPD patient.

FIVE PROJECTS

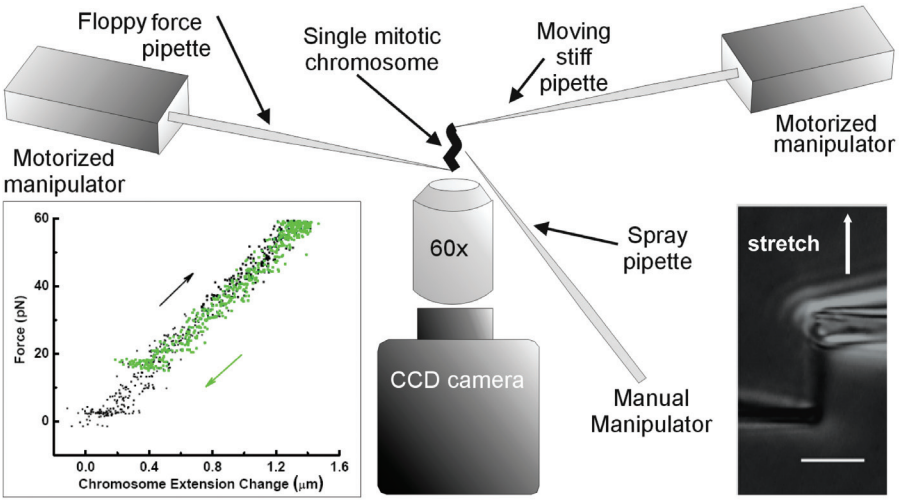
Projects 1 & 2: Projects 1 and 2, led by Rob Phillips (Cal Tech) and Jonathan Widom, respectively, are closely linked conceptually and methodologically. Using a "tethered particle motion" assay that measures DNA looping and atomic force microscopy to measure the trajectories of immobilized short DNAs of varying sequences, Project 1 examines how the mechanical properties of DNA, particularly flexibility, are influenced by its sequence. Project 2, in turn, investigates the influence of DNA sequence on nucleosome positioning. Nucleosome formation, the first level of DNA compaction, requires that DNA be flexible enough to bend around an octamer of histone proteins. Extending earlier findings from the Widom lab showing that nucleosome positioning in yeast is highly sequence-dependent, Project 2 has demonstrated that there is a genomic code for nucleosome positioning in humans as well. The relationship between nucleosome positioning and transcription factor accessibility is a crucial element in gene regulation, and investigators have developed an equilibrium thermodynamic model to examine this relationship. Because cancer-

"Our PS-OC investigates the molecular basis of information flow in normal cells and in cancer. We seek a quantitative and predictive understanding of fundamental mechanisms in genetic regulation and expression."

ous cells exhibit pervasive dysregulation of gene expression, a phenomenon that profoundly affects cell behavior, this work is highly cancer-relevant.

Project 3: Under the leadership of John Marko, a third team is studying how the higher-order organization of chromatin and chromosomes is altered in malignant cells and, possibly, in normal-appearing cells that reside in the vicinity of a tumor. Team members are using a number of important physical sciences-based approaches, some of which have been developed in their laboratories, to examine the physicochemical properties of the chromosome in such cells. To image chromatin domains, investigators are employing high-resolution electron microscopy coupled with the use of metal nanoparticles functionalized with antibodies to specific chromosomal proteins. Additionally, novel light scattering techniques developed in the laboratory of Vadim Backman are being used to examine changes in chromatin nanoarchitecture. Dr. Backman and colleagues have recently shown that seemingly histologically normal cells residing at a distance from a tumor scatter light differently than cells not in the vicinity of a tumor. This change in light scattering properties may be one manifestation of the "field effect," putative precancerous changes in cells within a field of cells that includes a frank tumor. As part of Project 3, Backman's team is examining whether changes in chromatin nanoarchitecture are responsible for these alterations in optical properties. These studies have important implications for tumor detection.

Project 4: Led by William Kath, Project 4 is developing quantitative predictive models of the regulatory networks associated with a given epigenetic state. Like genetic alterations, epigenetic changes are ubiquitous in cancer cells. Since changes in epigenetic states result from modifications in gene expression rather than from permanent changes in the genes themselves, these states should be susceptible to perturbation. The ultimate goal of this project is to use dynamical network modeling to identify compensatory perturbations in key regulatory networks that can return the malignant phenotype to a pre- or non-malignant state. One of several experimental systems that will be utilized in this work is matched multiple myeloma cells that express either high or low levels of the putative oncogene MMSET. Multiple myeloma cells from approximately 15% of patients with this cancer bear a chromosomal transloca-



Chromosome-stretching experiment conducted by the laboratory of John Marko for Project 3. A single mitotic chromosome is suspended between two pipettes on motorized manipulators in an aqueous tissue culture. Lower right shows micrograph of chromosome and pipettes. Lower left shows force-extension data for a human chromosome.

