National Cancer Institute

PHYSICAL SCIENCES in ONCOLOGY

PERSPECTIVES

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

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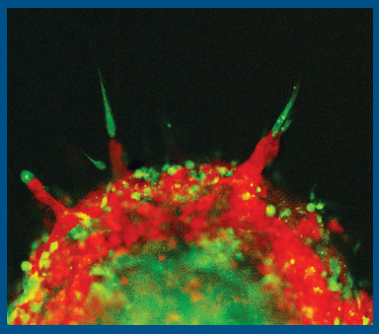
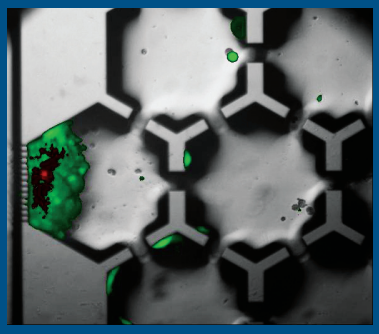
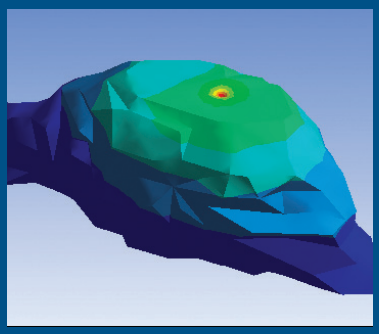
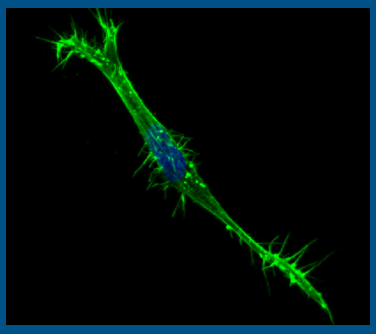
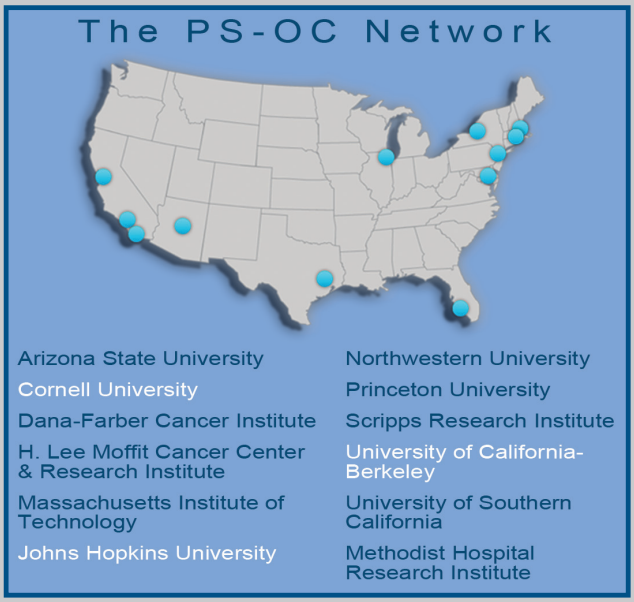








NCI Perspective: Mechanics of Cancer



During metastasis, cancer cells encounter several obstacles such as negotiating extracellular matrices, invading surrounding cells and tissues, and traversing in/out of vasculature and lymphatic vessels. To overcome these barriers, cancer cells undergo transitions that perturb cell processes such as surface receptor expression, cytoskeleton reorganization, and directional polarity, which ultimately contribute to enhanced cell migration/motility. These problems represent major barriers to progress against cancer – but represent areas of major strength in the physical sciences. Physicists and engineers are major resources for sophisticated investigation of cell migration and to define the magnitude of the extreme compressive and tangential forces that these cells encounter. Being able to detect mechanical changes will allow for new understanding of the disease and the development of novel therapeutic solutions targeted specifically at mechanical biomarkers of cancer cell mechanics.

The Physical Sciences – Oncology Center (PS-OC) program funds teams of trans-disciplinary scientists to facilitate the convergence of physical sciences with cancer research and bring a fresh perspective to cancer research. This issue of *PS-OC Perspectives* highlights the use of advanced tools and approaches from the physical sciences that expand the understanding of the role of mechanics in cancer at all length scales. The issue emphasizes the team science that is abundant within each Center and across the PS-OC Network and how these connections are leading to innovation.

Elucidating the Function of Isocitrate Dehydrogenase Mutations in Aberrant Epigenetic Programming

By Ariosto S. Silva and Robert H. Austin

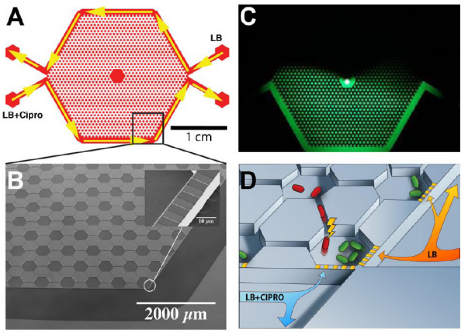
Multiple Myeloma (MM) is an incurable disease in which malignant plasma cells proliferate uncontrollably in the bone marrow (BM) causing debilitating symptoms like dysfunctional hematopoiesis, bone erosion leading to fractures, and renal failure. Most patients initially respond well to therapy; however, they inevitably become refractory to treatment, resulting in relapse and death. Thus, the fast rate of evolution of drug resistance is the major cause of treatment failure[1].

The bone marrow has an unevenly distributed vascularization, with hypoxic regions believed to be niches for hematopoietic stem cells[2] and vessel-rich sinusoids which facilitate the traffic of cells to and from the bloodstream. Irregular vasculature also generates uneven chemotherapy distribution during treatment, and these gradients may be a major factor for minimal residual disease by sparing part of the MM population exposed to sub-lethal drug concentrations.

Our previous work with bacteria (figure at right, this page) showed that exposure to gradients of DNA-damaging agents accelerates the evolution of drug resistant mutants to ciprofloxacin by increasing the mutation rate by four orders of magnitude. We hypothesize that the same may be true in the myelomatous bone marrow, where the irregular tumor vasculature creates gradients of chemotherapy perfusion and hypoxia[3], both having DNA-damaging effects.

In bacteria, this accelerated evolution occurs in regions where the drug concentrations are not highly cytotoxic, but sufficient to accelerate the mutation rate by inducing sub-lethal DNA damage. We call these regions "goldilocks", and they might be key for the accelerated evolution of drug resistance in MM.

In this project, we study the evolution of drug resistance in MM cell lines and MM patient cells, under stable gradients of DNA damaging agents (Doxorubicin and Melphalan), as well as hypoxia, in microfluidics chips designed in the Princeton PS-OC. These chips, dubbed "Death Galaxy", are composed of hundreds of microhabitats, which are interconnected chambers, fitting approximately 100 mammalian cells each, and allowing cell traffic and diffusion. A stable gradient of soluble factors, like chemotherapy, is established from one extreme to the other of the chip, providing a tool to study the evolution of drug resistance in a heterogeneous microenvironment.



