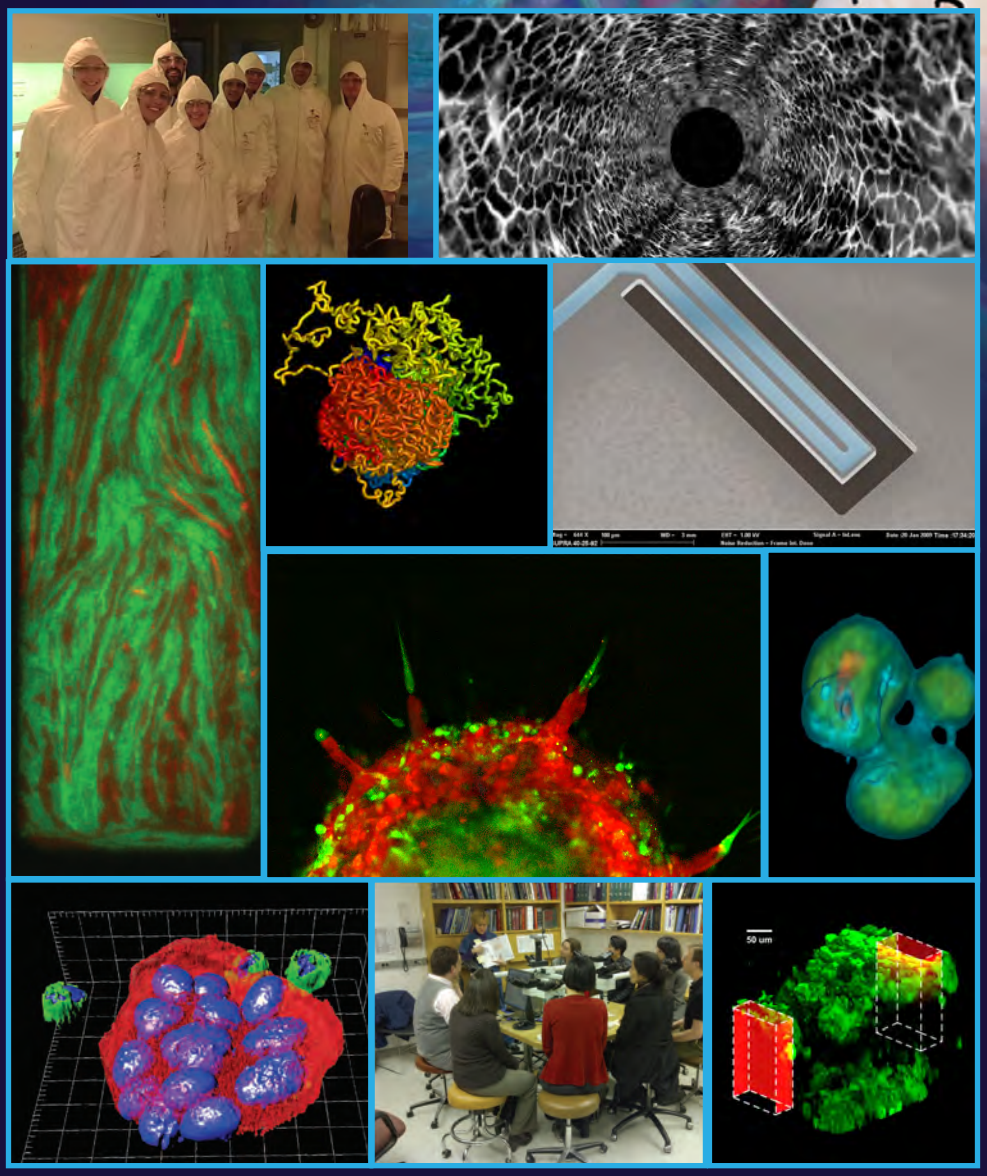
National Cancer Institute



PHYSICAL SCIENCES-ONCOLOGY CENTER PROGRAM

Program Update: Year Three

Fall 2012

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

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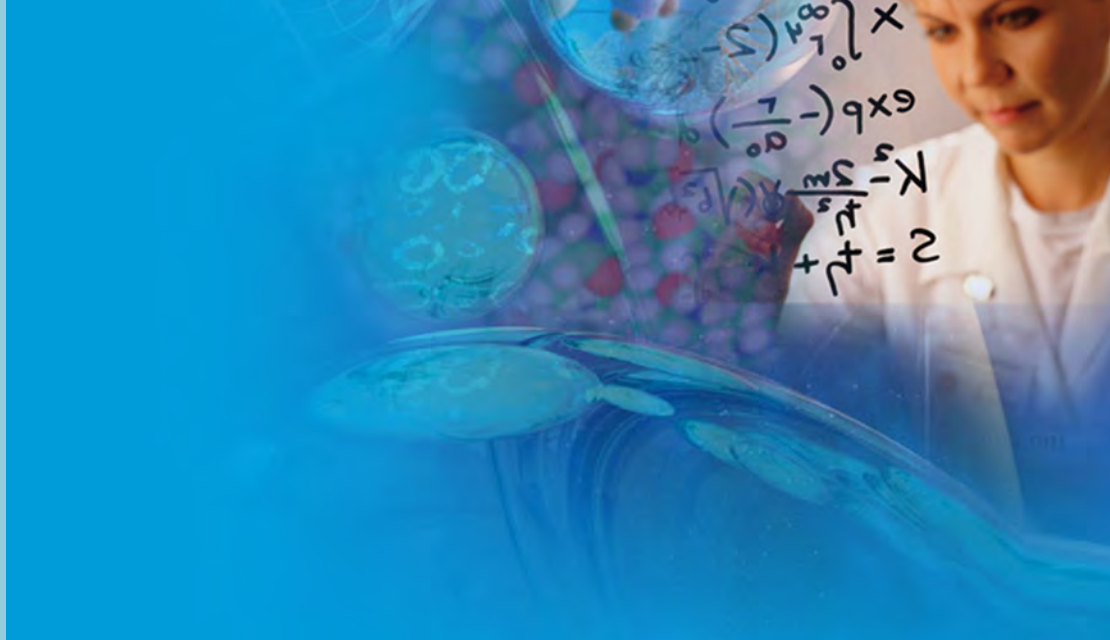


1. Executive Summary

The history of science suggests that leaps in scientific progress occur when researchers reevaluate existing dogma in light of new perspectives or paradigms. With this in mind, the National Cancer Institute's (NCI's) Physical Sciences-Oncology Centers (PS-OC) Program was initiated to study the problems of cancer research from a "physical sciences perspective." Building on recent initiatives that focus more on quantitative biology, nanotechnology, systems biology, and multidisciplinary education, the NCI engaged scientific teams from the fields of physics, mathematics, chemistry, computational science, and engineering to examine cancer using new, perhaps non-traditional approaches and theories from the physical sciences. A series of scientific workshops in 2008 identified four areas in cancer research that could benefit by engaging physical scientists: (1) physical laws and principles of cancer, (2) evolution and evolutionary theory of cancer, (3) information coding, decoding, transfer, and translation, and (4) de-convoluting cancer's complexity. In 2009, the NCI launched the PS-OC Program, a Network of 12 Centers funded through U54 cooperative agreements, to bring more physical scientists to cancer research and address these thematic areas at various length scales ranging from genomics, through cellular/tissue, up to the patient/population. Each Center conducts transdisciplinary research, education, and outreach activities integrating the perspectives of physicists, mathematicians, chemists, engineers, computer scientists, cancer biologists, and oncologists to examine cancer using approaches and theories from the physical sciences.

This report describes the status of the PS-OC Program at the end of its third year of operation. It highlights the infrastructure of the PS-OC Program that was established, as proposed and described by strategic documents approved by the NCI advisory boards and committees (RFA-CA09-009), as well as the programmatic tools and events developed to enable synergistic project collaborations. More importantly, the report describes the early outcomes of the PS-OC Network from the standpoint of scientific output, project highlights of key advancements, collaborative research, and leveraged funding. In summary, PS-OC Program investigators have reported the following information in semi-annual progress reports to NCI in the first three year of operation.

- 538 peer-reviewed publications with an average impact factor of 9.18
- 600 trainees involved in the PS-OC Program
- 500 collaborations among PS-OC investigators
- 23 patent disclosures

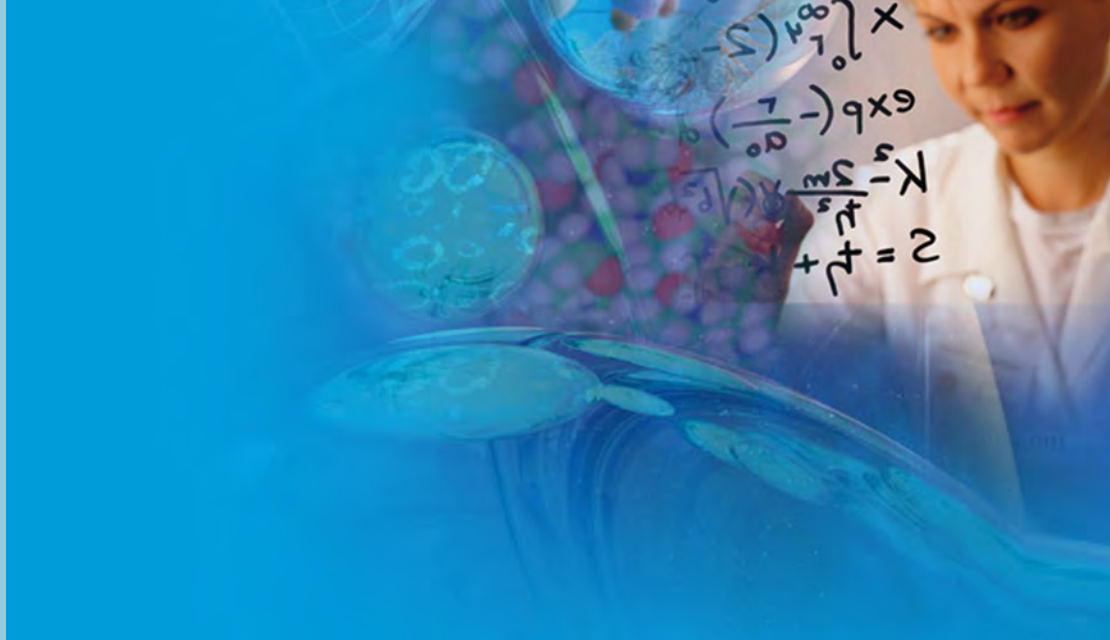
Examples of research highlighted include the following: (1) understanding mechanisms behind the generation of mutations in cancer genomes based on 3-D architecture and polymer physics, (2) optimizing dosing strategies for lung and brain cancer treatment using computational physics and evolutionary theory, and (3) making progress toward understanding the emergent mechanical properties in tumor progression and metastasis using physical sciences tools and principles.

The report also outlines the PS-OC Network activities, such as the Trans-Network Projects, the Annual PS-OC Network Investigators' Meetings, working groups, and workshops that aim to foster a collaborative environment within the Network. New projects and theories have emerged from these collaborative efforts, including a new Trans-Network project investigating the heterogeneity of cytoskeletal architecture as an indicator of treatment response and a new theory introducing the existence of "spreaders" and "sponge"-like tumors during metastasis.

Finally, the report describes the unexpected acceleration of the PS-OC Program to clinical research. Scientific advances generated within the PS-OC Program have catalyzed the genesis of or been incorporated into at least five clinical trials. In addition to these trials, there has been an increase in the number of PS-OCs, from 6 to 12, using clinical samples, tissue, or retrospective clinical trial data.

We are excited to share the accomplishments of the PS-OC Program with you. It is encouraging to witness the emergence of the physical sciences perspectives in cancer research, the gradual embrace of this approach by the cancer community, and the clinical implications that are emerging as this new field matures.

Physical Sciences-Oncology Centers Program Staff
Office of Physical Sciences-Oncology
Center for Strategic Scientific Initiatives
Office of the Director
National Cancer Institute
Bethesda, Maryland
September 2012



2. Physical Sciences-Oncology Program Organization

2.1. Introduction

The interactive organization of the Physical Sciences-Oncology Centers (PS-OC) Program was designed, based on the recommendations of workshop participants and the National Cancer Institute (NCI), to foster collaborations between physical scientists and cancer researchers with the goal of expanding knowledge of cancer through innovative and perhaps unorthodox perspectives of the disease. The Program supports a Network of 12 PS-OCs, each containing multiple projects, cores, and training and outreach units guided by an overarching scientific Framework with a physical sciences perspective (PSP). Several unique aspects of the Program distinguish it from other NCI programs, including (1) the requirement of a Principal Investigator (PI) with a physical sciences degree that works closely with a Senior Scientific Investigator (SI), who has formal training as a cancer biologist or a clinician in oncology; (2) Pilot and Trans-Network set-aside funds to support high-risk innovative research ideas sprouting from collaborative discussions and results; and (3) a focus on training a transdisciplinary group of scientists at the intersection of physical sciences and oncology. At the start, the PS-OC Program contained approximately 200 investigators and 150 trainees. In three short years more than 300 investigators and 250 trainees became affiliated with the PS-OC Network. The Program office maintains close interaction with these investigators and trainees to support meritorious science and is involved in ongoing monitoring of the scientific progress. Working groups, PS-OC Network activities, and resources established by investigators and PS-OC Program staff are designed to promote and support transdisciplinary collaborations within each Center and across the PS-OC Network.

The following section gives a detailed description of the PS-OC Program mission, history, infrastructure, operation, resources, tools developed toward effective progress monitoring, and groups organized to support interactions of the PS-OC investigators.

2.2. OPSO Mission

The mission of the NCI Office of Physical Sciences-Oncology (OPSO) is to facilitate the development of innovative ideas and new fields of study that converge perspectives and approaches of physical sciences and engineering with cancer biology and clinical oncology. By fostering a culture that encourages different perspectives and serving as a nexus for the development and implementation of physical sciences-based initiatives, the OPSO supports and nurtures new transdisciplinary environments and cancer research for NCI as well as its integration across trans-NIH and inter-agency activities. Through the use of various funding mechanisms and outreach activities, the OPSO aims to unite these often disparate areas of science to better understand the physical and chemical forces that shape and govern the emergence and behavior of cancer at all levels that will lead to exponential progress against cancer.

"The United States can anticipate comparable world-changing innovations in the 21st century if we adapt our education and research funding strategies to capitalize on new opportunities emerging at the convergence of the life sciences with the physical sciences and engineering."

Susan Hockfield, President of the Massachusetts Institute of Technology

"The Next Innovation Revolution," Science 2009

"The mission of the National Cancer Institute (NCI) Office of Physical Sciences-Oncology (OPSO) is to facilitate the development of innovative ideas and new fields of study that converge perspectives and approaches of physical sciences and engineering with cancer biology and clinical oncology."