"Extending earlier findings from the Widom lab showing that nucleosome positioning in yeast is highly sequence-dependent, we have demonstrated that there is a genomic code for nucleosome positioning in humans as well."

tion that results in overexpression of MMSET. MMSET is a histone methyltransferase and its overexpression has been shown by team member Jonathan Licht to induce epigenetic changes in multiple myeloma cells, modifying both the overall histone methylation pattern and the gene expression profile. Investigators have manipulated these multiple myeloma cells to create a matched set of cells that expresses either high or low levels of MMSET. Current efforts focus on comparing nucleosome positioning, histone modifications, and gene expression levels for genes that are bound and regulated by MMSET in the matched cell sets. These experimental data will provide the foundation upon which regulatory network models of cell state can be developed.

Project 5: Project 5 leader Andreas Matouschek and his team are using physical sciences methodologies to interrogate the interactions of the proteasome, a molecular machine that degrades cellular proteins, with its substrates. Importantly, the proteasome does not always completely degrade its substrate and can sometimes generate biologically active protein fragments. Such is the case for NF-kappaB, a constituent of a pathway frequently altered in cancer. The proteasome has become a target for anti-cancer drugs because of its central role in cellular regulation. Recently, a general proteasome inhibitor, bortezomib/Velcade, has been approved for treatment of multiple myeloma and mantle cell lymphoma. Understanding the mechanism of proteasome action may allow the development of more specific, targeted therapeutic approaches to interfere with the signaling pathways that promote or sustain cancer growth. Team members are examining how substrates move through the proteasome, what forces are exerted on the substrate by the proteasome, and how substrates react to these forces. These questions are being addressed using three different techniques that can measure the interaction of isolated proteasomes with single substrate molecules: total internal reflection fluorescence degradation assays, magnetic tweezer force measurements, and atomic force microscopy.

EDUCATION AND OUTREACH

The Northwestern PS-OC has established a dynamic Education and Outreach Unit whose mission is to attract and train a new generation of scholars working at the intersection of the physical sciences and cancer research. In the past year, the Unit has organized a variety of educational activities, including a journal club; a seminar series; a two-day workshop on the fundamentals of tumor biology; two summer research programs for undergraduates; and several center-wide research forums. The Unit currently serves roughly 100 trainees, and more than 1000 members of the Northwestern scientific community have participated in its functions.

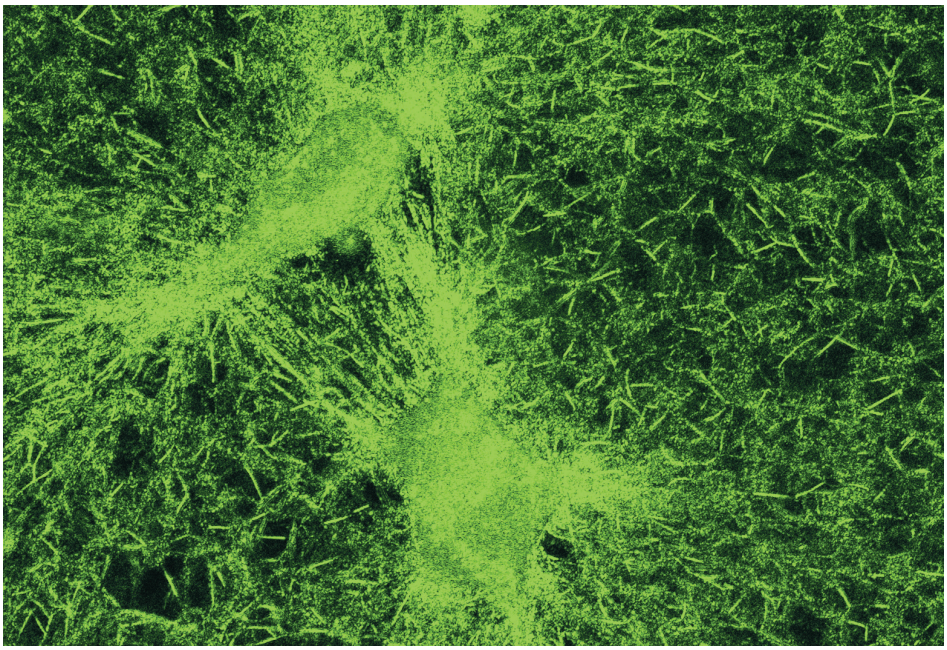
The Center's most widely attended educational event of the current academic year took place last November when its inaugural symposium attracted a combined internet and live audience of more than 200 people. Several symposium talks, including lectures by three invited speakers—Kevin White, Milan Mrksich, and Marcus Peter—have been uploaded to the video archives on the Center's website at www.psoc.northwestern.edu. These lectures are part of a growing video repository that has helped draw more than 1000 unique visitors per month to the Center's website during the first quarter of 2011.