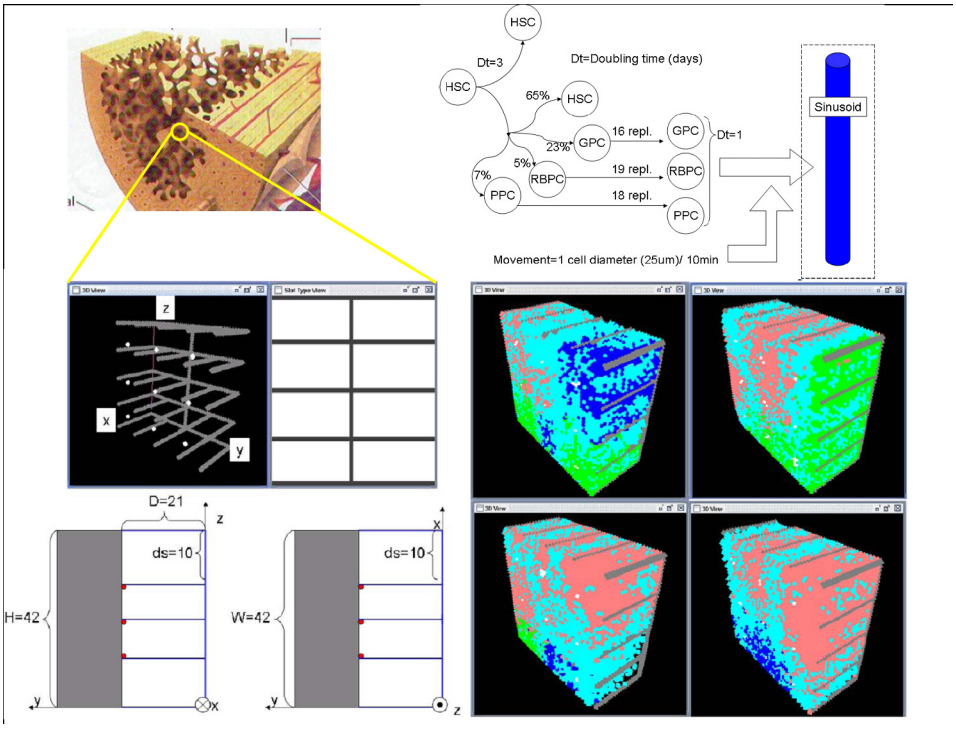
(A) An overview of the entire micro-ecology, showing the flow of the nutrient streams and the nutrient+cytotoxin containing streams. (B) SEM image of the area of the array in (A) outlined by the box. (C) Image of the expected cytotoxin concentration using the dye fluorescein as a marker. (D) The design of our micro-ecology creates high population gradients using constriction of nutrient flow via nanoslits in the presence of a cytotoxin gradient.

We use fluorescent live microscopy to quantify cell death in MM cell lines. This approach allows us to build drug dose response curves in both concentration and duration of exposure, unlike classic assays which vary drug concentration but not exposure time. The cells recovered from the microfluidics chip at the end of the experiment will be analyzed for DNA sequencing to quantify the mutation rate.

"Our agent-based spatial computational model incorporates the geometry of the marrow's bone and vessels, healthy stroma and myelomatous cells, and can be used to simulate the free growth or response to therapy."

We expect to observe both faster emergence of drug resistant clones and an increased heterogeneity in this resistant population, as compared to classical methods of selection of drug resistant cells. This heterogeneous population thus will have higher chances of being cross-resistant to other drugs, which also has an important clinical impact, as cross-resistance to drugs is a major cause of fatality in cancer[4].

The data from dose response of the initial and selected population of patient cells, and their rate of mutation will be incorporated in the computational model of the bone marrow (Virtual Bone Marrow[5]) developed in the Moffitt PS-OC (figure at left). Our agent-based spatial computational model incorporates the geometry of the marrow's bone and vessels, healthy stroma and myelomatous cells, and can be used to simulate the free growth or response to therapy. It will be deployed to estimate the rate of evolution of drug resistance and tumor re-growth in patients, based on the *in vitro* data.



The red marrow in an adult is mostly concentrated in the trabecular bone, which is a sieve-like structure permeated by a network of branching sinusoids. In our spatial computational model we consider each of these sub-units independently, consisting of ten hematopoietic stem cells with different probabilities of asymmetric and symmetric division. Upon each asymmetric division, there is a different probability of a daughter cell being from one of the three main blood cell lineages (granulocytes, erythrocytes and megakaryocytes). Simulations of these subunits yield to always different distribution of number of cells for each lineage, but overall the entire body marrow produces a stable number of cells for the three lineages.

References

(1) Meads, M. B.; Hazlehurst, L. A.; Dalton, W. S. *Clin Cancer Res* 2008, 14, 2519.

(2) Parmar, K.; Mauch, P.; Vergilio, J. A.; Sackstein, R.; Down, J. D. *PNAS* 2007, 104, 5431.

(3) Wilson, W. R.; Hay, M. P. *Nature Reviews*, 11, 393.

(4) Blade, J.; Feliu, E.; Rozman, C.; Estape, J.; Milla, A.; Montserrat, E. *Cancer* 1983, 52, 786.

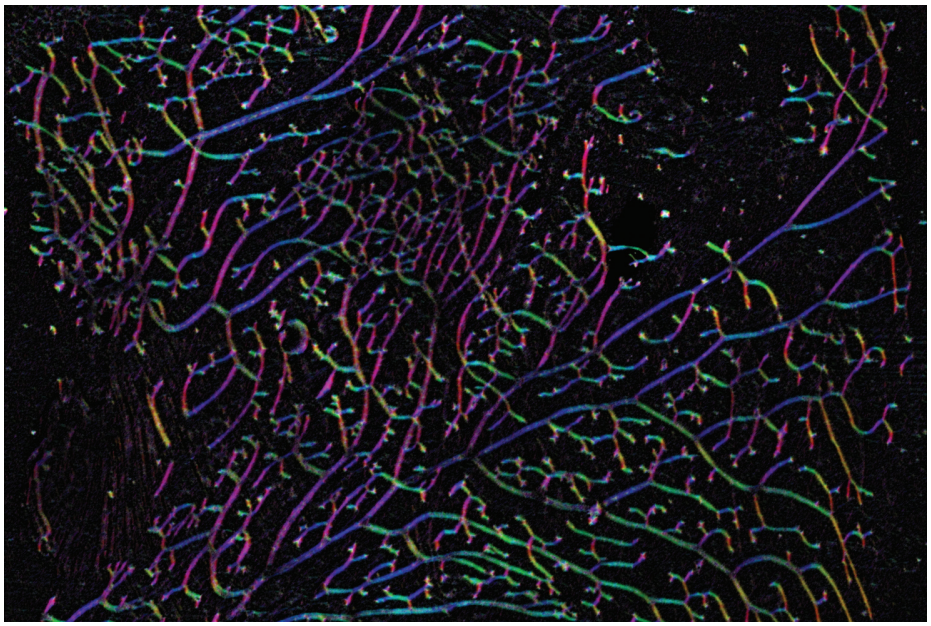
(5) Silva, A.; Anderson, A. R.; Gatenby, R. *Math Biosci Eng*, 8, 643.

Ariosto Silva is a research scientist at H. Lee Moffitt Cancer Center PS-OC and Robert Austin is the Principal Investigator for the Princeton PS-OC.

The Mechanobiology of Tumor Progression: The Bay Area PS-OC

By Saheli Datta

Ancient Egyptians noticed tumors were often harder than surrounding tissue; the Greek word for "hard mass" is the root of oncology. The Bay Area PS-OC focuses on the mechanobiology of tumor progression, emphasizing breast cancer. Math, electrical and bioengineering, physics, chemistry & biophysics at UC Berkeley intertwine with life sciences at LBNL and bioengineering and oncology at UCSF. Most of our labs are already intrinsically interdisciplinary. We aim to exploit existing connections in the Bay Area and to foster new collaborations.



Mouse mammary gland with nuclei stained green and collagen stained red. The mouse lacks the gene for collagen fiber formation, changing the way the gland mechanosenses the ECM. (Credit: Bissell Lab)

The Bay Area PS-OC personnel focus on understanding how tumor progression and plasticity are affected by mechanics (stress, strain, pressure, viscosity, stiffness, rotation, tension, shearing, and mechanical transduction) and physical organization (membrane-protein arrangements, cell-to-cell contacts, multicellular structure orientation, extra-cellular matrix composition, collagen density), while searching for potential mechanics-directed clinical interventions.