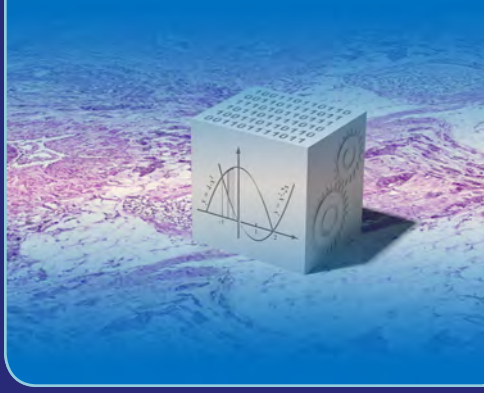


Figure 2.1. A list of physical sciences principles, system variables, and equations compiled by the PS-OC Physics Working Group in order to describe a physical sciences perspective. The word colors do not have any implications.

2.3. Program History

In September 2009, the PS-OC Program funded 12 cooperative agreement specialized Centers that together create a virtual Network. The PS-OC Network brings together expert teams from the fields of physics, chemistry, mathematics, computational science, engineering, cancer biology, and oncology to assemble and develop capabilities and research programs that will enable the convergence of the physical sciences with cancer biology. The strategic goal of the Program is to have the PS-OCs, both individually and collectively, support and nurture a new transdisciplinary research environment that (1) originates and tests novel, non-traditional physical sciences-based approaches to understanding and controlling cancer; (2) generates orthogonal sets of physical measurements and integrates them with existing knowledge of cancer; and (3) develops and evaluates theoretical physics approaches to provide a comprehensive and dynamic picture of cancer.

2.3.1 Overview of Spring 2008 Think Tank Meetings

The genesis for the Physical Sciences in Oncology initiative started in February 2008 with the first of three NCI-sponsored strategic "Think Tanks." These meetings brought together more than 300 extramural thought leaders from the fields of physical sciences and engineering with leaders in the fields of cancer biology and clinical oncology to ascertain how the NCI could more effectively engage the physical sciences in cancer research. The first NCI-sponsored meeting, "Integrating and Leveraging the Physical Sciences to Open a New Frontier in Oncology," was held in Washington, D.C., February 26-28, 2008, and highlighted four thematic areas that emerged from the meeting in which physical sciences approaches and principles could profoundly influence and improve our knowledge of cancer biology:

- *Physics (Physical Laws and Principles) of Cancer:* Defining the role(s) of thermodynamics and mechanics in metastasis and determining how this knowledge might be employed in new intervention strategies

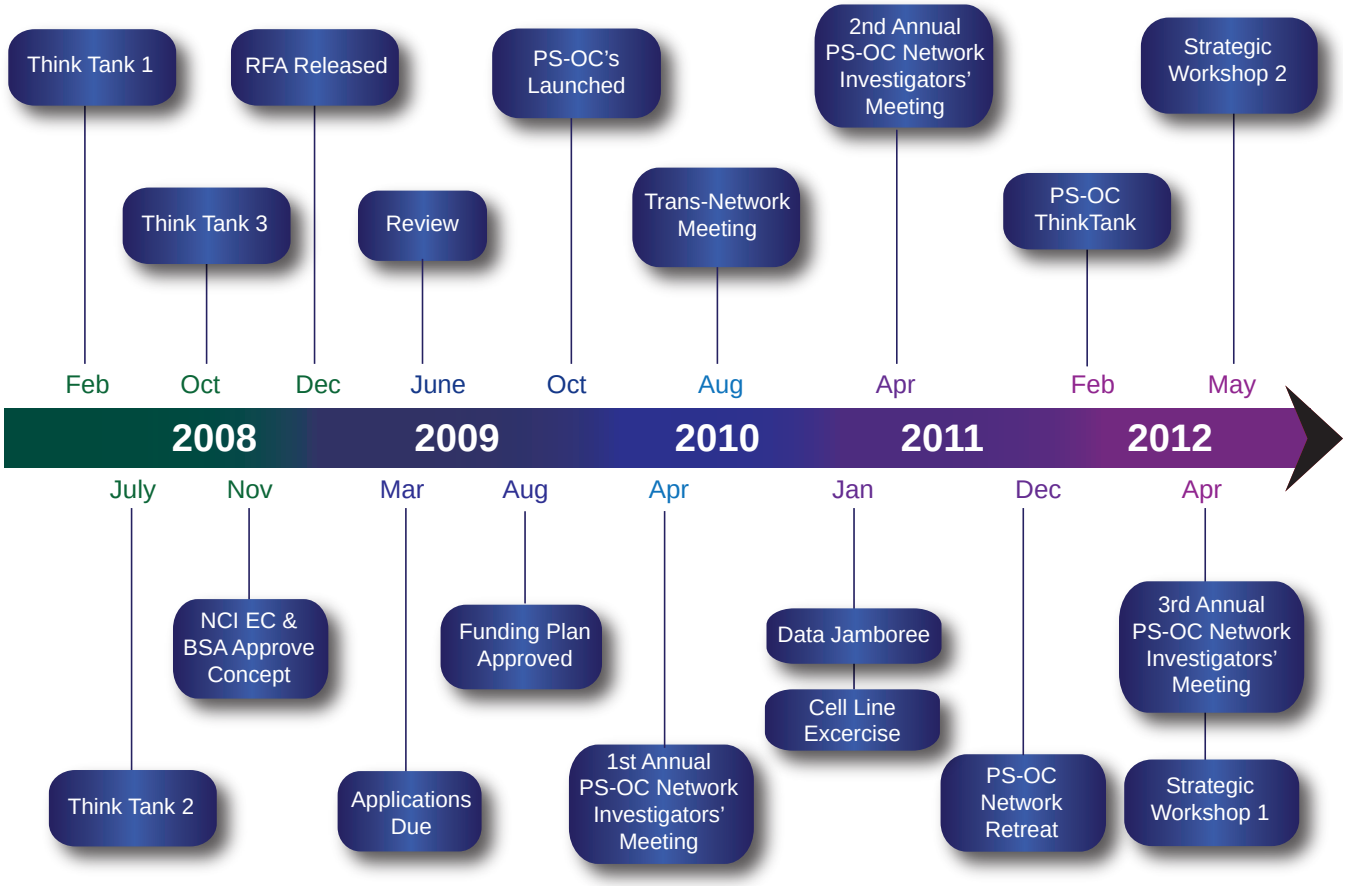


Figure 2.2. Timeline of Major Events in the Development of the PS-OC Program.

- *Evolution and Evolutionary Theory of Cancer:* Developing a comprehensive theoretical inclusive construct that would provide a foundation for understanding and predicting cancer heterogeneity
- *Information Coding, Decoding, Transfer, and Translation in Cancer:* Pursuing theoretical and supportive experimental approaches that define what information is and how it is decoded and managed in terms of cell signaling and contextual information translation in cancer
- *De-convoluting Cancer's Complexity:* Pursuing theoretical and experimental approaches from the physical sciences to cancer complexity that will inform a new fundamental level of understanding of cancer that may facilitate the prediction of viable pathways to develop novel interventions

Subsequent Think Tank meetings delved more deeply into specific thematic areas. The second Think Tank, "A New Look at Evolution and Evolutionary Theory in Cancer," identified a number of the major research questions in the field and helped refine a number of "grand challenges" that, if met, would significantly improve our understanding of the role of evolution in cancer. The role of information and information theory in cancer, specifically those changes that confer selective advantages, emerged as an area for which a great deal of knowledge is needed to elucidate the role of information flow at all scales in understanding the emergence of the malignant phenotype and resistance to therapy. This realization triggered the last Think Tank, "Physical Sciences-Based Frontiers in Oncology: The Coding, Decoding, Transfer, and Translation of Information in Cancer," which better defined this emergent and complex field relative to its potential role in understanding and controlling cancer.

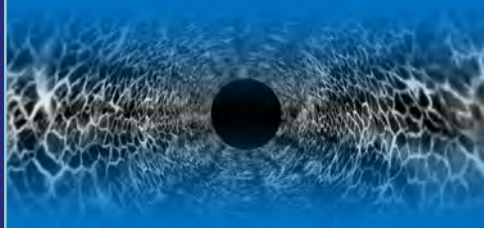
PS-OC Scientific Themes

Physics (Physical Laws and Principles) of Cancer

Evolution and Evolutionary Theory of Cancer

Information Coding, Decoding, Transfer, and Translation in Cancer

De-convoluting Cancer's Complexity:



2.3.2 Program Development and Funding History

Following the three extramural Think Tanks, a request for concept approval was presented to the NCI's Executive Committee and Board of Scientific Advisors (BSA) in the fall of 2008 (Figure 2.2). The concept to support the development of PS-OCs was approved at the 41st meeting of the NCI's BSA and the resulting Request for Applications (RFA-CA09-009) was distributed to the public in December 2008. A total of 35 applications were received on March 13, 2009, in response to the RFA, and one of the largest study sections assembled by the NCI convened June 29-30, 2009. The large number of reviewers was needed for several reasons: (1) the number of applications received; (2) the multicomponent nature of the proposed Center; and (3) the diverse subject matter expertise needed to properly review all the applications.

When the PS-OC Program launched in the fall of 2009, eight applications were funded utilizing appropriated funds ($22.5 million in total costs), and American Recovery and Reinvestment Act (ARRA) funding ($7.6 million in total costs) was utilized to support four additional Centers at significantly reduced levels. ARRA funding was utilized strategically to achieve appropriate length scale coverage across the four themes. These meritorious applications represent innovative and high-risk areas of research at the interface of physical and life sciences that are aligned with the NCI's strategic vision of accelerating cancer research and advancing innovations through ARRA support. The funded Centers are distributed across the four themes defined by the workshops as well as length scales from DNA to the Patient (Figure 2.3).

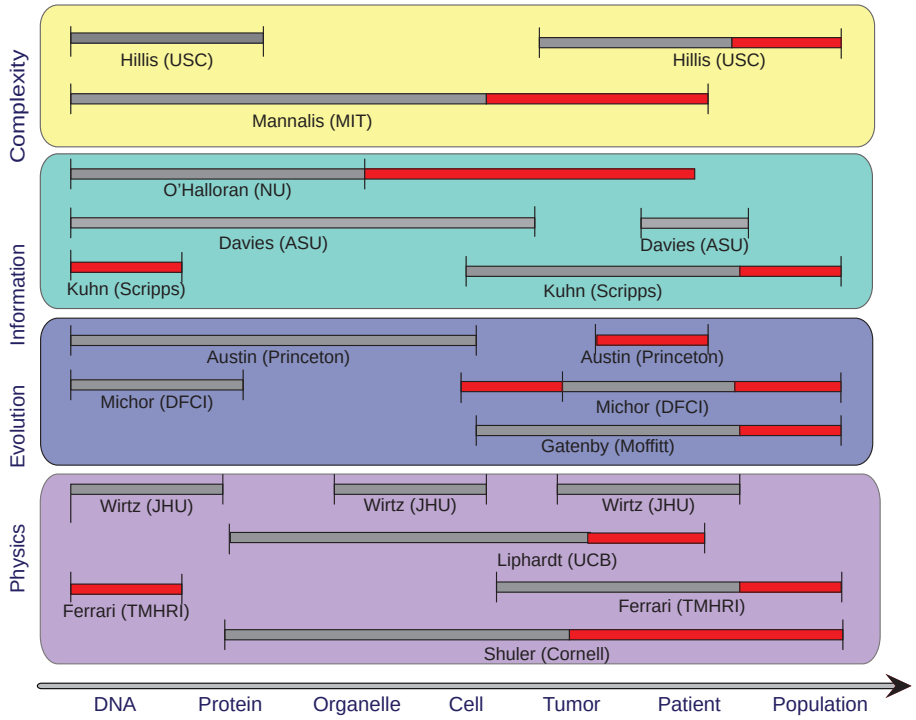


Figure 2.3. PS-OC by Theme and Length Scale. The PS-OCs (by PI and institution) are arranged by thematic area (Y-axis) and length scale (X-axis). Appropriated PS-OCs are indicated in green and ARRA-funded PS-OC are indicated in purple. The transition in length scale during the three years of PS-OC operation is indicated by the red bar.

2.4. Strategic Approach and Objectives

The three strategic Think Tanks were paramount in guiding the Framework of the PS-OC Program. While the distinctions between the physical sciences and life sciences disciplines have been noted (e.g., *A New Biology for the 21st Century*, National Academies Press [2009]; Research at the Intersection of the Physical and Life Sciences, National Academies Press [2010]), there was consensus among the participants on the need to establish transdisciplinary Centers comprising of integrated physical sciences-oncology teams in order to overcome the traditional barriers (silos) that have existed between these two scientific communities. The Think Tank participants also agreed that if the NCI's goal was to bring the physical science perspective (PSP) to cancer research, the Centers should be led by a physical scientist with a senior investigator (SI) from the oncology or cancer biology field. In addition to establishing an integrated team of physical scientists/engineers with cancer biologists/oncologists within each Center, Think Tank participants supported the idea that PS-OC investigators should also be closely integrated with investigators from different PS-OCs, forming a comprehensive and integrated PS-OC Network.

2.4.1 A Focus on Addressing "Big Questions" in Oncology

Starting with the Think Tank meetings and throughout the duration of the PS-OC Program, asking (and re-visiting) the "Big Questions" in cancer has been a strategic component of this Program at various levels. First, each PS-OC is governed by an organizing Framework or "school of thought" that defines the overall research direction of the Center and targets the physical science perspective to address a major barrier/question in cancer research rather than narrow questions pertaining only to a specific disease or model system. Additionally, the organizing Framework serves to focus the Center's activities on one or more of the Program's thematic areas in an effort to produce paradigm-shifting science that furthers our understanding of the laws that govern the emergence and progression of cancer.

On another level, the OSPO has asked investigators in the Network to pose "Big Questions" that the Network, as a whole, can make progress in addressing. The Network is continually refining and adapting the questions of interest. The following are examples of questions that have been posed at the Think Tank meetings and by the PS-OC Network investigators.

Examples of "Big Questions" Inspiring the PS-OC Program

- Can novel therapeutic strategies be developed based on increasing the genetic load of mutations in a cancer cell population that will lead to extinction of this population?
- How does higher-order chromatin structure and cellular context impact chromosome stability and gene expression?
- Is the fluid phase biopsy of solid tumors an accurate real-time representation of the disease over the course of the patient's lifetime?
- How does the heterogeneity of a tumor impact drug response?
- What properties mediate tumor dormancy and regrowth?
- Is a mechano-therapy of cancer possible?
- "Follow the genes" is the dominant paradigm. Can we develop a complementary "follow the physics" approach?
- What is the role of forces in metastasis?
- Is the transport oncophysics of the microenvironment what really matters?
- What is the energy budget of a cancer cell?
- How can we change the physical microenvironment (selective pressures) to prevent cancer?
- Is cancer curable? Can it be controlled through manipulation of the microenvironment?
- Why do tumors ultimately make a phase transition to a metastatic phenotype?
- What makes a microenvironment permissible for tumor growth?