Benette Phillips, PhD, is the Co-Director of Education and Outreach for the Northwestern PS-OC.

Cells Studied in 3-D May Reveal Novel Cancer Targets

By Mary Spiro

A study led by Johns Hopkins University Physical Sciences-Oncology Center researchers says cells studied in 3-D yield more accurate information that could help develop new drugs to prevent the spread of cancer.



Reflection confocal micrograph of collagen fibers in a 3-D matrix with cancer cells embedded. Image by Stephanie Fraley of the Wirtz lab at Johns Hopkins University.

"Finding out how cells move and stick to surfaces is critical to our understanding of cancer and other diseases, but most of what we know has been learned in the 2-D environment of Petri dishes," said Denis Wirtz, director of the Johns Hopkins PS-OC. "Our study demonstrates that how cells move inside a three-dimensional environment is fundamentally different from that seen in conventional flat lab dishes."

The study implies that results produced by common high-speed methods of screening drugs to prevent cell migration on flat substrates could be misleading, Wirtz said. "Our study identified possible targets to dramatically slow down cell invasion in a three-dimensional matrix." When cells are grown in two-dimensional environments, he added, they form long-lived focal adhesions on surfaces that can last several seconds to several minutes. The cell also develops a broad, fan-shaped lamella along its leading edges, which moves it forward. "Focal adhesions, if they exist at all, are so tiny and so short-lived they cannot be resolved with microscopy." Wirtz said.

The study's lead author, Stephanie Fraley, a Johns Hopkins doctoral student in Chemical and Biomolecular Engineering, said the shape and mode of movement for cells in 2-D are merely "artifacts of their environment" that could produce misleading results when testing the effect of different drugs.

"It is much more difficult to do 3-D cell culture than it is to do 2-D cell culture," Fraley said. "Typically, any kind of drug study that you do is conducted in 2-D cell cultures before it is carried over into animal models," she added. "Sometimes, drug study results don't resemble the outcomes of clinical studies. This may be one of the keys to understanding why things don't always match up."

Wirtz suggested part of the reason for the disconnect is that even in studies that are called "3-D," the top of the cells still protrude above the matrix. "Most of the work has

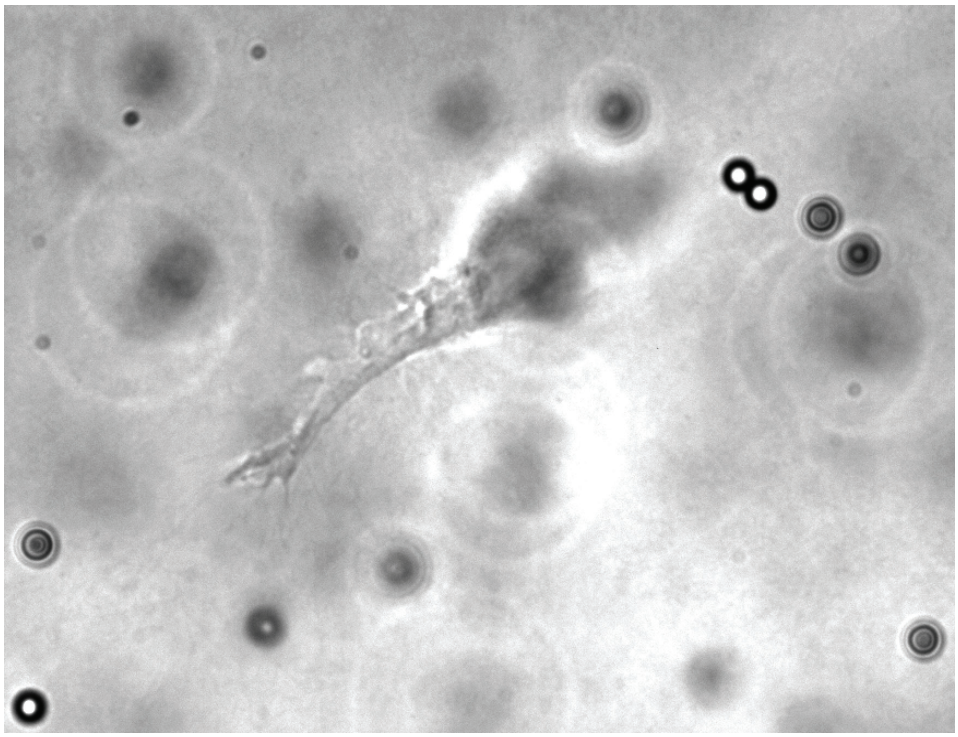
"Our study demonstrates that how cells move inside a three-dimensional environment is fundamentally different from that seen in conventional flat lab dishes."