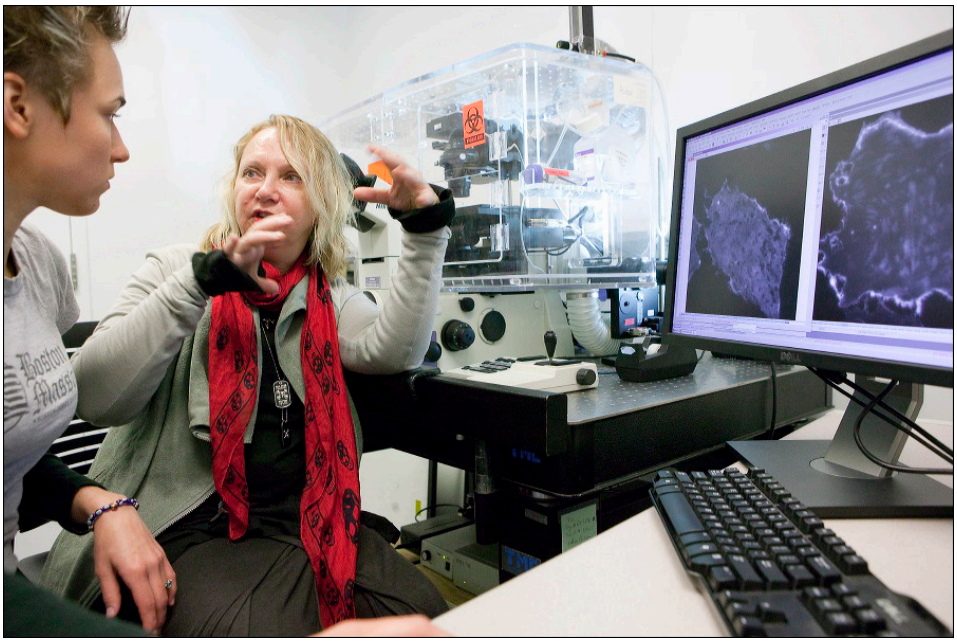
Projects are often described by length scale, interrogation method, and biological system. Length scales include sub-cellular and cellular, multicellular, and tissue scales. Interrogation methods include optical microscopies such as fluorescence, second harmonic generation, and super-resolution microscopy. Manipulation and control methods include atomic force microscopy and patterned membrane restricting substrates. Systems include traditionally cultured mammalian and human cancer cell lines, 3-D cultured cells and acini, and mouse and human breast tissue. Modeling and theoretical work is being conducted at the genomic, cellular and multicellular levels. While individual projects tend to focus on specific parts of the length-system-method-discipline parameter space, together the Center's work is designed to merge and provide an integrated view of both tumor development and progression.

Significant effort is directed towards experimental systems that reflect the importance of the extra-cellular microenvironment and the physical microenvironment of the cells. Ongoing collaborations have made it simpler for physical scientists to work with clinically relevant tissue samples. Joe Gray advises the center on 'omics' and corralling information.

"We are increasingly convinced that a mechanics-centric view of cancer can generate distinct and powerful new detection and intervention strategies, certainly for breast cancer and perhaps also for other malignancies."

Research Projects

Formally, the Center's research efforts are divided into three Projects. Project 1 is "Fundamental Mecha-no-Chemical Signaling of Cancer," headed by Jay Groves and including Frank McCormick and Clair Tomlin. Project 2 is "Mechanobiolgy of Acinar Stability," headed by Valerie Weaver and including Hana El-Samad, Sanjay Kumar, and Gerard Marriott. Project 3 is "Dynamics in the Tissue State-Space: From Normal to Tumor and Back," headed by Jan Liphardt and including Mina Bissell, James Sethian, Dan Fletcher and Sylvain Costes.



Valerie Weaver, leader of project 2 for the Bay Area PS-OC, discusses an experiment with one of her graduate students.

Project 1: Project 1 focuses on how the geometric organization of proteins on the cell surface affects oncologic phenomena. Jay Groves has a special system for studying the physical chemistry and organization of cell-cell interactions mediated by membrane proteins. His group grows self-assembling lipid bilayers on an inorganic substrate that allows microscopy from directly beneath the bilayers, which in turn include key signaling membrane proteins. Cultured cells placed on the bilayers interact with the proteins, stimulating reorganization of signaling molecules that can be tracked by the microscope below the surface membrane. Clair Tomlin has been working on building a computational model of these dynamics, and the McCormick lab has been working with Xiaolin Nan to apply a new technique, photo-activated localization microscopy (PALM), to document and analyze the distribution and density of Ras signaling proteins at a resolution of ~20 nm, and has created experimental systems to test the relationship between Ras cluster size and downstream signaling.

Project 2: This project studies how force and structure control spatial organization of signaling complexes to regulate tissue organization and tumorigenesis. Valerie Weaver's group investigates how intrinsic cell force and the physical parameters of the extra-cellular matrix impact the structural organization of integrin adhesion complexes to regulate epithelial tissue structure/function. Leading actors in their studies are acini, the small raspberry-like balls of cells which are the cultured equivalents of the breast alveoli, the basic milk-producing units in the breast and the starting point for many tumors. The Weaver lab has been building *in silico* and experimental models to understand how intrinsic and extrinsic force promotes tumor development. For example, they have a novel fluorescence interference approach to

"The Weaver lab has been building models to understand how intrinsic and extrinsic force promotes tumor development...The group has been able to visualize tumor cell invasion within three-dimensional matrices and tissues."

studying the role of the glycocalyx, a network of mechanically responsive glyco-proteins surrounding the acinar cells. By combining imaging modes across multiple length scales, the Weaver group has visualized tumor cell invasion within 3-D matrices and tissues. They have recently created high resolution maps of the surface topology of acinar structures in different mechanical environments. The El-Samad and Weaver groups have developed ways to control the force microenvironment experienced by acini, documenting its mechanical reactions. Meanwhile, Sanjay Kumar's team has been using laser microablation to selectively 'snip' actin fibers in cells within acini, using the recoil to map tension under different conditions. Kumar used pharmacological agents to study links between acinar mechanics and cytoskeletal remodeling enzymes such as the Rho-activated Kinase, that regulates acinar behavior. Marriott's group in turn developed novel polymer systems to stimulate conditions in the extra-cellular matrix by controlling stiffness with light and has collaborated with Kumar to apply these novel materials to study the mechanoresponsiveness of tumor cells.

Project 3: Project 3 is specifically focused on reversion, ways in which the physical environment can influence cells that seem to have embarked on the path to cancer to revert to a benign phenotype. Project 3 is closely related to Project 2. For instance, the Weaver team collaborates extensively with the Liphardt lab, and much of the work in the Bissell lab is undertaken by a postdoc trained in Kumar's group. As in project 2, acini are currently the main experimental and theoretical unit of discussion. The Bissell group has been using high-resolution imaging to watch mammalian epithelial cells form ordered hollow-lumen acini, and analysis has revealed that healthy cells use coherent rotations of groups of cells to order themselves; this ordering mechanism is lost in malignant structures and regained under reversion. The mathematicians of the Sethian group have been building physical models of this process. The Fletcher lab and the Liphardt lab are developing novel microscopy and combinations of optical imaging and active manipulation to interrogate these reversion systems. The major direction of these efforts is to be able to simultaneously manipulate and monitor groups of cells as they develop, disorganize, and, sometimes, revert.

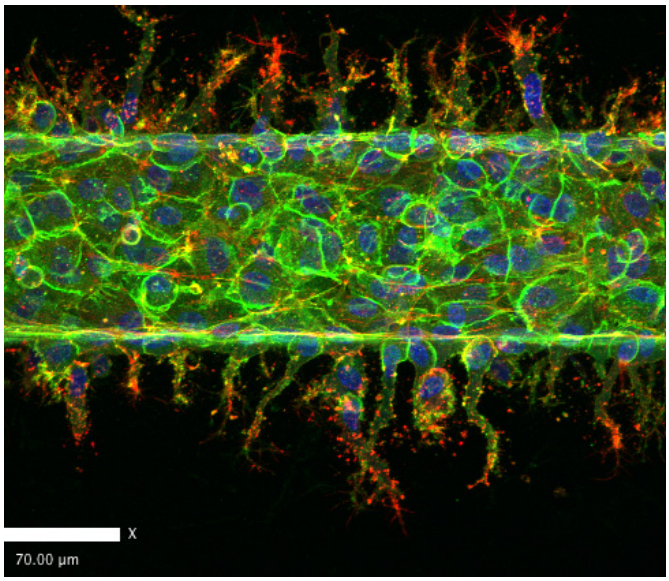
Dr. Daniel Fletcher (right), an investigator for Project 3, leads a recent imaging workshop sponsored by the Bay Area PS-OC.

Saheli Datta is a member of the Outreach and Dissemination Unit for the Bay Area PS-OC.

The Center on Microenvironment and Metastasis: The Cornell University PS-OC

By Teresa Porri

The Cornell Center on the Microenvironment and Metastasis is organized both to de-convolve cancer's complexity and to understand the interaction of mechanical forces and chemical cues in cancer metastasis. We use experimental and theoretical approaches, derived from the physical sciences, to address major questions and barriers in our understanding and treatment of cancer.



Confocal image of a portion of an in-vitro model blood vessel used to study angiogenesis and the effectiveness of anti-angiogenic cancer therapy. Actin (red), CD-31 (green) and nuclei (blue), with sprouting angiogenesis visible as a result of culture under pro-angiogenic conditions.