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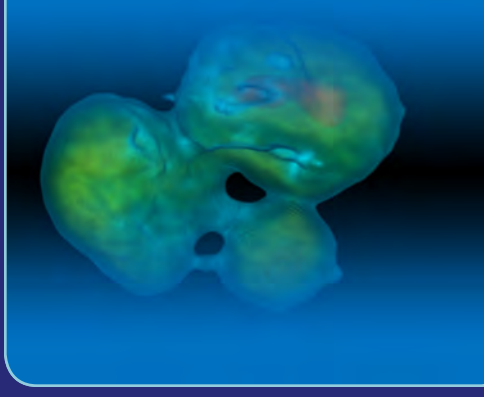

2.4.2 Structure of the PS-OC Network

The concepts and ideas that emerge from a Center's school of thought can be adopted and tested by investigators from other PS-OCs. Such transparency and openness necessitate an integrated and dynamic Network of PS-OCs that promotes interaction and flexibility yet maintains scientific excellence. A cooperative specialized research Center (i.e., U54) mechanism was selected because this mechanism offers the ability for a high degree of integration and coordination among researchers from the disparate fields represented in the PS-OCs. Furthermore, unique and selected resources that exist in the physical sciences community are shared among the PS-OCs; the exchange of computational model constructs as well as experimental protocols are important to maximize the effectiveness of each of the Centers. Moreover, the active involvement and guidance of experienced program managers with relevant backgrounds in physics, engineering, and genetics who also have training in cancer biology have been instrumental assisting the development and maturation of the Centers and the evolution of the Network. The interactions with Program staff accelerate facilitation of multiscale, multilevel methodologies, both within and across Centers, as well as to provide linkages to complementary programs at the NCI.

2.4.3 Overall Goals of the PS-OC Program

The primary objective of the PS-OC Program is *to unite the fields of physical science with cancer biology and oncology* through the development of transdisciplinary teams and infrastructure with the goal of better understanding the physical and chemical forces that shape and govern the emergence and behavior of cancer at all levels.

The PS-OC Program fosters the development and testing of innovative approaches to understanding cancer processes and new fields of study based on knowledge of both biological and physical laws and principles that define normal and tumor systems at all length scales. This is conducive to enhancing the possibility for *paradigm-shifting* science with the potential to generate exponential progress against cancer. The Program has five main goals designed to meet this objective.

- Establish an unprecedented Network of Centers and transdisciplinary teams focused on solving cancer problems.
- Train a new generation of transdisciplinary scientists in the area of physical sciences in oncology.
- Develop innovative (assumption challenging) physical sciences-centered experimental approaches to gain new knowledge of cancer initiation and progression.
- Develop and test new hypotheses/theories/models in cancer research.
- Collaboratively disseminate information to the cancer research communities and the public.

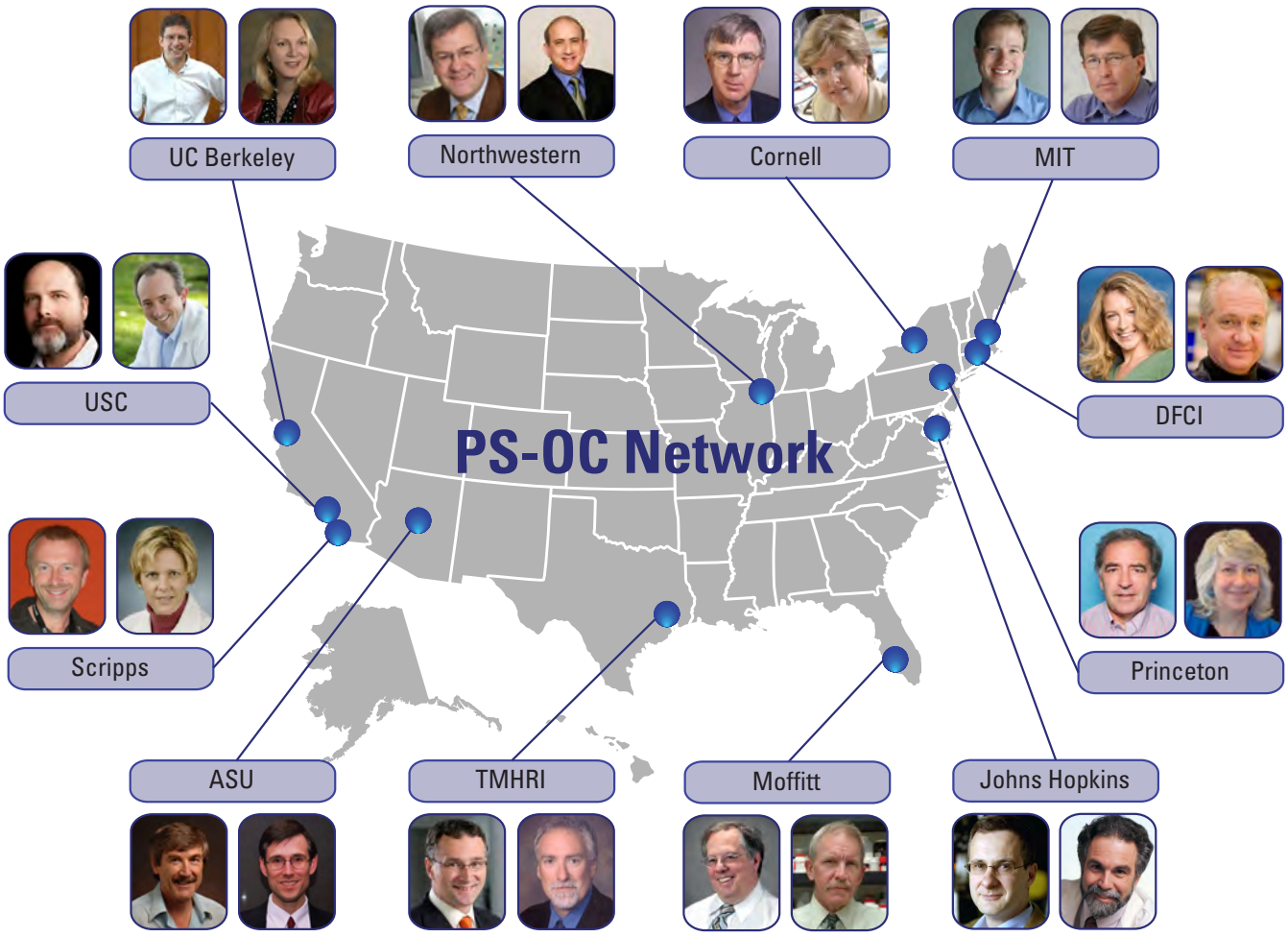

Figure 2.4. The PS-OC Network as of 2012. Each of the 12 PS-OCs brings together expert teams from the fields of physics, mathematics, chemistry, and engineering in conjunction with researchers in cancer biology and clinical oncology to assemble and develop the capabilities and research programs required to enable team research to converge disciplines of physical sciences/engineering with cancer biology/oncology. The PI and SI for each PS-OC are shown.

2.5. Program Infrastructure

The PS-OC Program is a Network of Centers (Figure 2.4) that aims to bring a physical sciences perspective to bear on critical questions and roadblocks in cancer research. To ensure that the NCI achieves the most from this unique opportunity and to help integrate a set of diverse disciplines, a variety of components and activities were incorporated into the Program to facilitate accomplishing programmatic goals and producing the best science.

2.5.1 Introduction to the 12 PS-OCs

Arizona State University Physical Sciences-Oncology Center

PI: Paul Davies, Ph.D. SI: William M. Grady, M.D.

The Arizona State University Physical Sciences-Oncology Center's (ASU PS-OC's) foremost aim is rigorously questioning the central tenets of cancer biology and creating innovative paradigm shifting tactics that challenge the barriers of contemporary cancer research and treatments. This team hypothesizes that cancer progression is linked to systematic physical differences in cells. Pioneering methods (e.g., single-cell tomography) to survey these physical changes are being

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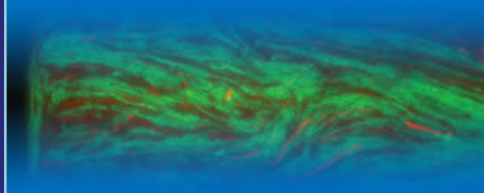


employed, and theoretical evolutionary models are applied to establish the evolution of a metastatic cancer cell from a physical context.

Cornell University Physical Sciences-Oncology Center

PI: Michael Shuler, Ph.D. SI: Barbara L. Hempstead, M.D., Ph.D.
PI: Harold Craighead, Ph.D. (2009)

The Cornell University Physical Sciences-Oncology Center (Cornell PS-OC) uses its expertise in manufacturing nano- and microfluidic devices to devise and assemble a three-dimensional tumor model to delve into the impact of physicochemical factors in tumor vascularization and cancer progression. This design imparts spatial and temporal resolution far greater than can be obtained by conventional two-dimensional tissue culture models. This platform facilitates the monitoring of non-linear responses to a combination of physical, chemical, genetic, and epigenetic stimuli and will lead to a better understanding of the signaling pathways that regulate the angiogenic switch.

Dana-Farber Cancer Institute Physical Sciences-Oncology Center

PI: Franziska Michor, Ph.D. SI: Eric C. Holland, M.D., Ph.D.

The Dana-Farber Cancer Institute Physical Sciences-Oncology Center (DFCI PS-OC) intertwines the physical sciences with cancer biology and oncology by engaging evolutionary theory to address several critical issues concerning cancer research. Tumors can be viewed from an evolutionary standpoint as collections of cells that accumulate genetic and epigenetic changes, which are then subjected to the selection pressures within a tissue. These normally heritable variations can lead to adaptations of the cells such as induction of angiogenesis, evolution of resistance or evasion of the immune system. Iterative modeling is being employed to study the evolution of brain, lung, and hematopoietic tumors.

H. Lee Moffitt Cancer Center & Research Institute Physical Sciences-Oncology Center

PI: Robert A. Gatenby, M.D. SI: Robert J. Gillies, Ph.D.

The H. Lee Moffitt Cancer Center & Research Institute Physical Sciences-Oncology Center's (Moffitt PS-OC) mission incorporates physical science concepts into the investigation of carcinogenesis. Both genetic alterations and microenvironmental selection pressures need to be deciphered in order to impede somatic evolution. Applied mathematical modeling is being used to determine whether oncogenesis is regulated by the escape from tissue homeostasis and provide further insight into the complex problems associated with cancer.

Johns Hopkins University Physical Sciences-Oncology Center

PI: Denis Wirtz, Ph.D. SI: Gregg L. Semenza, M.D., Ph.D.

The Johns Hopkins University Physical Sciences-Oncology Center (JHU PS-OC) explores the mechanical forces in cancer that bolster the tumor metastatic cascade. The team is studying and modeling cellular mobility and the assorted biophysical forces involved in the metastatic process. One such pressure includes hypoxia located within

the tumor. Hence, the effects of increased levels of HIF-1 on the mechanical properties of the extracellular matrix and the impact of hypoxia on cellular signaling are being evaluated. Micropatterned extracellular matrix is also used to uncover the dynamics of cell migration.

Massachusetts Institute of Technology Physical Sciences-Oncology Center

PI: Scott Manalis, Ph.D. SI: Tyler Jacks, Ph.D.
PI: Alexander van Oudenaarden, Ph.D. (2009-2012)

The Massachusetts Institute of Technology Physical Sciences-Oncology Center (MIT PS-OC) employs innovative technology and analytical and computational tools to explore the process of carcinogenesis and better understand the complexity of cancer at the single-cell level. This team utilizes pioneering single-cell mRNA counting techniques to model stem cell differentiation and reprogramming signaling networks as well as to probe the connection between cell growth and the cell cycle. Gene expression of various transcripts in individual cells is being surveyed over time to measure the quantity and pattern during these processes as well as to establish computational models of neoplastic progression.

Northwestern University Physical Sciences-Oncology Center

PI: Tom O'Halloran, Ph.D. SI: Jonathan Licht, M.D.
PI: Jonathan Widom, Ph.D. (2009-2011)

The Northwestern University Physical Sciences-Oncology Center (Northwestern PS-OC) probes the molecular basis of information flow within a malignant cell and is providing a basic understanding of how normal gene expression is calibrated and how the epigenome and proteome are regulated. Scientists are studying the diverse characteristics of gene expression and storage by exploring the 3-D organization of the genome and the higher order chromatin structure using leading-edge physical techniques. Insight into chromatin structure modifications in malignant cells has the potential to expedite the development of tools for the early diagnosis of cancer.

Princeton University Physical Sciences-Oncology Center

PI: Robert H. Austin, Ph.D. SI: Thea D. Tlsty, Ph.D.

The Princeton University Physical Sciences-Oncology Center (Princeton PS-OC) focuses on how to control the evolution of cancer resistance to chemotherapy by understanding its origin and dynamics. Using the basics of physics to evaluate stress response mechanisms in both fundamental and clinically relevant studies, the team hypothesizes that evolution in a small, stressed microenvironment will generate the rapid emergence of resistance. Microfabricated microenvironments and single-cell genomic analysis are used to evaluate metabolic and mechanical stressors and determine whether stress can alter the types of mutations accumulated by cells.

The Methodist Hospital Research Institute Physical Sciences-Oncology Center

PI: Mauro Ferrari, Ph.D. SI: Steven A. Curley, M.D.

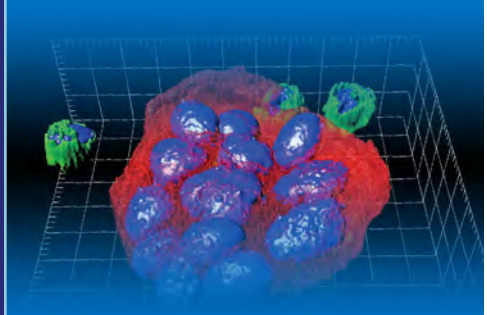
The Methodist Hospital Research Institute Physical Sciences-Oncology Center (TMHRI PS-OC) integrates advanced computational multiscale modeling, innovative engineered transport probes, and state-of-the-art imaging to elucidate the transport physics of various physical and biological barriers related to tumorigenesis and drug delivery. Notably, this transdisciplinary team is studying the physical barriers to the evolution of liver metastasis from colorectal cancer and the administration of novel carriers to surpass these barriers. Ultimately, these studies will provide a clearer grasp of the function and physics of biological barriers and in turn accelerate basic discovery and the design for potential therapeutics.

The Scripps Research Institute Physical Sciences-Oncology Center

PI: Peter Kuhn, Ph.D. SI: Kelly J. Bethel, M.D.

The Scripps Research Institute Physical Sciences-Oncology Center (Scripps PS-OC) is pursuing the mechanisms that regulate the survival of circulating tumor cells and probing the biophysical factors implicated in the endurance of individual circulating tumor cells while in the bloodstream and in their progression to metastatic disease. Fluid phase biopsies from epithelial cancers are being employed to assess

"In order to effectively integrate the physical sciences and cancer biology perspectives, cross-train young investigators in this emerging field, and grow the field outside the PS-OC Network, each PS-OC is coordinated by an Administrative Unit and an overarching Framework and comprises Projects, Resource Cores, and Education and Outreach Units."



and model the physical attributes (e.g., cell size, mechanical properties, ultrastructural complexity) of these tumor cells over the course of the disease across various body compartments.