been for cells only partially embedded in a matrix. We would call this a 2.5-D study," he said. "Our paper shows the fundamental difference between 3-D and 2.5-D: focal adhesions disappear, and the role of focal adhesion proteins in regulating cell motility changes."

Wirtz believes that "because loss of adhesion and enhanced cell movement are hallmarks of cancer," his team's findings should radically alter the way cells are cultured for drug studies. For example, the team found that in a 3-D environment, cells possessing the protein zyxin would move randomly, exploring their local environment. When the gene for zyxin was disabled, cells traveled in a rapid and persistent, almost one-dimensional, pathway far from their place of origin.

"It turns out that zyxin is misregulated in many cancers," Fraley said, therefore an understanding of the function of proteins like zyxin in a 3-D cell culture is critical to understanding how cancer spreads, or metastasizes.

"We think the same focal adhesion proteins identified in 2-D situations play a role in 3-D motility, but their role in 3-D is completely different and unknown," Wirtz said. "There is more we need to discover."



A high magnification image of an HT-1080 cell embedded in 3-D collagen. The cell is shown protruding toward beads in the focal plane. Image by Stephanie Fraley of the Wirtz lab at Johns Hopkins University.

Co-investigators on this study from Washington University in St. Louis included Gregory D. Longmore, a professor of medicine, and his postdoctoral fellow Yunfeng Feng, both of whom are affiliated with the university's BRIGHT Institute. Additional Johns Hopkins authors, all from the Department of Chemical and Biomolecular Engineering, were Alfredo Celedon, a recent doctoral degree recipient; Ranjini Krishnamurthy, a recent bachelor's degree recipient; and Dong-Hwee Kim, a current doctoral student.

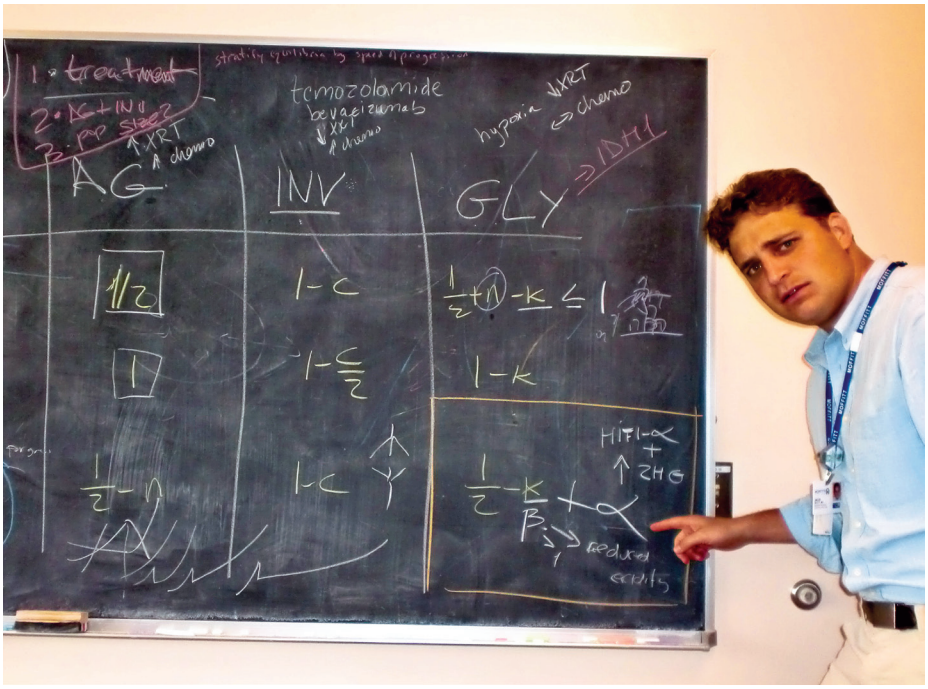
This study appeared in the June 2010 issue of *Nature Cell Biology*. A related video can be viewed on Youtube through the Johns Hopkins University Nanobiotechnology Channel: http://www.youtube.com/user/JHUINBT.

Mary Spiro is the Science Writer and Media Relations Director for the Johns Hopkins University Physical Sciences-Oncology Center.