Research Projects

Project 1: This project, led by Claudia Fishbach-Teschi and Vivek Mittal, integrates physical sciences and cancer biology approaches to enhance understanding of the cellular and molecular mechanisms that underlie tumor vascularization. This process is largely mediated by the microenvironmentally-controlled angiogenic potential of tumor cells and figures prominently in the growth of primary tumors and cancer metastasis. Our current understanding of tumor vascularization indicates that blood vessel invasion is stimulated by hypoxia in growing tumors. Hypoxia acts to induce pro-angiogenic factors, and, finally, endothelial cell sprouting from nearby blood vessels. However, new experimental evidence indicates that bone marrow-derived endothelial (EPCs) and hematopoietic progenitor cells (HPCs) play an important role in this process. These bone marrow cells contribute significantly to tumor neo-angiogenesis and selective targeting to impair their recruitment to and incorporation within the developing tumor vasculature has broad implications for the development of antiangiogenic cancer therapies. However, therapeutic modalities targeting the tumor vasculature have relied primarily on a "candidate gene" approach; our proposal, to identify in an unbiased manner extracellular mediators and intracellular signaling pathways of bone marrow cell recruitment represents a major advance.

Recent Publications:

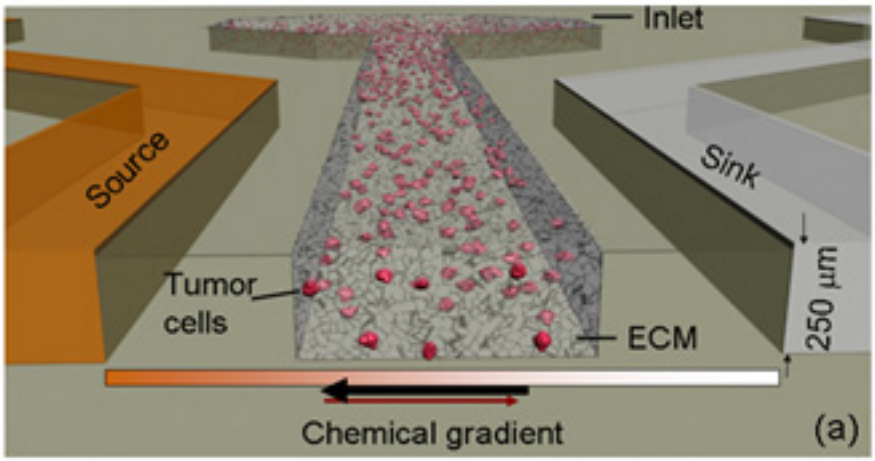
E M Chandler et al. *Physical Biology*, 2011, Feb;8(1):015008. Epub 2011 Feb 7.

Nayak S, Siddiqui J and J. Varner (2011) *IET Syst. Biol.* 5:2-14.

Cross VL, et al. *Biomaterials*, 2010 Nov;31(33):8596-607.

Project 2: Headed by Cynthia Reinhart-King and Paraskevi Giannakakou, this project focuses on understanding how chemical and mechanical forces in the tu-

"The Cornell Center on the Microenvironment and Metastasis is organized both to de-convolve cancer's complexity and to understand the interaction of mechanical forces and chemical cues in cancer metastasis."



Project 2 Microfluidic device which creates stable chemotactic gradients in 3-D matrices for the study of cell migration used in Project 2.

mor microenvironment enable and enhance cell migration during metastasis. Migration is a fundamentally physical process characterized by the coordinated movement of cells through the matrix, dominated by chemical and mechanical gradients within the microenvironment. We bring to bear a multidisciplinary approach to exert exacting control over the mechanics and chemistry of the microenvironment to study cancer cell migration as a function of chemical gradients and matrix stiffness mimicking the tumor environment. We propose to utilize our strengths in micro- and nanofabrication and high-resolution 3-D imaging to create tumor-mimicking microenvironments and monitor cell migration in real time. Our results will answer the questions: (i) What are the key extracellular physicochemical attributes that contribute to aggressiveness and tumor cell migration? (ii) Does the tumor microenvironment contribute to decreased cell-sensitivity to chemotherapeutics during relapse?

Recent publications:

Kraning-Rush, C.M. et al. *Physical Biology*, 2011, 8(1):012009.

Mak, M., C.A. Reinhart-King and D. Erickson, *Plos One*, 2011, 6(6):e20825.

Project 3: Operating under the leadership of Michael King and David Nanus, this project focuses on understanding the fundamental physical mechanisms of circulating tumor cell adhesion to inflamed endothelium under flow, as a critical step in the hematologic spread of metastatic cancers. This complex problem is inherently multidisciplinary and its investigation requires expertise in fluid physics across multiple length and time scales as well as a quantitative understanding of chemical reaction kinetics that are strongly coupled to externally applied force. At Cornell University, microscale fluid mechanics and advanced optical microscopy have been applied to develop powerful enabling technologies for the study of the molecular and cellular basis of metastatic cancer, while at Weill Medical College and SUNY Buffalo the biological components and their behavior can be studied in detail in the context of animal studies or clinical phenotypes.

Recent Publications:

Geng Y, et al. *Cell Molec. Bioeng.* 2010 Dec; 3(4):361-8.

Yin X, et al. *Carbohydr. Res.* 2010 Nov 2; 345(16):2334-42.

Research Cores

The Microfabrication Core is composed of the Cornell Nanobiotechnology Center and Nanoscale Science and Technology Facility. The facilities integrate nano- and microfabrication capabilities with biochemical processing and analysis

"Our multidisciplinary approach exerts control over the mechanics and chemistry of the microenvironment to study cancer cell migration as a function of chemical gradients and matrix stiffness mimicking the tumor environment."

tools in an environment designed to enhance interdisciplinary research. Facility staff members offer extensive support in problem solving as well as training in the use of tools and equipment.

The Epigenomics Core develops processes and devices to rapidly evaluate the molecular state of a few selected cells and work toward analysis of individual cells. The Core will enable scientists to understand the distribution of properties and to then consider the consequences of this heterogeneity in the context of various microenvironments.

Education and Training

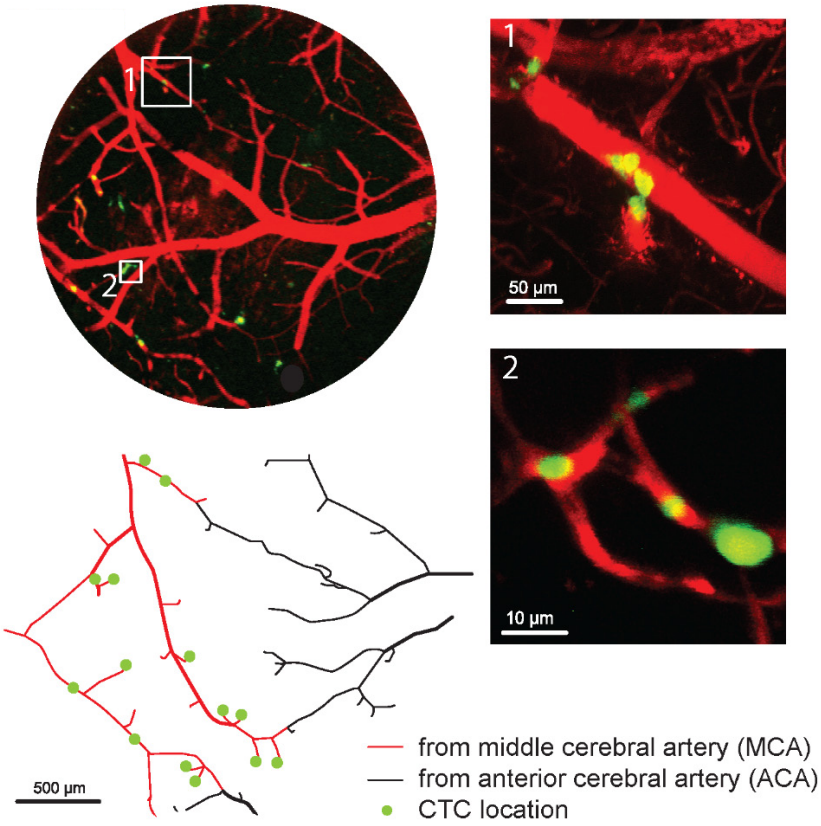
The goals for our education and training program include enhancing the process through which graduate students, postdocs, and medical scientists are trained at the interface of physics, engineering, and cancer biology; providing education and training modules that integrate the latest research discoveries of the Center and meet the changing needs of students and medical scientists; and disseminating the integrative training modules of the PS-OC.