University of California, Berkeley Physical Sciences-Oncology Center

PI: Jan Liphardt, Ph.D. SI: Valerie M. Weaver, Ph.D.

The University of California, Berkeley Physical Sciences-Oncology Center (UCB PS-OC) is determining how mechanobiology influences tumorigenesis in breast cancer by examining how the malignant phenotype is maintained by exchanges with its microenvironment and how reversion can occur if these pressures are normalized. This Center also examines how mechanical signals trigger genetic changes that induce tumorigenesis via the integration of state-of-the-art tools in the physical, theoretical, and biological sciences that will cultivate models of various interactions of model systems with their microenvironment.

University of Southern California Physical Sciences-Oncology Center

PI: W. Daniel Hillis, Ph.D. SI: David B. Agus, M.D.

The University of Southern California Physical Sciences-Oncology Center's (USC PS-OC) overall goal is to thoroughly understand therapeutic response by establishing a predictive model of cancer that can be utilized to determine tumor steady state growth and drug response, particularly those involved in the hematological malignancies of acute myeloid leukemia and non-Hodgkin's lymphoma. Furthermore, multiscale physical measurements are being conducted under unified conditions to facilitate the development of a model that can derive the tumor's traits during its growth and after any distress, such as chemotherapeutic treatment.

2.5.2 Organization and Policies of Individual PS-OCs

Each PS-OC is a virtual Center headed by a PI and an SI and comprises research facilities typically from two or more collaborating institutions. To begin merging the perspectives of physical sciences and oncology within each Center from the top down, the PIs are trained and have significant experience in the physical sciences while the SIs are trained and have significant experience in areas of basic and/or clinical cancer research. Each PS-OC consists of a collaborative transdisciplinary research team of investigators with complementary abilities focused by an organizing construct that addresses major questions/barriers in cancer research. In order to effectively integrate the physical sciences and cancer biology perspectives, cross-train young investigators in this emerging field, and grow the field outside the PS-OC Network, each PS-OC is coordinated by an Administrative Unit and an overarching Framework and comprises Projects, Resource Cores, and Education and Outreach Units (Figure 2.5).

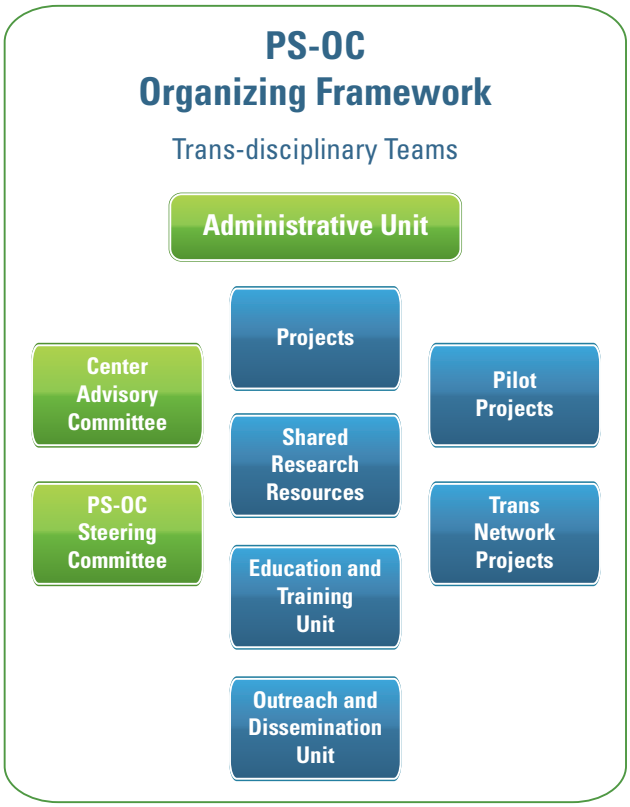


Figure 2.5. Center Organization. Each PS-OC is organized beginning with an overarching Framework designed to identify and address major questions in cancer research. Beneath the Framework sits an Administrative Unit that organizes and ensures progress of the Center as a whole. Within the Center there are a number of scientific Projects, Resource Cores, an Education and Training Unit, and an Outreach and Dissemination Unit. Other features of the PS-OCs include both Pilot Project and Trans-Network Project funds, which allow the Centers to meet their changing needs by quickly adding additional projects or partnering with other Centers.

Framework: A physical science-based overarching organizing Framework focuses the scientific efforts of a Center toward addressing major questions and barriers in cancer in innovative ways.

Projects: Each PS-OC consists of three to five major projects that combine one or more thematic areas described above and test, integrate, and support the overall PS-OC's overarching organizing Framework.

Shared Research Resources (Cores): These Cores support and/or provide expertise to the PS-OC and are available to all PS-OC Network investigators as either a physical or virtual infrastructure (e.g., fabrication, biological specimens, computational physics modeling, mathematical theory development).

Administrative Unit: The Administrative Unit roles are to (1) develop an individual Center administration, including the Center Advisory Committee (CAC) and (2) participate in overall Network activities, including the PS-OC Steering Committee. This Unit provides administrative support, coordinates Center activities, and assists the PI in interfacing with NCI Program Directors and Project Scientists.

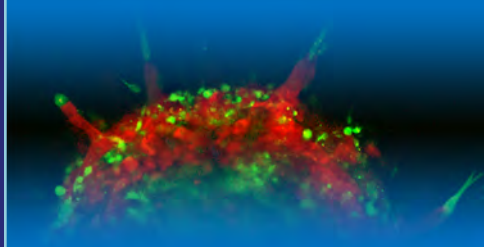
- *Center Advisory Committee (CAC):* Each PS-OC is governed by a CAC consisting of four voting key Center personnel of whom one is the PI (with two investigators representing physical sciences and two representing cancer biology or clinical sciences), one voting OPSO project scientist, and non-voting external scientific advisors. The CAC acts to ensure Center scientific progress and to develop Pilot Project processes.

- *PS-OC Center Pilot Projects:* Each PS-OC includes approximately 5 percent of total Center direct costs to be allocated specifically for individual PS-OC Pilot Projects. Pilot Projects are solicited, evaluated, and awarded by the CAC.

Outreach and Dissemination Unit: This Unit develops Outreach Programs (e.g., seminar series, workshops, advocate programs, student exchanges, and Web sites) to disseminate information to cancer biology and physical sciences communities about PS-OC capabilities, projects, and advances (approximately 2.5 percent of total Center direct costs). This Unit also develops strategies to solicit Outreach Pilot Projects (approximately 2.5 percent of total Center direct costs) to bring in expertise outside the individual PS-OC that will enhance specific PS-OC's efforts in its overarching Framework and invoke the engagement of the broader scientific community.

Education and Training Unit: This Unit develops modules for integrative training of graduate students and postdocs that include programs to develop a knowledge base relevant to cancer biology and the physical sciences (e.g., graduate programs, courses, seminars, and workshops) (approximately 2.5 percent of total Center direct costs). This

"The Steering Committee meetings have hosted important discussions including those on (1) the pros and cons of using model systems versus primary samples; (2) implementing systems for data sharing and data analysis; (3) how to best facilitate interaction within the Network; and (4) developing collaborative projects that leverage the broad expertise of the Network."



Unit also develops and oversees mechanisms to share and exchange graduate and post-doctoral trainees and junior and senior investigators among participating PS-OCs (approximately 2.5 percent of total Center direct costs).

Annual Site Visits: OPSO program officials conduct annual administrative site visits that are one full day in length. During the annual site visits all PS-OC Project/Core/Unit leaders are required to attend and present their scientific and programmatic progress (background, results, future directions, and red-flags/concerns). In addition, the site visits provide the CAC with the opportunity to meet face-to-face.

2.6. Organization and Activities of the PS-OC Network

To facilitate dialogue and collaboration among investigators throughout the PS-OC Network, a number of mechanisms described below were built into the Program that encourage and reward collaboration.

2.6.1 PS-OC Steering Committee

The PS-OC Steering Committee serves as the main governing board for the PS-OC Network and is responsible for ensuring the scientific progress and oversight of the Network. The Steering Committee was established jointly by and consists of (a) two representatives from each awarded Center (PI and SI) and (b) OPSO program officials (Figure 2.6). Each Center and each program official have one vote. This structure allows OPSO program officials to facilitate and promote inter-Center collaborative Pilot

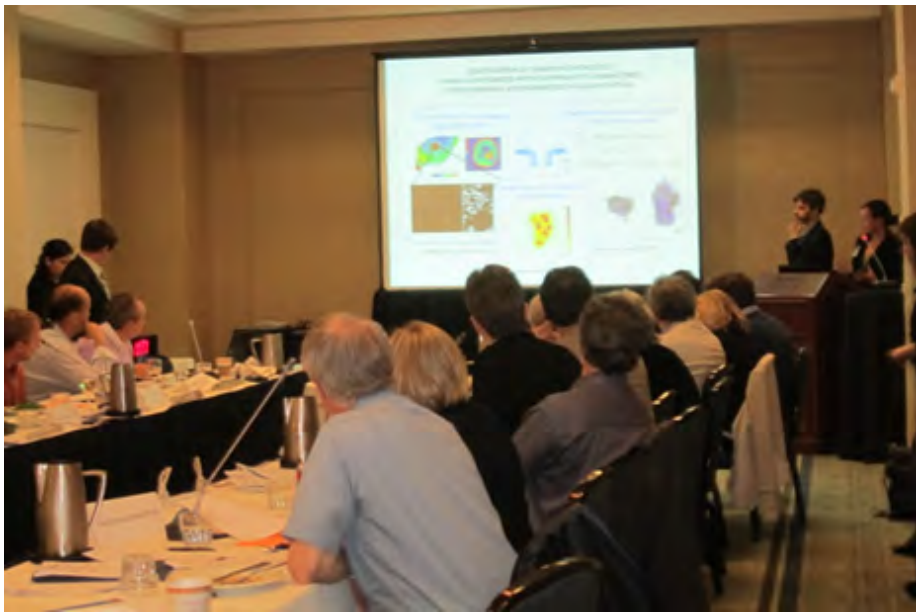


Figure 2.6. Face-to-Face Steering Committee Meeting at the Second Annual PS-OC Network Investigators' Meeting in La Jolla, California. The Steering Committee is listening to trainees presenting their Young Investigator Trans-Network proposals.

Projects based on synergistic Center expertise and projects. Additional expertise is solicited from non-voting external scientific members as needed.

The PS-OC Steering Committee meets bimonthly to discuss both critical Network policies and scientific progress. This forum serves as a critical point of interaction between members of each Center and between physical scientists and cancer biologists. The Steering Committee meetings have hosted important discussions including those on (1) the pros and cons of using model systems versus primary samples; (2) implementing systems for data sharing and data analysis; (3) how to best facilitate interaction within the Network; and (4) developing collaborative projects that leverage the broad expertise of the Network. Additionally, the Steering Committee has initiated and implemented a number of key Network-wide activities including the Cell Line Pilot Study, the Trans-Network Projects Program, and the Network Data Sharing Agreement and Pilot Data Coordinating Center.

2.6.2 Data Sharing

Scientific research depends on the free flow of information and ideas. The PS-OC Network is committed to establishing both an environment that promotes sharing of data and a mechanism to disseminate data both within the Network and to the broader scientific community.

Network Data Sharing Agreement

The PS-OC Network is unique in the manner in which it collaborates with broad cross-sections from both the cancer biology/oncology and physical sciences/engineering communities. With each PS-OC actively engaged in data generation, characterization, and analysis, the integration of these datasets will accelerate orthogonal exploration by all PS-OC investigators to help generate answers to some of the major questions and barriers in cancer research and support the development of clinical advances.

The PS-OC Steering Committee established, and all 12 of the PS-OCs have signed, a Data Sharing Policy to achieve two high-level goals:

- Facilitate collaboration between PS-OCs for purposes of achieving the goals of the Program and establishing a robust PS-OC Network.
- Disseminate PS-OC results to PS-OC investigators in a format that can be utilized efficiently and harmoniously, and, after public dissemination, to the broader research community.

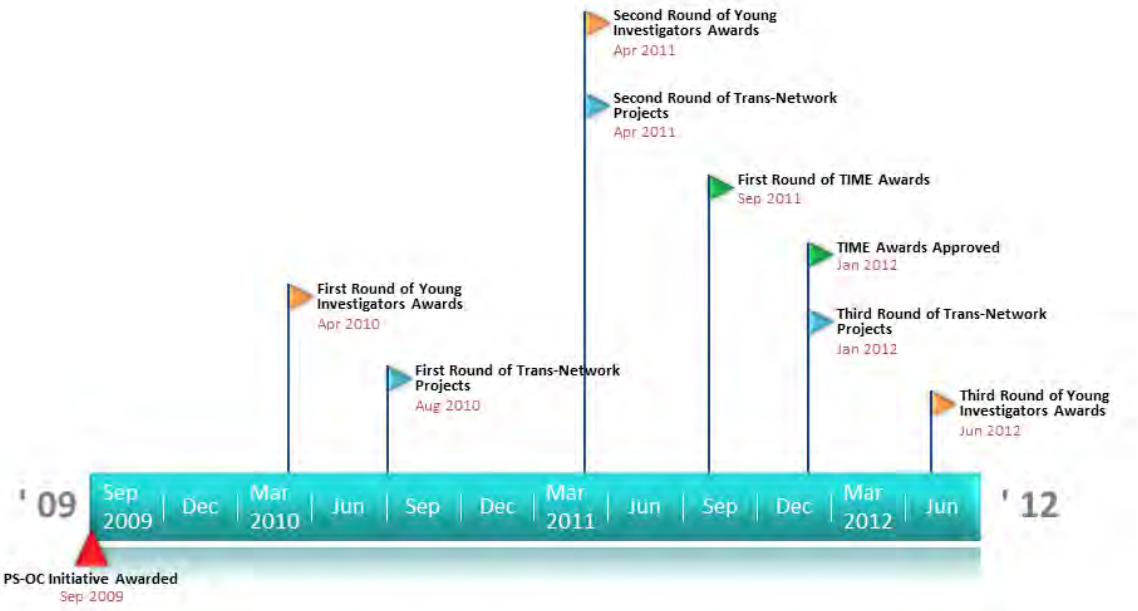
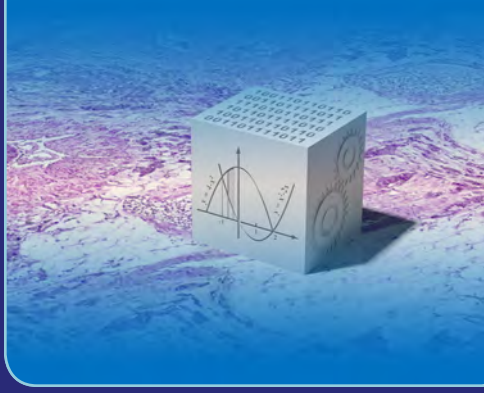


Figure 2.7. Timeline for the development and implementation of Trans-Network Pilot Projects, TIME Awards, and Young Investigators Trans-Network Awards.