Physicist and Physician: Mutually Exclusive Terms No More

By Jacob Scott

As a radiation oncologist in training, I have come to recognize metastatic disease as the bane of my existence. Once a patient has disseminated disease, my local therapies are largely useless when it comes to curative intent. So when I was looking for a research subject during my clinical residency, metastasis was an obvious choice. As I slowly began to turn my attention to this problem, I was surprised to find that our understanding of this process is not very robust—there is a lot of hand waving.



Jacob Scott (above), a resident physician at the Moffitt Cancer Center, and Chris McFarland, a graduate student at MIT, played a key role in forming a trans-network collaboration between their respective PS-OCs.

A physicist in a previous life, I thought that a mathematical model would be the best way to start deconvoluting such an untenable biological process as metastasis. It was my search to find a collaborator for this venture that led me to discover the Moffitt Cancer Center PS-OC, which is partially composed of a mathematical modeling group, Integrative Mathematical Oncology (IMO), headed by Sandy Anderson and Robert Gatenby. Even though I had no prior experience and was a clinician by training, I was enthusiastically welcomed into the group and invited to talk about my ideas. The early work that I performed with these collaborators culminated in the opportunity to present a poster at the inaugural PS-OC Investigators' Meeting. It was at this event that I met a grad student from the MIT PS-OC, Chris McFarland, who presented a beautiful model of primary tumor growth from a population genetics perspective. After meeting Chris and seeing his mentor, Leonid Mirny, present more of their work, we began to discuss how to bring their genotype-scale model of primary tumor growth to metastasis. Sadly for me and Chris, we met on the last day of the meeting, by which time all of the Young Investigator Trans-Network funds had been allocated. So, we did what any grad student and resident team would do—we worked at night and on the weekends.

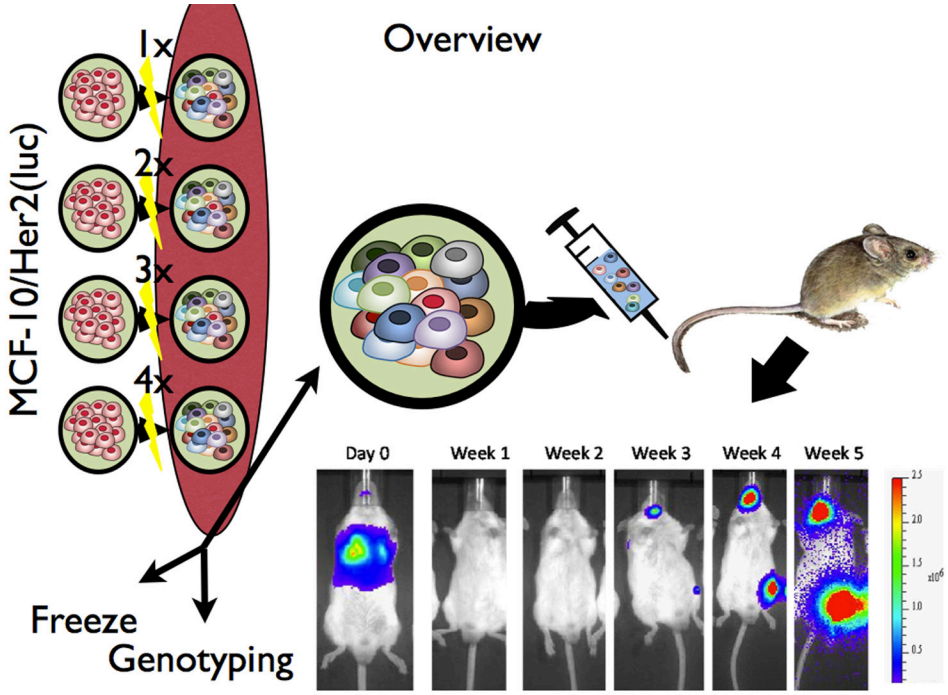
Modifying the MIT model of primary tumor growth with metastasis in mind, Chris and I—along with our mentors Leonid Mirny, Sandy Anderson and David Basanta—produced some interesting *in silico* results and a provocative hypothesis: higher mutational load leads to less metastatic fitness. While presenting these results at MIT, we developed an idea to test this hypothesis

"Mankind has been investigating this disease from a purely biological perspective for over a hundred years while making only marginal gains. The time to think differently has arrived."

experimentally by leveraging the strength of both PS-OCs—Moffitt's microenvironmental and evolutionary focus and MIT's expertise in deep sequencing and population genetics. We planned to impose extrinsic-graded mutagenic forces on identical cell lines to create clones with different numbers of mutations from a common starting point—like Galapagos sparrows—and then inject them into mice and see how many metastases formed. The mutations would be assayed and analyzed at MIT and the Broad Institute while metastatic fitness would be analyzed at Moffitt utilizing the Center's biological and imaging groups. The marriage of the two modeling groups—one a genotype shop (MIT) and the other a phenotype shop (Moffitt)—also gave us hopes of better defining the elusive genotype-to-phenotype map.