The Education Unit's Cancer Brainstorming Series is a discussion group designed to promote creativity and critical thinking among early-stage scientists while fostering dialogue across the physical sciences and biology. Our nano-biotechnology course is webcasted to multiple sites and new participants are welcome.

Outreach and Dissemination

Our Center's Outreach and Dissemination Unit provides critical links to the external research community by making knowledge, inventions, and new capabilities created by our research accessible across the PS-OC network and in the broader research community. The unit provides these links through its seminar series, annual symposium, research meetings, and training provided in conjunction with the microfabrication core.

Teresa Porri leads the Outreach and Dissemination Unit for the Cornell University PS-OC.

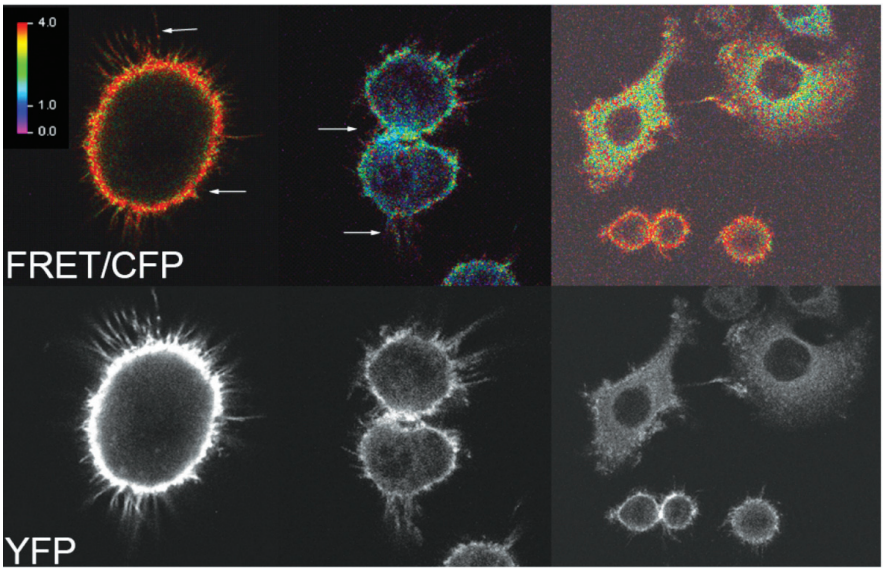


In vivo imaging of circulating tumor cell arrest in rodent brain. Multiphoton imaging through a cranial window of intra-arterially injected tumor cells (green) shows that cancer cells arrest in arterioles (inset 1) and capillaries (inset 2).

Probing the Forces that Cause Metastasis: The Johns Hopkins University PS-OC

By Mary Spiro

The Johns Hopkins Physical Sciences-Oncology Center aims to unravel the physical underpinnings driving the growth and spread of cancer through the study of cellular and molecular dynamics and physical forces. The PS-OC is sometimes referred to as the Johns Hopkins Engineering in Oncology Center.



Enhanced activated RhoA at the mitotic cell cortex of a live cancer cell as measured using a genetically encoded FRET pair. Credit: Prashant Mali (Searson/Wirtz labs)

Denis Wirtz, PhD, the Theophilus H. Smoot Professor of Chemical and Biomolecular Engineering in the Whiting School of Engineering, directs the Center. The Johns Hopkins' PS-OC draws upon the expertise of 11 Hopkins researchers from varied disciplines to study the role of physical forces involved in the development and spread of cancer.

Gregg L. Semenza, MD, PhD, is the Center's associate director. Semenza is affiliated with the Johns Hopkins School of Medicine departments of Pediatrics, Medicine, Oncology and Radiation Oncology, and the McKusick-Nathans Institute of Genetic Medicine. He is the C. Michael Armstrong Professor in Medicine and founding director of the Vascular Program at the school's Institute for Cell Engineering.

Partners include Greg D. Longmore, a cancer cell biologist at Washington University in St. Louis; Timothy C. Elston, a theoretical and computational bio-physicist at the University of North Carolina, Chapel Hill; Yiider Tseng, an experimental biophysicist and biochemist at the University of Florida; and Charles W. Wolgemuth, a theoretical and computational biophysicist at the University of Connecticut.

Research Projects

Project 1: Semenza and Sharon Gerecht, an assistant professor of Chemical and Biomolecular Engineering, lead a project focused on analyzing the makeup and physical properties of the extracellular matrix, the three-dimensional (3-D) scaffold in which cells live.

"Normal cells live in a flexible scaffold, but cancer cells create a rigid scaffold that they climb through to invade normal tissue," Semenza said. "We study how this change occurs and how it is affected by the amount of oxygen to which cancer cells are exposed."

Project 2: Wirtz and Longmore study the physical basis for cancer cell adhesion and de-adhesion and how it increases the likelihood that cancer cells will break free, move into the bloodstream and migrate to other tissues.

"Mechanical forces inside the body,

"The Johns Hopkins University PS-OC aims to unravel the physical underpinnings driving the growth and spread of cancer through the study of cellular and molecular dynamics and physical forces."

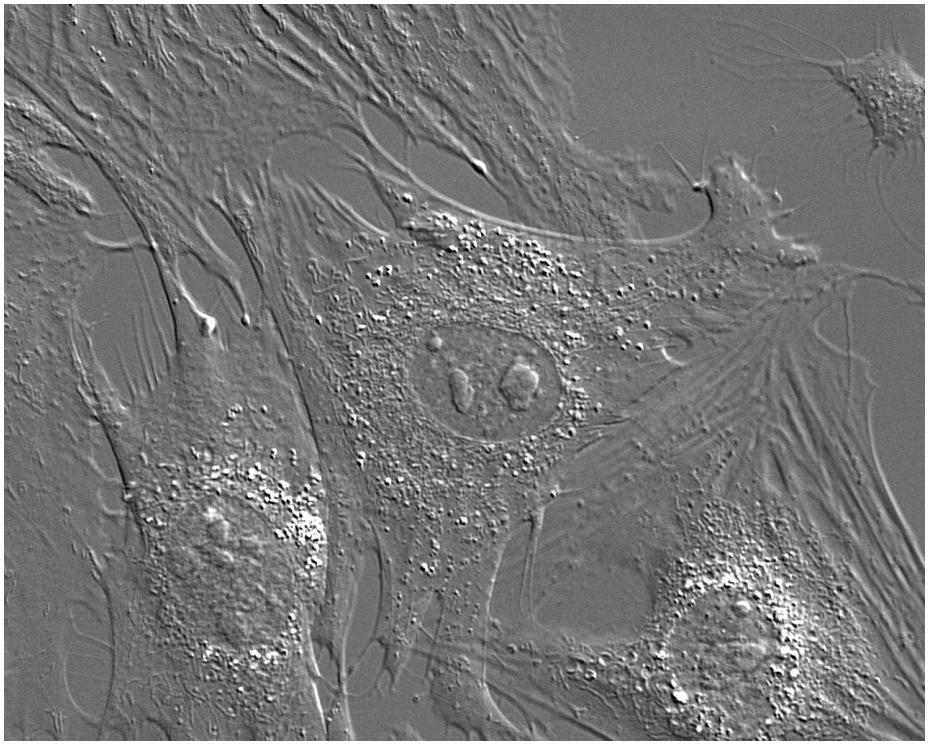
such as shear exerted by blood flowing through blood vessels, typically destroy the millions of cancer cells that are constantly shed from tumors." Wirtz said. "But the 'fittest' of cancer cells survive these Darwinian-like selective pressures and may become the culprits that spread cancer."

Project 3: Konstantinos Konstantopoulos, professor and chair of the Whiting School's Department of Chemical and Biomolecular Engineering, is working with Martin L. Pomper, who holds appointments in the School of Medicine's Department of Radiology and the Sidney Kimmel Comprehensive Cancer Center. They are investigating the effects of fluid mechanical forces at different oxygen tension microenvironments on tumor cell signaling, adhesion and migration.

Research Cores

Imaging Core: Through the Integrated Imaging Center, J. Michael McCaffery, associate research professor of biology in the Krieger School of Arts and Sciences, over-sees the Imaging Core and facilitates imaging resources for PS-OC faculty. The Imaging Core has capabilities in epifluorescence/atomic force microscopy; ultra-fast/sensitive dynamic live-cell imaging; total internal reflectance fluorescence microscopy; confocal/multiphoton microscopy; fluorescence correlation spectroscopy; multimode scanning electron microscopy; high-vacuum, low-vacuum, and environmental-SEM; conventional and high-resolution analytical transmission electron microscopy; fluorescence-activated cell sorting and analysis.