"All Trans-Network Projects are meant to (1) help generate answers to a major question or barrier in cancer research through unconventional physical sciences perspectives and approaches, and (2) enhance the PS-OC Network interaction through establishing and developing new collaborations."



2.6.3 Trans-Network Projects

A critical and unique component for realizing the full potential of the PS-OC Program is the availability of funds for Trans-Network Projects (Figure 2.7). Awarded within each of the Centers are restricted funds (~$100,000 direct costs) allocated specifically to support Trans-Network Projects. These funds provide each Center an opportunity to catalyze new perspectives and test research ideas through the development of robust collaborations within the PS-OC Network. All Trans-Network Projects are meant to (1) help generate answers to a major question or barrier in cancer research through unconventional physical sciences perspectives and approaches and (2) enhance the PS-OC Network interaction through establishing and developing new collaborations. These funds allow potential "outside the box" projects that originate from discussions between Network investigators an avenue to achieve funding. The the ultimate goal is to transition successful projects to independent funding when possible.

Trans-Network Pilot Projects

The PS-OC Steering Committee was tasked with developing Trans-Network programs to meet the needs of a developing transdisciplinary Network. A series of Trans-Network pilot programs have been developed that address both broad topics and specific questions alike. Over three rounds of funding, the Trans-Network Program has featured an open Call for Proposals on any topic, a Call for Proposals addressing questions in heterogeneity, and a call focused on a small number of "Big Questions." To date, these programs have funded 13 collaborative projects ranging in size from two to six participating PS-OCs and $100,000 to $600,000 in total costs. A number of these projects are highlighted in Section 5.1.

PS-OC Trans-Network Impact Experiment (TIME) Awards

The PS-OC Trans-Network Impact Experiment (TIME) awards were created by the PS-OC Steering Committee in May 2011 as a mechanism for investigators, from two or more Centers, to secure funding for innovative experiments, as opposed to lengthy specific aim-based projects. Applications were accepted on a rolling basis and evaluated within two weeks by a panel of four PS-OC PIs using the following criteria: (1) that the experiment leverages work already funded within the PS-OCs; (2) that it has the potential to make a broad scientific impact within the PS-OC Network; and (3) that it has clinical implications. TIME awards were capped at a maximum of $30,000 in total costs, and $360,000 of Trans-Network funds were allocated for this mechanism for one year. Successful applicants are required to interact regularly with a member of the TIME award panel who serves as a mentor for the project and presents the results of the work to the PS-OC Steering Committee. To date, three TIME awards have been funded.

Young Investigator Trans-Network Projects

A small portion of the total Trans-Network funds is utilized by the PS-OC Steering Committee to solicit proposals from young investigators (graduate students and postdocs) and award a select number of applications at the Annual PS-OC Network Investigators' Meeting. Over three years, twelve Young Investigator Trans-Network awards have been made. Five Young Investigator awardees have since moved on to independent assistant professor positions, and four of these are still actively involved in the PS-OC Network.

2.6.4 Annual PS-OC Network Investigators' Meeting

The Annual PS-OC Network Investigators' Meeting has participants from across the Network ranging from PIs to post-doctoral fellows and graduate students (Figures 2.8 and 2.9). The Annual PS-OC Network Investigators' Meetings highlight scientific efforts within the PS-OC Network, promote collaborations, and provide a venue for working group discussions to explore the physical laws and principles that shape and govern the emergence and behavior of cancer at all scales. The PS-OC Network Investigators' Meetings also host tutorial sessions and training groups to provide education and guidance to the next generation of young scientists in the many disciplines that constitute the PS-OCs. Finally, components of the Trans-Network Projects and Young Investigator Trans-Network Projects programs are implemented during the PS-OC Network Investigators' Meetings.

2.6.5 PS-OC Network Working Groups

Several working groups have been established within the PS-OC Network to foster collaboration and facilitate achievement of Program goals. Each working group is facilitated by at least one OPSO program official with Network investigators servings as chairs or co-chairs of the groups. Additional working groups may be established on the basis of

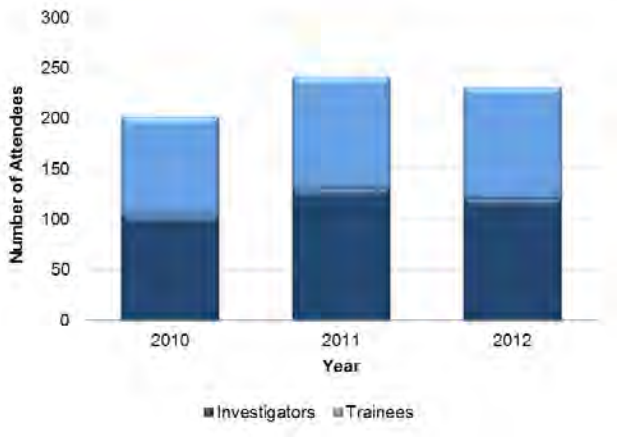
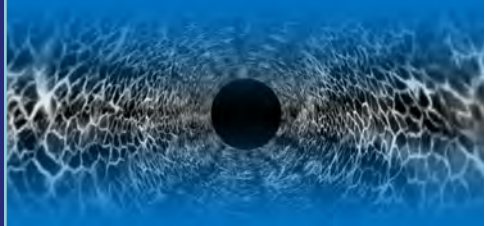


Figure 2.8. Total number and distribution of attendees at the Annual PS-OC Network Investigators' Meetings.

Figure 2.9. Second Annual PS-OC Network Investigators' Meeting, La Jolla, California.

"The Annual PS-OC Network Investigators' Meetings highlight scientific efforts within the PS-OC Network, promote collaborations, and provide a venue for working group discussions to explore the physical laws and principles that shape and govern the emergence and behavior of cancer at all scales."



need and interest. Working groups have been created for the following areas and are described in more detail in Sections 5.4, 7.2, and 8.1.

Physics

The Physics Working Group was initiated at the start of the PS-OC Program as a way to integrate several of the physics tools and principles implemented by the PS-OC Network. This Physics Working Group is made up of more than 40 investigators representing all 12 PS-OCs. Group leaders Claudia Fischbach-Teschl (Cornell PS-OC) and Denis Wirtz (JHU PS-OC) hold meetings twice yearly to initiate discussion and identify ways to integrate scientific approaches between PS-OCs. The Physics Working Group established several objectives designed to initiate communication and integration across the PS-OC Network and disseminate information as a group about the PS-OC Program. A subgroup has been initiated to integrate cell modulus measurements based on the working group discussions.

Evolution of Drug Resistance

The PS-OC Evolution of Drug Resistance Working Group serves to provide a forum for PS-OC investigators to discuss research and opportunities in the area of evolutionary theory and drug resistance in cancer. The working group has annual seminars at the PS-OC Network Investigators' Meetings and semi-annual conference calls, during which guest speakers are often invited to present their work. The working group and others contributed to a special theme issue on the evolution of drug resistance in cancer published in *Molecular Pharmaceutics* in December 2011.

Circulating Tumor Cell Transport

The mechanisms of circulating tumor cell (CTC) transport from a primary tumor to a metastatic lesion are not well understood, and our current assumptions of the process tend to overlook the physics and physical forces. Several of the PS-OCs are investigating the clinical implications of CTCs using a physical sciences perspective. To combine efforts in this area, the PS-OC Network initiated the PS-OC CTC Transport Working Group in the fall of 2011. Organized by Owen McCarty, a biomedical engineer at Oregon Health and Science University and project leader for the Scripps PS-OC, the group is made up of a team of seven investigators currently studying CTC transport mechanisms within the PS-OC Network. These members represent five PS-OCs and four different disciplines, including biomedical engineering, chemical engineering, oncology, and mathematics. The initial objectives of the group were to discuss hurdles to understanding the mechanisms of CTC transport, challenge current assumptions about physical science principles, and identify collaborative experiments to test new hypotheses in this area. Accomplishments to date include new Trans-Network projects, collaborations, student exchanges, and a special issue collection of publications (Therapeutic targeting of circulating tumor cells) related to the topic. See http://www.frontiersin.org/Cancer_Molecular_Targets_and_Therapeutics/researchtopics/Therapeutic_targeting_of_circu/750.

Education and Training

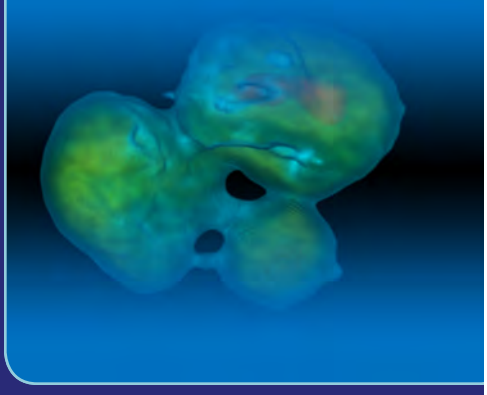
The goal of the PS-OC Education and Training Working Group is to facilitate future collaborations between physical scientists and cancer biologists by enhancing the cross-training of young investigators. The working group serves as a resource to help coordinate and share ideas, best practices, and insights for running PS-OC Education and Training Units and is made up of Education and Training coordinators from each PS-OC and OPSO program officials. The working group meets quarterly, including a face-to-face meeting during the Annual PS-OC Network Investigators' Meeting. During these meetings, group members present information on successful programs that they have run or courses that they have developed, discuss ideas for new programs or courses, and solicit advice on addressing problems or implementing programs.

Science Outreach and Dissemination

The Outreach and Dissemination Working Group is composed of leaders of the Outreach and Dissemination Units from each PS-OC, in addition to a representative from the OPSO. This working group was formed from the inception of the PS-OC Program and its members meet quarterly. The Outreach and Dissemination Working Group acts as a forum where the members inform one another of the outreach activities in their Centers, exchange ideas, and discuss how to reach out to the broader scientific community. The

Figure 2.10. Screen shot of the iTRAQR Interactive Network analysis tool depicting collaborations among PS-OC investigators.

"The initial objectives of the group were to discuss hurdles to understanding the mechanisms of CTC transport, challenge current assumptions using physical science principles, and identify collaborative experiments to test new hypotheses in this area. Accomplishments to date include new Trans-Network projects, collaborations, student exchanges, and a special issue collection of publications (Therapeutic targeting of circulating tumor cells) related to the topic."



Outreach and Dissemination Working Group also partners with relevant professional societies (e.g., to generate editorials and "thought leader" pieces for professional society newsletters and journals) to promote PS-OC opportunities and advances. The accomplishment of the individual PS-OCs and their researchers are highlighted on regular basis in the PS-OC newsletter (print and HTML), which is developed and distributed by the Outreach and Dissemination Working Group.

PS-OC Steering Committee Operations Subgroup

The goal of the PS-OC Steering Committee Operations Subgroup is to ensure the efficient and uniform implementation of policies and procedures enacted by the Steering Committee. The PS-OC Operations Subgroup serves as a liaison between the Steering Committee, the individual Centers, and the broader PS-OC community and is made up of the Center Administrators from each PS-OC along with OPSO program officials. The Operations Subgroup has regularly scheduled quarterly teleconference meetings during which members of the group share information and discuss issues surrounding the implementation of PS-OC guidelines and policies. Specifically, the Operations Subgroup has served to address questions and clarify policies related to completing the annual and semi-annual progress reports, implementing Pilot Project and Exchange Programs, completing annual carryover requests, and preparing for annual site visits. The Operations Subgroup is an essential means of communicating a uniform consistent message to members of the PS-OC Network and making sure the needs of the Centers and the Program are addressed appropriately.

2.6.6 PS-OC Program Evaluation

The PS-OC Program is dedicated to building transdisciplinary teams and infrastructure to better understand and control cancer through the convergence of physical sciences and cancer biology. This is a new field, and there is no precedent on evaluation. Thus, we have built an infrastructure for a program evaluation allowing the PS-OC program officers to do an assessment of the program on an ongoing basis.

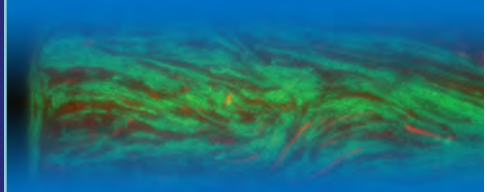
Based on an initial assessment of the program by the Science and Technology Policy Institute, a PS-OC Process/Outcome Evaluation was designed to assess the extent to which the PS-OC Program has been successful in reaching the goals listed in section 2.4.3 described in the initial RFA concept.

The recommended evaluation component is prospective data collection on activities and key outputs/outcomes. To help facilitate the collection of data, we have developed an Extended Scientific Reporting form that Network investigators use for NIH annual and NCI semi-annual progress reports. Aside from the traditional descriptions of scientific progress, investigators are asked to identify collaborations within and outside the Network, indicate red flags, and identify the Center's most novel findings for the reporting period. Collecting data on program activities and outputs prospectively serves the following purposes: (1) activities and outputs can be monitored by Program officials so that changes can be made as needed and (2) any errors or inadequacies

that are detected in the data can be addressed sooner rather than later.

In 2010, the PS-OC Program was awarded NIH Evaluation Set-Aside funds to perform a prospective evaluation of the Program that includes the construction of a real time tracking system for progress report data (iTRAQR) (Figure 2.10) and the development of unique indicators for analysis of collaborations, bibliometrics, and field convergence. These systems have been put in place to determine whether and how PS-OC funding builds infrastructure and sustains transdisciplinary science at awarded institutions. This system will also aid Program officers in identifying and maintaining the most successful components of the Program while adjusting or removing other components that are not effective. In 2012, PS-OC program officials released an online survey to collect additional information on program performance from PS-OC investigators, trainees, and administrators. The data from the survey have been used in combination with the data collected from the progress reports to provide a comprehensive assessment of the PS-OC Program.

"The PS-OC Network is unique in the manner in which it fosters collaborations across broad cross-sections from both the cancer biology/oncology and physical sciences/engineering communities. Each PS-OC is actively engaged in data generation, characterization, and analysis. Network investigators work collaboratively both within Centers and across the Network. The dynamics of the PS-OC Network require investigators to share data, knowledge, research materials, and other resources to support the goals and objectives of the PS-OC Program."