We hatched this plan without a moment to spare—just nine days before the Trans-Network grant application deadline. Testing the hypothesis that enough well placed energy can get the candle to burn in the middle—in addition to both ends—we wrote our proposal with David Morse, a Moffitt colleague who is interested in metastasis and *in vivo* imaging. Soon after, we found ourselves in Denver for the 40-hour grant gauntlet, and our fates—as well as our friendship—were sealed with the funding of our proposal.

This experience has not only forged a new collaboration, but it has also united two very disparate modeling groups who would otherwise have never worked together. The gamble the NCI is taking by bringing physical scientists into the realm of cancer research, has, for me, already paid off. Mankind has been investigating this disease from a purely biological perspective for over a hundred years while making only marginal gains. The time to think differently has arrived. And to think differently, we have to collaborate. Evolution doesn't happen if you just pollinate yourself.

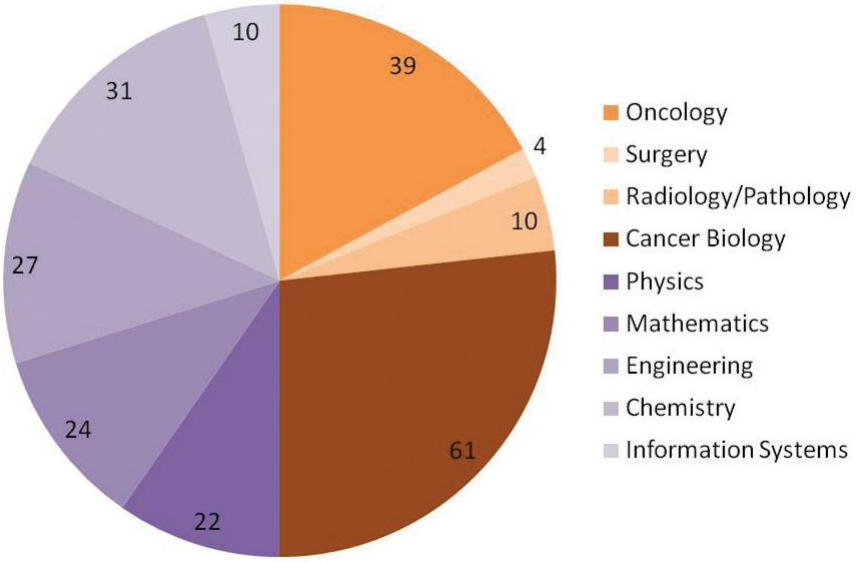


Overview of Moffitt-MIT Trans-Network Project. Differentially mutate a PS-OC cell line. Sequence the different resultant mutants. Assay these mutants for metastatic fitness.

Jacob Scott is a Resident Physician at Moffitt Cancer Center. He spends his free time working in Sandy Anderson and Robert Gatenby's Integrative Mathematical Oncology Group and plans to take time off clinical training to pursue a doctorate in Applied Mathematics at Oxford University next year.

A Snapshot of the PS-OC Network

By Karen Jo



The scientific diversity of the PS-OC Network is represented above. Drawing equally from the physical and biological sciences, the Network includes investigators from nine academic fields.

Seeking to cultivate new approaches to studying cancer, NCI established the PS-OC Network—which consists of twelve multidisciplinary research centers integrating concepts and methods from the physical sciences into the fields of cancer biology and oncology—in the fall of 2009. Following the Network's first anniversary, a great deal of useful information regarding its scientific diversity, training efforts, and unique Trans-Network initiative became available. Within the NCI Office of Physical Sciences-Oncology (OPSO), staff members have been diligently analyzing annual progress reports submitted by each of the PS-OCs to document the overall development of the Centers. Statistical measurements have been made regarding numerous reported data, and a comprehensive snapshot of the Network has emerged. The following overview will highlight some of these findings to illustrate the unique scientific environment and program elements characterizing the PS-OC enterprise.

SCIENTIFIC DIVERSITY

The PS-OC Network is designed to unite scientists from disparate disciplines so they can share knowledge and collaborate in developing new paradigms to investigate cancer. To highlight the depth of scientific knowledge in the Network, the OPSO compiled a list of investigators sorted by discipline. The results reveal that an enormously diverse group of investigators operates within the twelve PS-OCs. Of the investigators currently engaged in Network projects, roughly half identify themselves as physical scientists and half are self-identified as biological scientists. While the majority of the investigators trained in biological sciences are experts in cancer biology and oncology, the Network's physical scientists specialize primarily in the fields of engineering, mathematics, physics, and chemistry. All told, nine areas of specialization are represented within the PS-OCs.