Microfabrication Core: Supervised by Peter Searson, the Joseph R. and Lynn C. Reynolds Professor of Engineering in the Department of Material Science and Engineering, the Microfabrication Core possesses a spin coater, UV lab, e-beam evaporator, mask aligner and wet chemical equipment for photolithography. The core has capabilities for microcontact printing, fabrication of microfluidic devices, and surface modification and engineering. It contains a 900 square foot nanoparticle synthesis and characterization laboratory and a 370 sq-ft Class 10,000 clean room.



Mouse embryonic fibroblasts in culture as imaged by differential interference contrast (DIC) microscopy. Credit Shyam B. Khatau (Wirtz Lab).

"Normal cells live in a flexible scaffold, but cancer cells create a rigid scaffold that they climb through to invade normal tissue. We study how this change occurs."

Education and Outreach

Johns Hopkins PS-OC has a multidisciplinary training program for predoctoral students and postdoctoral fellows. The training program is co-directed by Searson and the School of Medicine's Kenneth W. Kinzler, who ranks among the world's most-cited cancer biologists and serves as co-director of the Johns Hopkins Ludwig Center. Training and collaboration with investigators located at the four other research universities on the grant will occur through periodic onsite visits and via Web-based platforms.

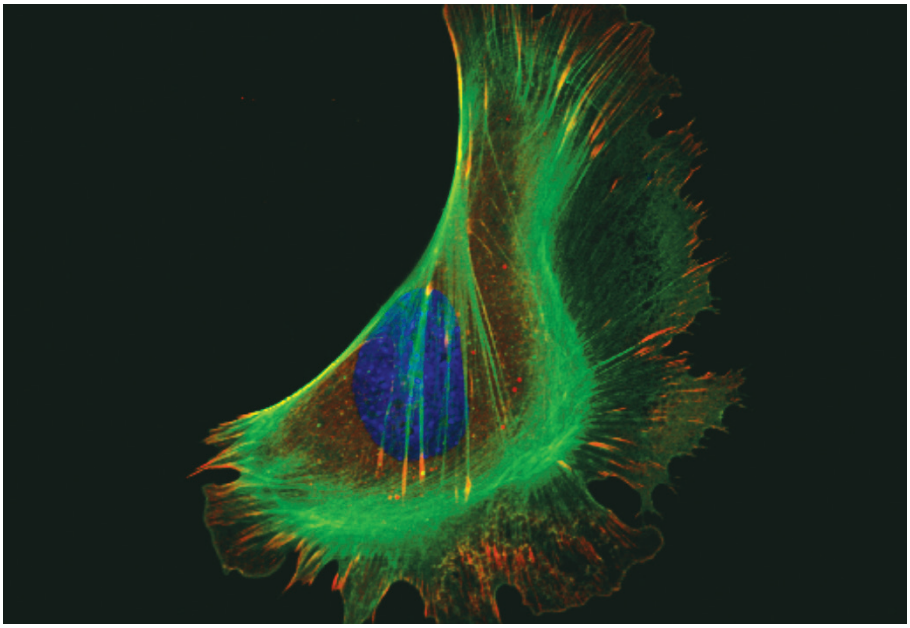
Future Directions

Gerecht Lab researchers are seeking to improve formation and ingrowth of blood vessels on polysaccharide-based hydrogel scaffolds commonly used therapeutically for the recovery of ischemic or wounded tissues. Decreasing the crosslinking density in the hydrogel resulted in reduced rigidity, increased swelling, and increased vascular endothelial GF (VEGF) release capability of the hydrogel structure. Decreasing crosslinking density also facilitated rapid hydrogel disintegration and tissue ingrowth.

The Konstantopoulos group has shown how hemodynamic forces affect receptor-ligand interactions related to the process of allowing tumor cells to roll and attach to blood vessel walls and eventually metastasize. They are studying how certain receptor-ligand pair combinations initiate transient binding.

The Wirtz lab compared micromechanical properties of human induced pluripotent stem cells (hiPS) with those of human embryonic stem cells (hES). Using a method they developed for particle tracking micororheology, they found fundamentally different viscoelastic properties of the cytoplasm between the hES and the hiPS. Wirtz and Searson also studied cortical retraction scenarios following mitosis. Following cortical retraction of high-motility cells (typically diseased cells), the resulting daughter cells lose actin guides from the original mother cell and can spread to new locations. Activation of RhoA resulted in kinase inhibition leading to greater reoccupation of the mother cells space, whereas RasV12 over expression resulted in greater daughter cell motility. High-motility daughter cells exiting from the original mother cell footprint are more likely to be relevant to metastasis.

Mary Spiro is science writer and media relations director for the Johns Hopkins PS-OC.

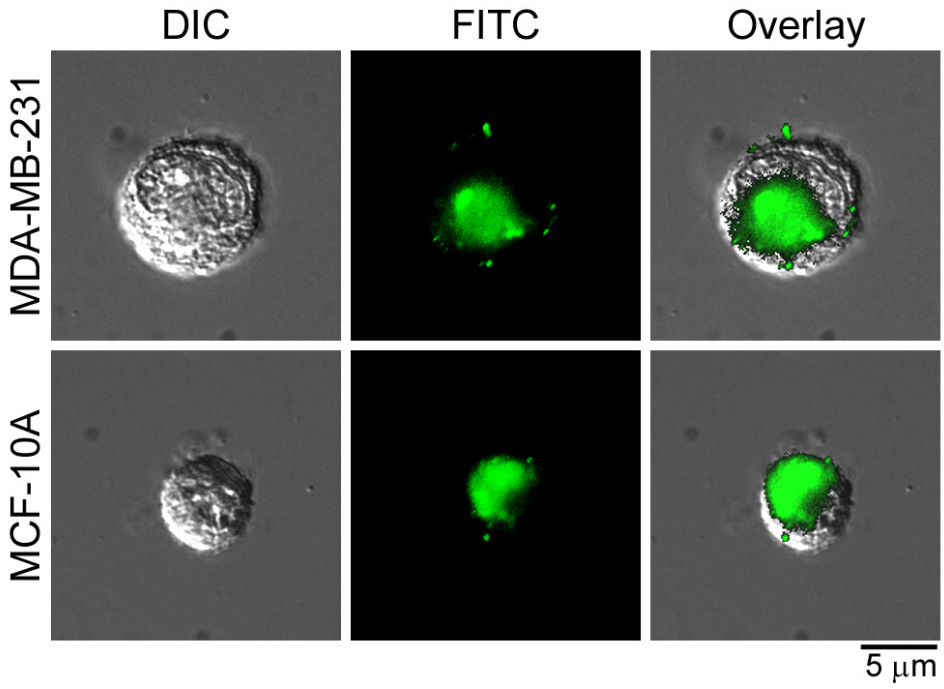


Actin fibers (green) terminated by vinculin-containing focal adhesions (red) in a mouse embryonic fibroblast plated on collagen. DAPI (Blue) stains nuclear DNA. Credit: Donghwee Kim (Wirtz Lab)

To Stick or Swim: Characterizing the Procoagulant Activity Of Circulating Tumor Cells

By Owen McCarty

Cancer metastasis is the process whereby cancer cells separate from the primary tumor mass, enter the vascular or lymphatic circulation, exit into a new tissue, and colonize the invaded microenvironment. 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. As a Project Leader in the Scripps PS-OC, I work as part of a team seeking to characterize the interactions of cancer in the third tumor microenvironment, the bloodstream. We seek to understand the variables involved in how blood cells, such as platelets, and blood proteins and coagulation factors may function as a transient 'tumor stroma' for metastasizing cells, affecting how they conduct their migration through the circulation.



Cultured breast epithelial cells bind thrombin under procoagu-lant conditions. Human sodium citrate-anticoagulated plasma was incubated with the metastatic adenocarcinoma cell line MDA-MB-231 or non-metastatic breast epithelial cell line MCF-10A in the presence of OG-488 active-site labeled thrombin (1 μM). Coagulation was in-itiated by addition of 16.7 mM CaCl2. Samples were imaged by DIC and fluorescence microscopy. OG-488 thrombin fluorescence is indicated in green.