2.7. PS-OC Network Resources

2.7.1 Network Bioresources

PS-OC Network Bioresource Core Facility (PBCF)

The PS-OC Network Bioresource Core Facility (PBCF) was created in September 2010 following the initiation of the Cell Line Pilot Study, where one laboratory distributed two cell lines, the necessary cell culture reagents, and a detailed Standard Operating Protocol (SOP) to all Centers of the PS-OC Network. From the Cell Line Pilot Study it became apparent that utilization of cell lines and tissues with common reagents and SOPs is critical for cross comparison of data sets. Through a contract awarded to ATCC, the PBCF serves as a centralized biodistributor and biorepository providing PS-OC Network investigators with common stocks of authenticated cell lines and primary cells (non-malignant and cancerous), cell culture reagents, and related SOPs upon request. The PBCF has capability to prepare and distribute extracts of RNA, DNA, or protein from human cell lines, primary cells, and tissues. Moreover, any modified cell lines can be deposited by PS-OC Network investigators to the PBCF for authentication and distribution to collaborators within the PS-OC Network. The PBCF ultimately functions to increase both the time and cost efficiency of the transfer of biological specimens to and among PS-OC Network investigators.

Currently the PBCF houses 33 cell lines from ATCC stock and six cell lines that were deposited by PS-OC investigators (Table 2.1). All cell lines have been characterized and authenticated by ATCC. Detailed SOPs have been written which include growth curves and karyotype analysis for each cell line.

The PBCF currently offers cell lines in the biorepository at minimal cost. Going forward, the PBCF services will eventually expand to be a biorepository of human biological specimens, acquiring and authenticating tissues from PS-OC Network investigators in accordance with the NCI Best Practices for Biospecimen Resources guidelines established by the Office of Biorepositories and Biospecimens (OBBR). Movement in this direction will involve collaborations with other offices and divisions at the NCI as well as in-depth discussions with PS-OC Network investigators.

PS-OC Network Bioresource Core Facility

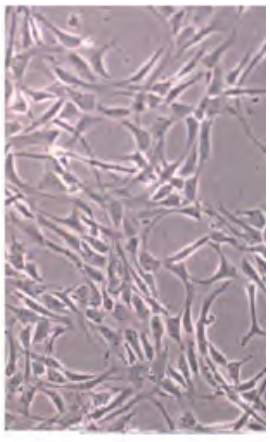
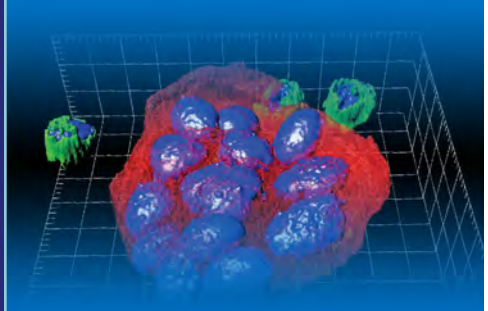


Table 2.1. PBCF cell lines available for distribution to PS-OC Network Investigators. Star * indicates a deposited third party cell line.

Tissue	Cell Line	Catalog #	Description
Breast	hTERT-HME1	CRL-4010	hTERT immortalized mammary epithelium, non-tumorigenic
Breast	MCF-7	HTB-22	weakly metastatic
Breast	T-47D	HTB-133	ductal carcinoma
Breast	ZR-75-1	CRL-1500	ductal carcinoma
Breast	MDA-MB-231	HTB-26	highly metastatic adenocarcinoma
Breast	DU4475	HTB-123	cutaneous metastatic nodule advanced breast cancer
Breast	HCC1937	CRL-2336	primary ductal carcinoma TNM grade IIB stage 3
Breast	MDA-MB-468	HTB-132	adenocarcinoma, triple negative
Breast *	MCF10A-JSB	NCI-PBCF-1000	non-malignant mammary epithelium
Breast *	MCF7-B7	NCI-PBCF-1001	Tamoxifen sensitive clone
Breast *	MCF7-G11-1	NCI-PBCF-1002	Tamoxifen resistant clone (1 μM)
Breast *	MCF7-G11-5	NCI-PBCF-1003	Tamoxifen resistant clone (5 μM)
Breast *	T47D-G11	NCI-PBCF-1004	Tamoxifen sensitive clone
Breast *	T47D-G9-1	NCI-PBCF-1005	Tamoxifen resistant clone (1 μM)
Prostate	RWPE-1	CRL-11609	HPV-18 immortalized prostate epithelium, non-tumorigenic
Prostate	22Rv1	CRL-2505	prostate carcinoma from primary site
Prostate	LNCaP	CRL-1740	metastatic prostate carcinoma from lymph node
Prostate	PC-3	CRL-1435	metastatic prostate carcinoma from bone
Prostate	DU 145	HTB-81	metastatic prostate carcinoma from brain
Prostate	VCaP	CRL-2876	metastatic prostate carcinoma from vertebrae
Brain	U-87	HTB-14	malignant glioblastoma
Brain	T98G	CRL-1690	glioblastoma multiforme
Ovary	HTB-75	Caov-3	ovarian adenocarcinoma from primary site
Ovary	SKOV-3	HTB-77	ovarian adenocarcinoma from ascites met
Ovary	NIH: OVCAR-3	HTB-161	ovarian adenocarcinoma from ascites met
Pancreas	hTERT-HPNE	CRL-4023	hTERT immortalized pancreatic epithelium, non-tumorigenic
Pancreas	Panc-1	CRL-1469	pancreatic epithelioid carcinoma
Pancreas	Capan-1	HTB-79	pancreatic adenocarcinoma from liver
Lung	NL20	CRL-2503	SV-40 large T antigen immortalized lung epithelium, non-tumorigenic
Lung	NCI-H2087	CRL-5922	non-small cell lung cancer adenocarcinoma lymph node metastasis (lung primary)
B-lymphoblast	NCI-BL2087	CRL-5965	B-lymphoblast (normal tissue) from NSCLC patient above
Lung	NCI-H2126	CCL-256	non-small cell lung cancer adenocarcinoma pleural effusion metastasis (lung primary)
B Lymphoblast	NCI-BL2126	CCL-256.1	B-lymphoblast (normal tissue) from NSCLC patient above
Colorectal	HT-29	HTB-38	colorectal adenocarcinoma; CpG island methylator phenotype
Colorectal	HCT116	CCL-247	colorectal adenocarcinoma; microsattelite unstable
Colorectal	Caco-2	HTB-37	colorectal adenocarcinoma; microsattelite stable
Colorectal	LoVo	CCL-229	colorectal adenocarcinoma; microsattelite unstable
Colorectal	SW480	CCL-228	colorectal adenocarcinoma; microsattelite stable
Colorectal	SW620	CCL-227	colorectal adenocarcinoma; microsattelite stable – derived from lymph node metastasis

"To promote the sharing of data and ideas both within and beyond the PS-OC Network, the Steering Committee has both established a PS-OC Data Sharing Policy and solicited the formation of a centralized PS-OC Data Coordination Center (DCC). The PS-OC DCC is being developed to facilitate (1) compliance with the NIH Data Sharing Policy, (2) sharing of data in a coordinated and timely fashion within and beyond the PS-OC Network, and (3) establishment of metrics and standards to promote uniformity and integration across the Network."



2.7.2 Data Collection and Coordination

The PS-OC Network is unique in the manner in which it fosters collaborations across broad cross-sections from both the cancer biology/oncology and physical sciences/engineering communities. Each PS-OC is actively engaged in data generation, characterization, and analysis. Network investigators work collaboratively both within Centers and across the Network. The dynamics of the PS-OC Network require investigators to share data, knowledge, research materials, and other resources to support the goals and objectives of the PS-OC Program. Furthermore, the integration of diverse data sets can facilitate orthogonal exploration by all PS-OC investigators to help generate answers to some of the big questions and barriers in cancer research and support the development of clinical advances.

PS-OC Intranet Site

The development of an intranet site was mandated by the PS-OC Steering Committee in October 2009 and launched approximately two months later in late December 2009. The site launched with 40 initial users and has grown to 200 registered users. To date, the site has been used as a calendar to post PS-OC meetings and events, announce conferences and symposia being presented by PS-OC members, and host the agendas, slides, and minutes from the PS-OC Steering Committee meetings. Additionally, the intranet site has played a critical role in (1) the PS-OC Cell Line Pilot Study, hosting data, presentations, and manuscript drafts, and (2) the Trans-Network Projects Program, where it hosts proposals in an open Wiki environment that enables collaborative proposal generation and commenting by other investigators. This site has served as a precursor to the more robust PS-OC Data Coordinating Center (PS-OC DCC) currently being developed.

PS-OC Data Coordination Center

To promote the sharing of data and ideas both within and beyond the PS-OC Network, the Steering Committee has both established a PS-OC Data Sharing Policy and solicited the formation of a centralized PS-OC Data Coordination Center (DCC). The PS-OC DCC is being developed to facilitate (1) compliance with the NIH Data Sharing Policy; (2) sharing of data in a coordinated and timely fashion within and beyond the PS-OC Network; and (3) establishment of metrics and standards to promote uniformity and integration across the Network. An initial solicitation to develop the DCC was issued in August 2011, and a number of bids were received. A team from the University of Tennessee, Knoxville (UT) and Oak Ridge National Laboratories (ORNL) was awarded a contract in April 2012 to work with the PS-OC Network in developing the DCC. The UT-ORNL team is currently working with PS-OC investigators to identify critical DCC needs and use cases to establish a DCC that will be a valuable asset to the Network.

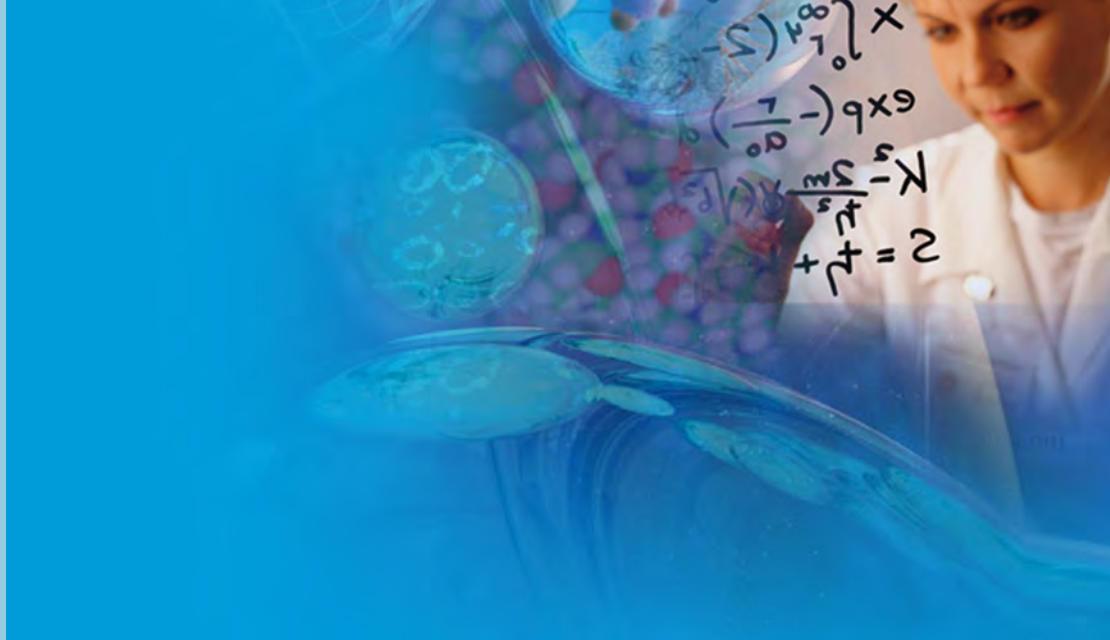
2.7.3 Genomic Characterization

To enhance the value of the PBCF cell line panel, facilitate downstream analysis of experiments, and allow for integration with and comparison to the vast amounts of genomic analysis being conducted, a detailed genomic characterization of the cell line panel will be conducted and the data will be made available to the PS-OC Network and the broader scientific community. The analysis will include:

- Exome sequencing of each cell line
- SNP and copy number analysis for each cell line
- RNA-sequencing based mRNA and miRNA expression analysis for each cell line

For a subset of the cell line panel (two cell lines) the analyses will be performed on early and late passages.

Several responses to the request for quotes have been received and are being reviewed, and work is expected to start on the project in the winter of 2012. The cells to be characterized will be acquired directly from the PBCF, and the team performing the analysis will work with the PBCF and PS-OC Network investigators to ensure that the cells are grown under conditions using the appropriate reagents and protocols. Additionally, the resulting data will be uploaded to the PS-OC DCC. There will be cross-talk between the PBCF and the DCC so that investigators ordering cell lines from the PBCF will be aware of the genomic characterization data associated with the cell lines of interest.



3. Program Performance

3.1. Reported Scientific Outputs and Research Highlights

Launched in fall 2009, the PS-OC Program consists of 12 specialized Centers that form a virtual Network of scientists spanning the fields of engineering, physics, mathematics, chemistry, cancer biology, and clinical oncology to explore new and innovative, perhaps unorthodox, approaches aimed at better understanding and ultimately controlling cancer. While there are many challenges in merging these disparate fields, the PS-OC Network has reported a large number of high-quality publications, patent applications, leverage funds, and unexpectedly started to inform development of secondary objectives in clinical trials. Many of these advancements are in parallel with the formation of new transdisciplinary collaborations at the Network and Center levels.

Since the start of the PS-OC Program, metrics have been collected on PS-OC Network collaborations, publications, patents, conferences, courses, and trainees in order to monitor the ongoing performance and make adjustments where needed to foster the best collaborative environment for highly innovative transdisciplinary science and training. The extent and scope of the scientific and training accomplishments of the PS-OC Network are reflected in these metrics. In just three years, the PS-OC Program has reported 538 publications, with an average impact factor of 9.18 and an average of more than 5.5 first year citations; more than 500 new collaborations resulting in a 17 percent increase in the number of transdisciplinary authorships on publications between PS-OC investigators since the start of the Program; more than $70 million in non-NIH leveraged funds; and 55 PS-OC courses taught or developed. These metrics are highlighted in more detail in the PS-OC Program Metrics–Year 3 Update report and are summarized in the table below.

The PS-OC Program: Year 3 Update

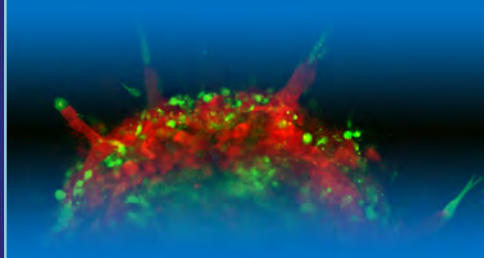
PIs New to NCI	8 of 12
Reported Publications	538
Average Impact Factor	9.18
Average First-Year Citations	6.54
Increase in Transdisciplinary Authorships	17%
Patent Disclosures	23
Non-NIH Leveraged Funds	$73 M
Trans-Network Publications	10
PS-OC Related Courses	55
PS-OC Trainees	684
PS-OC Related Clinical Trials	5

Throughout this report, key scientific advances, including unpublished work, that have contributed to these metrics are highlighted. These key advances span scales from the individual project level to the Center level to the Network level. This section emphasizes key scientific advances in each of the four thematic area of the PS-OC Program. The following sections of the report focus on scientific output from collaborative and Pilot Projects across Centers and the PS-OC Network. Network-wide advances, including Trans-Network funded projects selected by the PS-OC Network, are described in more detail in Section 5. Infrastructure built at the Center level to foster interactive environments for science and training as well as for approaching challenging questions from multiple length scales is summarized in Section 6.