TRAINEES

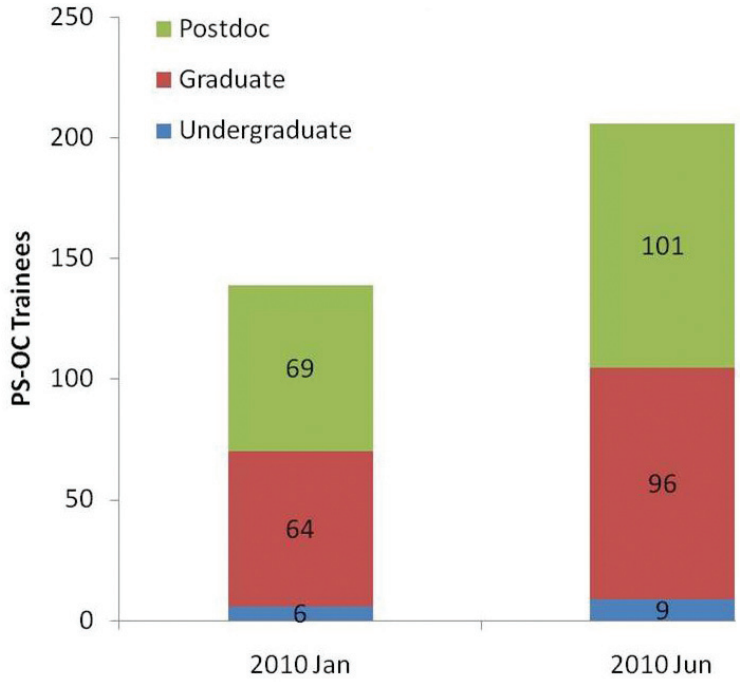
In 2004, the Committee on Facilitating Interdisciplinary Research within the National Academy of Science argued that "it is not the faculty who are in the best position to spur interdisciplinary research, but rather the 'lab rat' who is actually doing experiments." This statement supports the OPSO's belief that trainees will play a vital role in undertaking the interdisciplinary research that is essential to the Network's success. Providing them with out-

"An enormously diverse group of investigators operates within the Network. Its physical and biological scientists span a broad range of fields, including cancer biology, engineering, mathematics, physics, and chemistry."

standing interdisciplinary learning opportunities will thus become a priority for the Network as it matures. Trainee research areas and accomplishments are highlighted in Center progress reports and have been synthesized by the OPSO. Data from these reports show that the Network boasts a significant number of trainees, mostly postdoctoral fellows and graduate students, from both the physical and biological sciences. Most important, though, is the fact that this number dramatically increased from 139 to 206 in the Network's first year and is expected to continue rising. As the PS-OC trainee cohort expands, the OPSO will continue tracking its composition by discipline and institutional status while also monitoring career trajectories for departing students and postdocs.

TRANS-NETWORK PROGRAM

A unique element of the PS-OC program is its use of an annual funding competition to encourage the formation of multi-center teams seeking answers to fundamental questions within cancer research. Open to all network investigators, the competition encourages a progressive strengthening of proposals throughout a multi-stage application process. Prior to last summer's Trans-Network meeting in Denver, proposals from nine teams, each consisting of investigators from at least two PS-OCs, were posted online for the entire Network to help improve. At the meeting, each team presented updated proposals to five distinguished panelists. Following the presentations, teams again revised their proposals based on panelist comments prior to final submission the following day. It was noted by our staff that many presentations evolved substantially based on comments provided throughout the application process. After final review by panelists and the PS-OC Steering Committee, the Network funded the two highest-scoring proposals: "Development of Models of Penetration of Resistance" (Dana-Farber Cancer Institute, Princeton, and USC) and "Genotypic Determinants of Metastatic Fitness-a Delicate Balance of Passenger and Driver Mutations" (MIT and H. Lee Moffitt Cancer Center). Thanks to all who participated in last year's meeting. We look forward to another successful event at the April PI Meeting in La Jolla.



Distribution by institutional status of trainees participating in PS-OC research.

Karen Jo is a NIH/NCI Cancer Research Training Award (CRTA) Intern in the NCI Office of Physical Sciences-Oncology.