Activation of blood coagulation and platelets frequently occurs in cancer patients. Several lines of experimental evidence suggest that the activation of platelets play a crucial role in the metastatic progression of cancer, the most convincing of which is the inhibition of metastasis by experimental thrombocytopenia and the restoration of metastatic potential by platelet infusion.[1,2] In collaboration with Dr. Konstantopoulos of the JHU PS-OC, we demonstrated that the rolling and tethering of circulating tumor cells to platelets, and the formation of heteroaggregates between tumor cells and platelets, can be mediated by P-selectin, a transmembrane cell adhesion molecule expressed on activated platelets.[3,4] We hypothesize that the formation of circulating tumor cell platelet clusters within the circulation may facilitate tumor cell arrest and extravasation from the bloodstream.

Metastatic cancer has long been linked to coagulopathies such as thromboembolism, a leading cause of death in cancer patients. Causal association between thrombosis and cancer was first recognized by Bouillard in the 1820's, then developed by Trousseau in the

"Our future goal is to take advantage of circulating tumor cells isolated from cancer patients over the course of varying disease states to provide more conclusive data linking coagulopathies and metastatic potential."

1860's, who, observing his own disease, described that patients who present with migratory superficial thrombophlebitis are likely to have underlying pancreatic cancer.[5,6] Studies have demonstrated an association between elevated levels of circulating tissue factor (TF) and thrombosis in cancer patients.[7] TF is a key protein in the initiation of blood coagulation, assembling with the proteolytic enzyme activated factor VIIa (FVIIa) on blood cell membranes with exposed negatively charged phosphatidylserine. Using models of coagulation under shear conditions, we recently demonstrated that the aggressively metastatic MDA-MB-231 breast tumor cells can promote coagulation and occlusive thrombus formation in a TF-dependent manner.[8] Our studies support the notion that the prothrombotic potential of circulating tumor cells may be, in part, a consequence of TF expression. Our future goal is to take advantage of circulating tumor cells isolated from cancer patients over the course of varying disease states to provide more conclusive data linking coagulopathies and metastatic potential.

"Metastatic cancer has long been linked to coagulopathies such as thromboembolism, a leading cause of death in cancer patients. Studies have demonstrated an association between elevated levels of circulating tissue factor (TF) and thrombosis in cancer patients. TF is a key protein in the initiation of blood coagulation, assembling with the proteolytic enzyme activated factor VIIa on blood cell membranes with exposed negatively charged phosphatidylserine...We recently demonstrated that the aggressively metastatic MDA-MB-231 breast tumor cells can promote coagulation and occlusive thrombus formation in a TF-dependent manner. Our studies support the notion that the prothrombotic potential of circulating tumor cells may be, in part, a consequence of TF expression."

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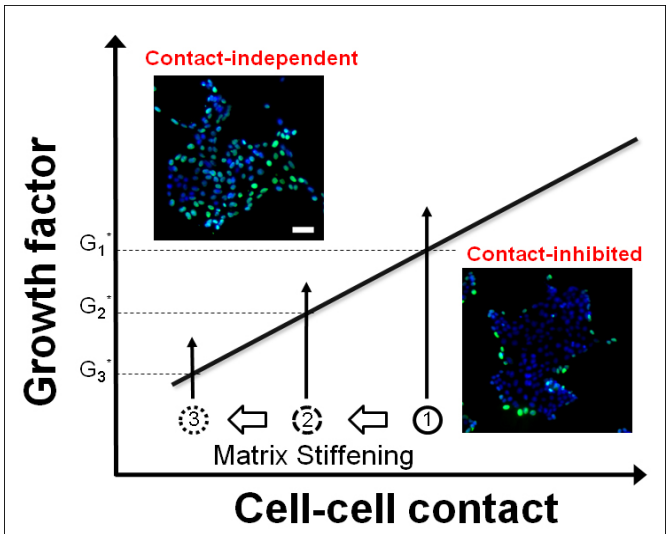
Covertly Nudging cells to the tipping point: ECM Stiffening, EGF Sensitivity and Contact-Inhibition of Proliferation

By Anand Asthagiri

With the physical microenvironment established as a key driving force in cancer development, an emerging challenge is to parse the interplay between physical factors and classical molecular cues in cancer progression. Understanding this interplay can help guide combination therapeutic strategies that target physical and molecular factors. Based on recent work in our lab, a system where such combination strategies might prove important involves the interplay between the physical stiffening of the ECM and the molecular oncogenic signal, EGF.

Leveraging support from the USC PS-OC, our lab undertook an analysis of spatial and dynamic patterns in epithelial cell proliferation in response to systematically tuning the microenvironment.[1,2] These studies revealed that normal epithelial cells can be shifted from contact-inhibited to contact-independent proliferation when EGF levels are increased above a threshold amount.[1]

Thresholds are, of course, quite common in biology. It is a key paradigm in developmental biology: cells that receive sub-threshold levels of a signal choose one fate and those exposed to supra-threshold level choose another fate.[3] Thresholds provide a way for spatial gradients in signal to achieve patterns in cell fate choices.



A state diagram model of contact-inhibition. Non-transformed epithelial cells reside in two states: contact-inhibited with proliferation occurring at the periphery of clusters, or contact-independent with proliferation occurring throughout the cluster. Fluorescence images are shown of BrdU (green) and DAPI (blue) stains of representative epithelial clusters in the contact-inhibited and contact-independent modes of proliferation. (Scale bar, 50 m.) Matrix stiffening shifts normal cells closer to the transition line to contact-independence (1 -> 2 ->3). Although such perturbations may not have a phenotypic effect (i.e., cells remain contact-inhibited), they reduce the EGF threshold (G¬1 -> G2* -> G3*) needed to induce contact-independent growth. Figure reprinted from Kim and Asthagiri, 2011.*

The intriguing aspect of the EGF threshold uncovered in our work is that it was not “hard wired”. The threshold depended on another microenvironment variable: cell-cell adhesion.[4] Enhancing cell-cell interactions using micropatterns increased the threshold; meanwhile, dampening cell-cell interactions using RNAi targeted to E-cadherin decreased the threshold. Thus, the greater the interaction between cells, the higher the threshold of EGF level, making it more difficult to shift cells into a contact-independent mode of proliferation.

This quantitative view of contact-inhibition of proliferation is captured in a state diagram model (see fig-

"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."

ure). An implication of this model is that contact inhibition can no longer be viewed simply as a qualitative phenotype associated with normal cells. It is also possible to quantify how strongly a normal cell system is contact-inhibited. That is, by measuring the threshold of EGF, one can quantify the extent to which a cell system is contact-inhibited.

Putting a number on contact-inhibition opens the door to testing new hypotheses: are there cancer-promoting perturbations that nudge a cell system closer to the transition point without actually pushing cells over to contact-independence? Such perturbations would have no overt effect on contact-inhibition but could latently alter the extent of contact-inhibition. This latent effect, according to the model, could be detected by quantifying the threshold of EGF.

We tested this idea by systematically perturbing a third variable of the tumor microenvironment: the stiffness of the ECM.[2] We discovered that stiffening the ECM markedly reduced the EGF threshold and attenuated cell-cell interactions. The effect is potent: a 4.5-fold increase in the matrix stiffness can yield a 100-fold drop in the EGF threshold.

Perhaps even more striking than the magnitude of the effect is that at low levels of EGF, matrix stiffening has no apparent effect on the proliferation phenotype: cells remain contact-inhibited and proliferation occurs just at the periphery of a cluster. Although the phenotype is unchanged, a significant quantitative effect accrues as the threshold amount of EGF needed to induce contact-independent proliferation drops.