The clinical implications and potential translation of PS-OC results is an unexpected outcome of the early stages of the Program. The interactions of physical scientists with clinicians have led to an increase in the number of projects using clinical samples and the translation of physical sciences based studies to clinical trials. The translation of mathematical models, technology, and physical parameters to the clinic has started and is documented in this section. To emphasize the translation potential of this research, these projects are marked with a green star. We anticipate this area to grow as new results are generated and the collaborations mature as an outgrowth of the PS-OC Network.

Under the umbrella of evolution and evolutionary theory, advances have included ... understanding how an evolutionary and mathematical framework can be used to optimize treatment schedules.

Advances made in the understanding the physics of cancer include identifying coordinated cellular movements that facilitate the proper formation of mammary acini."



3.2. Key Scientific Advancements with a Physical Sciences Perspective

The focus and structure of the PS-OC Program was influenced and shaped by a series of NCI-sponsored strategic Think Tanks that engaged extramural thought leaders from a wide range of fields to explore how the NCI could more effectively engage the physical sciences in cancer research. These strategic Think Tanks identified four thematic areas in which physical sciences approaches and principles could profoundly influence and improve our knowledge and understanding of cancer biology and oncology:

- Evolution and Evolutionary Theory of Cancer
- Physics (the Physical Laws and Principles) of Cancer
- Information Coding, Decoding, Transfer, and Translation in Cancer
- De-convoluting Cancer's Complexity

Each PS-OC organized its Center Framework and research projects around one or more of these themes and over the first three years of the PS-OC Program exciting progress has been made on aspects of each theme. Examples of research highlights for each theme are described in detail in the following subsections.

3.2.1 Evolution and Evolutionary Theory

The concept of applying the physics perspective of evolution and evolutionary theory to problems in cancer research is one supported by the PS-OC Program. Indeed, cancer as viewed by the physical sciences may be considered as a complex adaptive system that is most appropriately studied in the context of evolution and evolutionary theory in order to understand the origin and behavior of cancer cells at multiple length scales. At the foundation of this theme are experimental and theoretical models that support the development of an evolutionary computational physics approach to understand, predict, and control the cancer process.

Highlighted in this section are results compiled from several PS-OC investigators who use evolution and evolutionary theory approaches in cancer research. Dr. Michor at the Dana-Farber Cancer Institute (DFCI) PS-OC led evolutionary cancer modeling efforts designed to optimize drug dosing for non-small cell lung cancer with Dr. Pao at the Vanderbilt Ingram Cancer Center and investigator with the DFCI PS-OC. Informed by Dr. Pao's in vitro growth kinetics data for EGFR-mutant non-small cell lung cancer cell lines and patient data, Dr. Michor generated mathematical models to predict tumor behavior and calculate the optimized dosing schedule to delay the onset of T790M-mediated drug resistance. Currently two clinical trials are being designed based on these dosing strategy findings, one of which would be the first trial to try alternative dosing schedules with kinase inhibitors in cancer. At the Princeton University PS-OC, Dr. Austin custom designed a microenvironment to examine the kinetics of evolution of

drug resistance in bacterial cells under the stress of antibiotic treatment. Four key mutations that lead to resistance in the population in about 10 hours were identified. These findings make strides toward uncovering the kinetics of drug resistance driven by evolution in response to stress in cancer cells. Dr. Gatenby and Dr. Gillies at the Moffitt PS-OC used a combined mathematical and experimental approach to examine how acid buildup in the tumor microenvironment promotes tumor growth and invasion and determined that buffering the pH of the tumor microenvironment is a potential treatment strategy. When treated early with sodium bicarbonate buffer, spontaneous cancer development is reduced in a mouse model for prostate cancer and an improved outcome is observed in melanoma mouse models treated with ipilimumab. Finally, Dr. Holland, a neurosurgeon at the Memorial Sloan-Kettering Cancer Center has been working with radiation oncologists, Dr. Michor, and other mathematical modelers to calculate optimized dosing schedules for radiation therapy in mouse models of glioma. Based on parameters derived from a mouse model and evolutionary cancer modeling, the investigators generated a model of glioblastoma stem and differentiated cells, which was used to derive optimized radiation schedules that were tested and verified in mice. The investigators intend to initiate a clinical trial based on their findings. These advances in applying evolutionary theory to address key questions in cancer biology are detailed below.

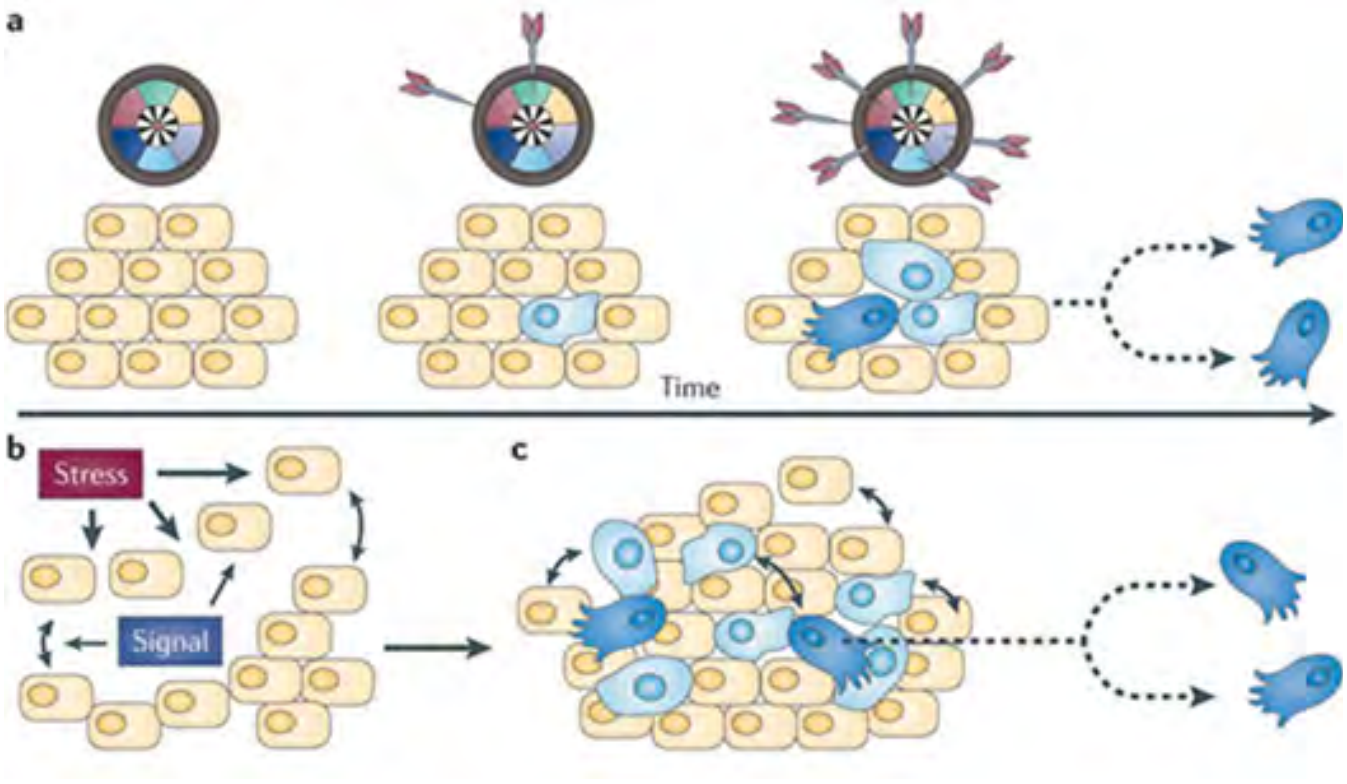


Figure 3.1. An example of a physical sciences perspective of evolution in cancer from the PS-OC Network. (A)The traditional view of cancer is as a cell-autonomous result of cumulative genetic mutations. Genes can be conceptualized according to their function as sectors on a dartboard that represent the hallmarks of cancer, and familial or acquired mutations can be thought of as randomly occurring dart strikes. (B-C) An alternative view of cancer as a collective stress response. Stress signals orchestrate the display of multiple adaptive phenotypes that are traditionally considered 'abnormal' and can include rapid proliferation and tumour cell dissemination. Normal and abnormal cells can coexist.

★ Optimization of Drug Dosing for Lung Cancer with Evolutionary Cancer Modeling

William Pao, Vanderbilt Ingram Cancer Center; Jasmine Foo, University of Minnesota; Franziska Michor, Dana-Farber Cancer Institute

Project Objectives and Significance

Non-small cell lung cancers (NSCLCs) that harbor mutations within the epidermal growth factor receptor (EGFR) gene are sensitive to the tyrosine kinase inhibitors (TKIs) gefitinib (Iressa) and erlotinib (Tarceva). Unfortunately, all patients treated with these drugs will acquire resistance, most commonly as a result of a secondary mutation within EGFR (T790M). Because both drugs were developed to target wild-type EGFR, the investigators hypothesized that current dosing schedules were not optimized for mutant EGFR or to prevent resistance. To investigate this further, Drs. Pao, Foo, and Michor developed isogenic TKI-sensitive and TKI-resistant pairs of cell lines that mimic the behavior of human tumors and used those pairs to show that the drug-sensitive and drug-resistant EGFR-mutant cells exhibited differential growth kinetics, with the drug-resistant cells showing slower growth. The investigators then incorporated these data into evolutionary mathematical cancer models with constraints derived from clinical datasets. These models predicted alternative therapeutic strategies that could prolong the clinical benefit of TKIs against EGFR-mutant lung carcinoma by delaying the development of resistance

Background

Gefitinib and erlotinib are first-generation EGFR TKIs that are clinically effective against those NSCLCs harboring mutations in exons encoding the kinase domain of EGFR. Unfortunately, lung tumors in all patients eventually develop acquired resistance to these two agents. The most common mechanism of resistance, observed in approximately 50 percent of cases, is a second site mutation within exon 20 of EGFR (T790M). This mutation alters binding of these drugs within the ATP pocket. Currently, targeted therapeutic options for T790M-harboring NSCLCs are limited.

Accomplishments and Scientific Advancements

Drs. Pao, Foo, and Michor hypothesized that because clinically available EGFR TKIs were developed against wild-type EGFR, current empiric dosing regimens were not optimally designed to inhibit the EGFR mutants in NSCLC or to minimize the development of drug resistance. In testing this hypothesis, the investigators identified differences in the growth kinetics of TKI-sensitive and TKI-resistant (T790M-containing) isogenic NSCLC cells and incorporated these findings, along with patient data, into evolutionary cancer models to generate mathematical models predictive of tumor behavior (Figure 3.2). The team modeled the drug-sensitive and drug-resistant cell populations as a multitype binary branching process. Application of the experimentally determined estimates of viable cells as well as apoptosis in the presence and absence of drug generated fitted curves describing the birth and death rates of both cell populations as a function of the concentration of erlotinib. These curves were used to predict the risk

Green star = clinical implications

Physical Sciences Perspective

The iterative development of experimental data from cell lines and patients and evolutionary theory-based mathematical frameworks was used to develop a computational model to predict the risk of resistance. The model was optimized over all possible dosing strategies to identify those predicted to lead to resistance least quickly.

Summary of Research Highlight

Evolutionary cancer modeling coupled with an understanding of the unique biological properties of TKI-sensitive and TKI-resistant cells has allowed this team to propose optimized dosing schedules for the treatment of EGFR-mutant lung cancer. This approach could be more generally applied toward the optimization of dosing strategies of other targeted therapies used against oncogene-driven cancers.

Implications for Cancer Research

Mathematical models based on the characteristics of diverse cancer cell types could offer clues for designing optimal treatment strategies for diverse cancer types with the potential to improve treatment outcomes.

of resistance and expected number of resistant cells for each dosing strategy and to optimize over all possible strategies to identify the ones predicted to lead to resistance the least quickly.This approach identified several strategies, validated in vitro and in vivo, to improve the treatment of EGFR-mutant NSCLC before and after the emergence of T790M-mediated acquired resistance.

Future Plans

This research team is planning to implement these insights into several new clinical trials. In the first trial (ongoing discussions with OSI Pharmaceuticals, the developers of erlotinib), the researchers will investigate the optimized dosing schedule—a low-dose continuous and high-dose pulsed combination—that should delay disease progression. This would be the first trial to try alternative dosing schedules with kinase inhibitors in cancer. In the second trial, sponsored by Genentech, patients with EGFR mutant lung tumors treated with first-line erlotinib will be randomized to chemotherapy alone (carboplatin/pemetrexed) or chemotherapy plus erlotinib.

Publications

Chmielecki, J., *et al*. Optimization of dosing for egfr-mutant non-small cell lung cancer with evolutionary cancer modeling. *Sci Transl Med* **3**, 90ra59 (2011).

Foo, J. & Michor, F. Evolution of resistance to targeted anti-cancer therapies during continuous and pulsed administration strategies. *PLoS Comput Biol* **5**, 6 (2009).

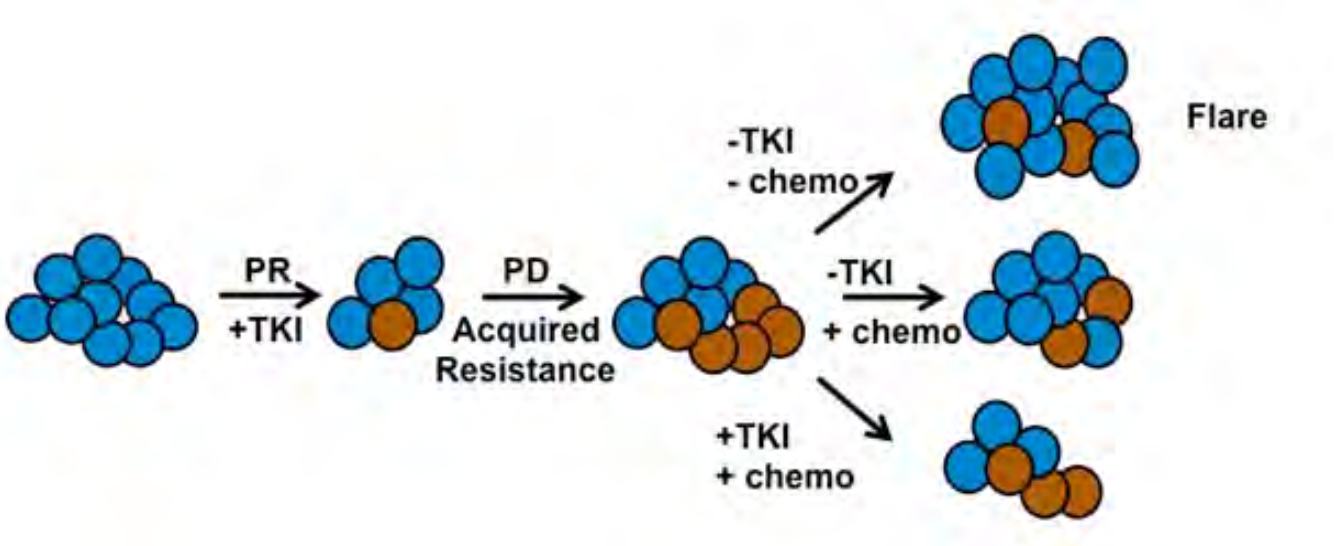


Figure 3.2. The natural history of some epidermal growth factor receptor (EGFR) mutant lung cancers can be explained by differential growth rates between drug sensitive/ resistant cells. In the schematic above blue circles represent tyrosine kinase inhibitors (TKI)-sensitive cells and brown circles TKI-resistant cells. Based on clinical data, there is usually an initial partial response (PR) to TKIs followed by acquired resistance and progression of disease (PD). Mathematical models suggest that continuation of TKIs with chemotherapy (chemo) at optimized dosing schedules may delay disease progression.