PS-OC Network Job Openings

Postdoctoral Fellowship, Dana-Farber Cancer Institute: Franziska Michor's lab at the Dana-Farber Cancer Institute in Boston has an opening for a postdoctoral fellow interested in Evolutionary Systems Biology of Cancer. Our lab investigates fundamental questions in cancer research by using mathematical, statistical, and computational techniques to approach them. In particular, we employ applied probability techniques, statistical and computational analyses, and dynamical systems. Applicants may have a background in applied mathematics, statistics, bioinformatics, or computational biology; an interest in cancer biology and experience in applied probability and statistics is necessary. Email michor@jimmy.harvard.edu for more information.

Postdoctoral Fellowship, Methodist Hospital Research Institute: A postdoctoral position is available in the Center for Transport Oncophysics PS-OC. A qualified candidate will be trained in the field of transport phenomena in tumor micro-environment and cancer nanotechnology. Experience in cell culture, mouse work, and conjugation chemistry is desired. Contact Biana Godin Vilentchouk (bgodin@tmhs.org) for more information.

Postdoctoral Fellowship, Stanford: Stanford University's Canary Center seeks a postdoctoral fellow with an interest in computational systems biology. The ideal candidate will be an energetic scientist/engineer with excellent communication skills and a background in the physical or biological sciences. Research will take place in a lab focused on the discovery and validation of molecular diagnostic markers and will concentrate on the computational modeling of cell state; modeling the processes by which tumor proteins are released from the tumor into the circulation; modeling the processes by which these proteins are maintained in the circulation; and testing the model against experimental results. Contact Pat Riley at periley@stanford.edu for more information.

Faculty Position, USC: The Center for Applied Molecular Medicine (CAMM) at USC has an opening for an assistant or associate professor (research or tenure line). CAMM seeks to discover and translate protein biomarkers indicative of a patient's likely response to existing therapies. The successful candidate for this position will help build the *in vitro* diagnostics component of the program. To be considered, applicants must have a PhD and significant experience in characterization of cancer therapeutic response using *in vitro* strategies (e.g., blood proteomics). Researchers in the areas of proteomics, novel *in vitro* sensor technologies, mouse models of cancer, systems biology, or bioinformatics related to cancer proteomics are particularly encouraged to apply. For a complete job description, please email hr@proteowizard.org.

Project Manager, USC: The USC PS-OC seeks a talented and energetic scientist/engineer with excellent communication skills to perform independent research and serve as a Program Manager (PM). A background in the physical or biological sciences is required. The PM will assist in the day-to-day operations of the center, facilitate communication, ensure that projects are on track for fulfilling milestones, and assist in preparing progress reports, site visit presentations, and funding agency communication. The ideal candidate will also leverage center funding by writing training grant proposals or proposals on research topics related to the center's goals. Senior candidates will be encouraged to develop an independent research program. For more details, email hr@proteowizard.org.

On the Cover

Figure 1: Confocal microscopic image of BCECF-labeled MDA-MB-231 breast cancer cells adherent to human umbilical vein. Endothelial cells were cultured to confluence within a 50 µm rectangular microfluidic channel in polydimethylsiloxane (a silicon-based polymer) and stained for focal adhesions (green), vascular endothelial-cadherin (red) and nuclei (blue). This image illustrates the feasibility of using endothelialized microfluidic channels of similar size on the *in vivo* microvasculature to study adhesive events between tumor cells and the endothelium *in vitro*. Image submitted by the Cornell University PS-OC.

Figure 2: Fibroblast imaged by Vito W. Rebecca, a cancer biology PhD student working in the laboratory of Dr. Keiran Smalley at the H. Lee Moffitt Cancer Research Center PS-OC. In the image, phalloidin is stained red; the nuclei are stained blue; and fibronectin is stained yellow.

Figure 3: This image shows an agressive, metastatic breast cancer cell (MDA-MB-231) exhibiting multilobular nuclear morphology with highly irregular nuclear membrane. The nuclear surface is colored blue and the cytoplasm gray. Image submitted by Vivek Nandakumar, Laimonas Kelbauskas, Roger Johnson, and Deidre Meldrum of the Arizona State University PS-OC and the Arizona State University Center for Biosignatures Discovery Automation.

Figure 4: High resolution imaging on a Zeiss LSM710 confocal microscope confirms association between circulating tumor cells (stained), white blood cells, and platelet-like structures (both unstained). Image submitted by the Scripps Research Institute PS-OC.

PS-OC Perspectives is edited by Anna Calcagno, Nicole Moore, and Will Kazmier. • The editors thank the PS-OC Outreach and Dissemination Working Group for their contributions to this newsletter. • This publication and the work included herein were supported primarily by the NCI Physical Sciences in Oncology initiative. • Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect those of the National Cancer Institute.