Thus, a physical perturbation in the microenvironment (ECM stiffening) has no perceptible effect on the proliferation phenotype even as it profoundly sensitizes non-transformed cells to respond more readily to a classical molecular pathway (EGF signaling). This interplay could have implications in cancer progression where even early stages of ECM stiffening may sensitize cells to amplification of the EGF pathway. During morphogenesis, local remodeling of the ECM may sensitize compartments of developing tissue to EGF. We are currently investigating these and other roles for the interplay between physical and molecular factors in cancer progression and morphogenesis.[4]

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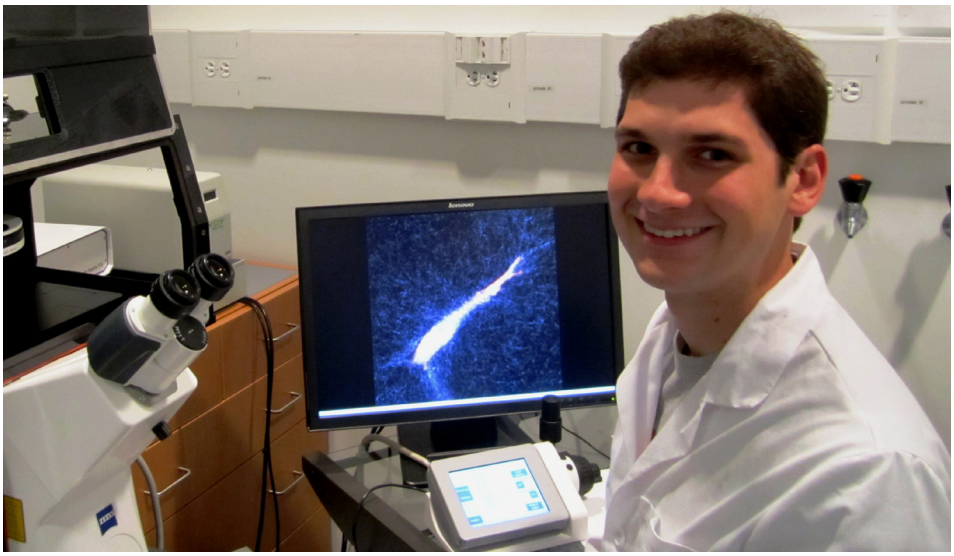
Anand Asthagiri is an Associate Professor in the Department of Chemical Engineering at Northeastern University and a member of the USC PS-OC.

Summer Immersion Term Experience at the Cornell PS-OC

By Shawn Carey

A critical component of my experience with the Cornell PS-OC has been the Summer Immersion Term, a program through which Biomedical Engineering graduate students spend seven weeks of the summer after their first year shadowing a clinical mentor at Weill Cornell Medical College in New York City. Last summer, to learn more about clinical oncology and pathology of breast cancer, I shadowed Dr. Sandra Shin, who is part of the Cornell PS-OC and serves as an Associate Professor of Pathology and Laboratory Medicine as well as Chief of Breast Pathology at Weill Cornell. This experience complemented my thesis research in the Reinhart-King lab, which is focused on exploring the intracellular and extracellular biophysical and biochemical factors that direct tumor cell migration during invasion and metastasis.



Shawn Carey, a member of Cynthia Reinhart-King's research group at the Cornell PS-OC, takes a brief break from his laboratory work.

Working with Dr. Shin, I was able to round out my clinical knowledge of breast cancer by following specimens from radiological detection during routine exams to biopsy and excision to gross and microscopic pathology. Because of the nature of my thesis research, I was particularly interested in learning about different variants of breast cancer and understanding how the type and degree of breast cancer dictates treatment. Toward this, I learned about the histopathological characterization of breast cancer, studied the basics of lesion grading and cancer staging, and contributed to two retrospective studies related to breast cancer diagnosis.

Finally, to complement my work in the Department of Pathology, Dr. Shin arranged for me to spend time shadowing several other clinicians. Thus, I was able to learn about the state of the art methods and technologies used in all aspects of breast cancer management: detection (Radiology), diagnosis (Pathology), and treatment (Surgery, Medical Oncology, and Radiation Oncology). This experience has helped define my thesis research, which fits into a larger thrust of work in the Reinhart-King lab to better understand the biochemical status and phenotypic behavior of tumors, with particular emphasis on the role of cellular biomechanics and interactions with the microenvironment. My summer immersion in Breast Pathology allowed me to explore the clinical side of cancer and develop new hypotheses regarding the onset, progression, and management of breast cancer.

Shawn Carey is a graduate student at Cornell University where he works in the laboratory of PS-OC investigator Cynthia Reinhart-King.

PS-OC Cell Line Exercise Data Jamboree

By Joe Alper

From its inception, the collaborative network of the Physical Sciences-Oncology Centers has engaged in a novel effort to apply the unique set of skills and methodologies housed in each center to the problem of characterizing two well-defined cell lines: the MCF-10A cell line and the MDA-MB-231 cell line. As this effort, known as the Cell Line Exercise, neared its winter 2010 conclusion, NCI staff and project leaders mapped out one approach to organizing the data for a publication in a leading research journal. On January 23-24, 2011, representatives from each of the research teams met in Scottsdale, Arizona, to present their findings and to begin preparing the network publication that would present the data generated during the Cell Line Exercise and show that such a multi-institutional and multi-disciplinary collaboration can succeed in generating new insights for further testing.

Participants in the PS-OC Data Jamboree, which was held in Scottsdale, Arizona.

Prior to the meeting, exercise participants formed a publication team consisting of Robert Austin (Princeton PS-OC), Parag Mallick (USC PS-OC), Owen McCarty (Scripps PS-OC), Thea Tlsty (Princeton PS-OC), Valerie Weaver (UC Berkeley PS-OC), and Denis Wirtz (JHU PS-OC). Over the course of several conference calls, this group established five sub-teams organized according to length scale ranging from the subcellular to the network scale. Each sub-team was charged with creating one figure for the publication.

At the Data Jamboree, the leader of each sub-team presented its multi-part figure and described the experiments that generated the data behind each component of the figure. From these presentations, it was clear that parallel measurements using a variety of techniques definitely reveal distinct differences between the two chosen cell lines that could be related to one another. In particular, the sub-teams concluded that proteomics data could serve as a unifying theme around which to structure data from other approaches.

The presentations at the Data Jamboree, each including data gathered by investigators at multiple institutions, showed without doubt that it is possible to create a working network of investigators spanning many disciplines and taking many research approaches. As Jerry Lee, deputy director of the NCI's Center for Strategic Scientific Initiatives, said, "People outside of the PS-OC Network are excited by the fact that the PS-OC has done this set of experiments and is demonstrating that a network can function in a way that's bigger than the sum of its individual laboratories and investigators."

Following the presentations and discussion, the sub-team leaders and

".....a network can function in a way that's bigger than the sum of its individual laboratories and investigators."

NCI staff held a late evening meeting at which they decided to organize the paper based on how given sets of data can be used to generate hypotheses based on physical science parameters of cells, such as modulus, migration, and morphology. The attendees broke into groups to reformulate the figures and then presented the new figures to the Data Jamboree attendees.

The sub-groups agreed to prepare new versions of their figures for review over the next month or two, and it was also agreed upon by PS-OC members and NCI staff that all data generated during the Cell Line Exercise would be deposited in a database that NCI is already in the process of creating for just this purpose.

As the Data Jamboree concluded, several participants commented that Cell Line Exercise started a large number of conversations and collaborations among PS-OC laboratories. These efforts, they predicted, will lead to very exciting results over the next couple of years.

Joe Alper is a science writer for parrotfish consulting group.

Two breakout groups focused on putting together a figure for the manuscript. Top: The "morphology" group, led by Owen McCarty (Oregon Health and Science University, Scripps PS-OC). Bottom: The "modulus and migration" group led by Cindy Reinhart-King (Cornell PS-OC).

On the Cover

Figure 1: Tumor cell spheroid comprised of MDA-MB-231 cells (green) and MCF10A cells (red) embedded in collagen. Image courtesy of the Reinhart-King Lab, Cornell PSOC.

Figure 2: An overlay image of bone marrow stromal cells (HS-5, expressing GFP) and myeloma cells (8226-S, expressing RFP) co-culture in the Death Galaxy (or Alcatraz death strip) chip at day 13. Cells aggregate in the microhabitats adjacent to nutrient source channel. Image courtesy of Amy Wu, Austin Lab, Princeton PS-OC.

Figure 3: Finite element (FE) mesh based on confocal data of a MDA-MB-231 cell was used to simulate cell deformation by atomic force microscopy. The FE mesh was created and the simulation was run by Sandor Kasas, EPFL, ASU PS-OC.

Figure 4: This is a best projection obtained from fluorescence confocal sections of a human fibrosarcoma cell fully embedded in a 3D collagen I matrix. The micrograph shows actin filament organization (green) in both mother protrusions that prolong the nucleus (blue) and daughter protrusions that stem from mother protrusions. Image courtesy of Anjil Giri, Wirtz Lab, JHU PS-OC

PS-OC Perspectives is edited by 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.