Physical Sciences Perspective

This work combined the use of a unique fabricated microenvironment with a heterogeneous fitness landscape to uncover the fundamentals by which rapid evolution occurs. This approach integrates perspectives from three disparate fields: physics of complex systems, evolutionary biology, and mechanistic molecular biology.

Summary of Research Highlight

The combination of fabricated microenvironments and stress gradients led to resistance fixing in a bacterial population within 10 hours of exposure to antibiotic. This work shows that this physics-based ecological process can indeed enormously accelerate evolution and has implications for understanding the development and penetration of resistance to cancer therapies.

Implications for Cancer Research

Structured microenvironments provide a more realistic approach to understanding the evolution of drug resistance in real-world environments that are spatially complex and full of chemical and nutrient gradients. The application of such approaches to understand the dynamics evolution of resistance to cancer therapies has the potential to revolutionize our understanding of this phenomenon.

Acceleration of Emergence of Bacterial Antibiotic Resistance in Connected Microenvironments

Qiucen Zhang, Guillaume Lambert, and Robert H. Austin, Princeton University; David Liao, University of California, San Francisco; Hyunsung Kim and Nader Pourmand, University of California, Santa Cruz; Kristelle Robin, Hong Kong University of Science and Technology; Chih-kuan Tung, University of Pittsburgh

Project Objectives and Significance

The emergence of bacterial antibiotic resistance and tumor anticancer drug resistance represent major limitations in the treatment of infection and cancer, respectively. Yet the variables that influence the rate of emergence of resistance are not well understood. Knowledge about the rapid emergence of antibiotic resistance in the heterogeneous conditions within the mammalian body may be helpful in understanding the emergence of drug resistance during cancer chemotherapy.

Background

Systematic emergence of antibiotic resistance in bacteria remains a persistent problem world wide. Genetic analyses following the isolation of resistant mutants have shed light on the biological processes that are altered in mutant bacteria, although such studies fail to probe how such mutations occur and spread within a population during antibiotic treatment. In particular, the importance of spatial heterogeneities and their effect on evolutionary processes during the emergence of antibiotic resistance is often overlooked. For example, the dynamics of the evolution of individuals on a heterogeneous fitness landscape can be accelerated if the population is broken into smaller populations with weak interchange of mutant individuals among the populations. A spatially complex environment may lead to an enhanced rate of evolution for two reasons. First, if a stress gradient is imposed on a connected network of populations, and if a mutant acquires some resistance to the local stress, the relative fitness of the mutant is increased if it moves to join a population exposed to even higher stress. Second, because there are fewer individuals in the region of higher stress, the mutant can fix more quickly in the smaller population.

Accomplishments and Scientific Advancements

This work identified four apparently functional single nucleotide polymorphisms (SNPs) that rapidly and repeatedly fix in a population. The 4 SNPs were found in 5 strains sampled from 3 independent experiments and arose and fixed within the population within 10 hours of exposure to antibiotic in a microfabricated device termed the "death galaxy" (Figure 3.3). A detailed understanding of the order in which the SNPs occur is essential, but it is unlikely that the four SNPs emerged simultaneously; in all likelihood they are sequential. Data obtained in the death galaxy device offer a template for exploring the rates at which bacterial antibiotic resistance arises in the human body. This provides a new framework for exploring rapid evolution dynamics in the development of cancer cell resistance.

Future Plans

There are fundamental questions to pursue in order to understand the surprising results produced by this work and how they can be incorporated into a consistent evolutionary framework: (1) Is the process time reversible? (2) Where are the hitchhikers (i.e., the passenger versus driver mutations)? (3) Is the process of rapid mutation a result only of replication errors? (4) What happens when agents compete in a high evolution rate environment? In the experiments conducted so far, a single strain of bacteria competes with itself, and a mutant emerges and fixes in the population. This is just a first step toward a true real-world evolution experiment in which bacteria with different phenotypes compete against each other in a true arms race of evolution.

Publication

Zhang, Q., *et al.* Acceleration of emergence of bacterial antibiotic resistance in connected microenvironments. *Science* **333**, 1764-1767 (2011).

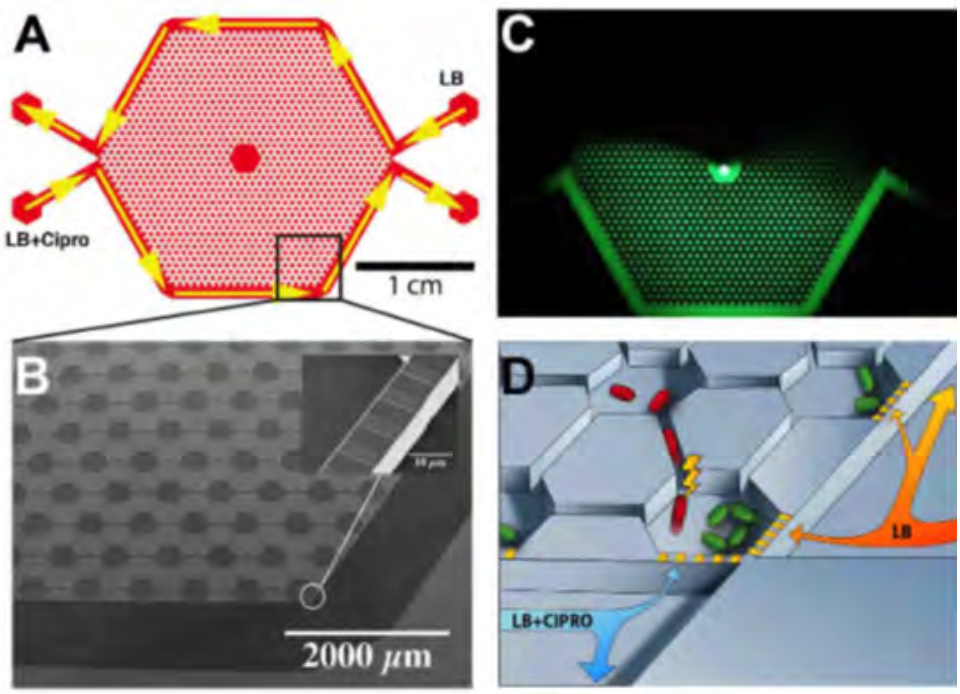


Figure 3.3. (A) An overview of the entire microenvironment, showing the flow of the nutrient streams and the nutrient + Ciproflaxin containing streams. (B) Scanning electron microscope (SEM) image of the area of the array outlined by the box in (A). (C) Image of the expected Ciproflaxin concentration gradient using the dye fluorescein as a marker. (D) The basic design of this micro-ecology creates high stress using constriction of nutrient flow via nanoslits in the presence of an antibiotic gradient.

★ Acid-Mediated Invasion and Therapy

Robert A. Gatenby, Robert J. Gillies, Arig I. Hashim, Veronica Estrella, Mark Lloyd, and Mark Robinson-Tessi, H. Lee Moffitt Cancer Center & Research Institute

Project Objectives and Significance

High glucose metabolism and poor perfusion in solid tumors result in a net production of acid that is then exported into the surrounding stroma. To evaluate the hypothesis that tumor-derived acid facilitates local invasion, this project combined mathematical modeling and empirical studies using a dorsal window chamber. Based on the results of these studies, the investigators are now developing acid-neutralizing therapies designed to reduce metastasis formation. If successful, this program will develop and characterize a novel suite of therapies to retard the formation of metastatic lesions and prevent the transition of in situ to invasive cancers.

Background

Conversion of a normal epithelium to metastatic carcinoma occurs through a series of stages accompanied by genetic and epigenetic changes. These changes represent steps along an evolutionary process and hence are successful responses to sequential environmental selections. It is self-evident that commonly observed traits, or "hallmarks," of cancer must represent successful adaptation strategies to commonly experienced environmental selection pressures. Elevated glucose uptake, as evidenced by FdG-PET scans of cancer patients, is also a "hallmark of cancer," as FdG avidity is elevated in almost all metastatic carcinomas. Importantly, this glycolytic phenotype is independent of tumor oxygenation. The end products of glucose metabolism are acids; hence, the extracellular pH (pHe) of solid tumors is significantly lower than that of normal tissues. There is accumulating evidence that this lower pH of the tumor relative to normal tissue enhances the ability of cancer cells to invade locally. Furthermore, neutralization of acids with oral buffers can inhibit transition from in situ to invasive cancer, local invasion of primary tumors, and metastasis formation.

Accomplishments and Scientific Advancements

Quantification of pH gradients and tumor growth has led to the remarkable observation that tumors are growing and invading into areas with the lowest pH. The dorsal window chamber (DWC) has been developed as a reliable and reproducible system for investigation of tumor and peri-tumoral acidosis at the microscopic level. This team has established a system in which tumors within a defined stromal matrix are implanted into a DWC on immunodeficient mice. This system can be interrogated with multiphoton or confocal microscopy and can deconvolute up to four different fluorescent dyes. With this system, tumor borders and growth can be accurately defined using cells expressing fluorescent proteins, while patency of vessels can be monitored using blue dextran. In addition, spatial changes in pH can be measured with ratiometric imaging of the pH-sensitive dye, SNARF-1, and high-resolution images of glucose uptake can be obtained using FdG with a novel-imaging technology. Quantification of pH gradients

Physical Sciences Perspective

The investigators combined mathematical modeling and empirical studies using a dorsal window chamber to investigate the role of the tumor physical microenvironment, the non-organic components of the microenvironment, in regulation of growth and invasion. More generally, this team's approach is based on the fundamental physics paradigm that complex systems have simple first principles.

Summary of Research Highlight

This work demonstrated that low extracellular pH stimulates tumor growth and invasion and that increasing intra- and peri-tumoral pHe using systemic buffers can inhibit local tumor growth and inhibit the transition from in situ to invasive cancer and metastases formation. These findings suggest that intra- and extra-tumoral pHe represent fundamental mechanisms by which many, perhaps all, tumors promote growth and invasion.

Implications for Cancer Research

This team has shown that it can delay and even prevent development of both primary and secondary cancers by altering the physical dynamics of the system. These results could have enormous impact on future therapy and prevention strategies.

Green star = clinical implications

and tumor growth has led to the remarkable observation that tumors are growing and invading into areas with the lowest pH (Figure 3.4). Tumor growth, invasion, and acidity in this system are inhibited when mice ingest water containing 200 mM sodium bicarbonate. The acid-mediated invasion hypothesis initially grew out of a mathematical model. The investigators are using this new data to create improved mathematical models to investigate the growth and invasion of acid-producing tumors.

Based on findings from the DWC experiments and the modeling work, the investigators have initiated work to translate these findings. This group examined the effects of buffer therapy on spontaneous cancers and immune function. These studies have shown that, if begun early, buffer therapy can prevent the emergence of spontaneous cancers in TRAMP mice and that buffer therapy improves outcome in melanomas treated with ipilimumab. Bicarbonate clinical trials in patients experiencing pain associated with pancreatic cancer were initiated. These trials showed some positive effect but were marred by low compliance and some adverse events, and they were suspended in late 2011. The bicarbonate has been reformulated in capsules, and the pain trial has been rewritten as a 3 + 3 phase I with phase II expansion at maximum tolerated dose.

Future Plans

This work demonstrated that low extracellular pH stimulates tumor growth and invasion and that increasing intra- and peri-tumoral pHe using systemic buffers can inhibit local tumor growth and inhibit the transition from in situ to invasive cancer and metastases formation.

Publications

Ibrahim-Hashim, A., *et al.* Systemic buffers inhibit carcinogenesis in tramp mice. *J Urol* **188**, 624-631 (2012).

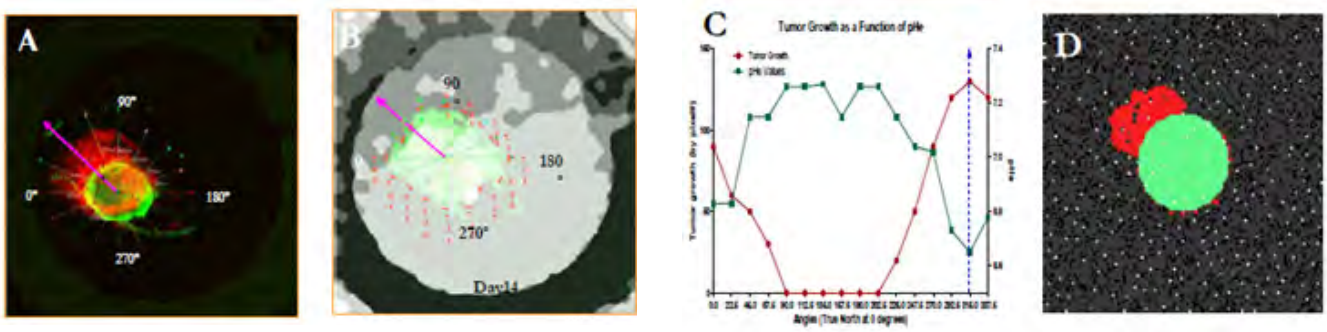


Figure 3.4. Acid mediated Invasion. (A) Growth of HCT116 tumors in DWC. Day 4 (green) and day 14 (red) tumor images are superimposed. (B) pHe map derived from SNARF-1 fluorescence ratios on day 14. (C) Growth and pHe data from A and B were plotted as a function of angle around tumor centroid. (D) Growth of tumors modeled using modified cellular automata based on acid-mediated invasion (Veronica Estrella and Mark Robertson-Tessi).

Physical Sciences Perspective

This work utilized evolutionary theory and a mathematical model based on a differential equation system describing the behavior of different subpopulations of cells during radiation therapy to predict the response rates to and efficacy of different administration schedules. Optimization over all schedules was performed using dynamic programming tools to identify the best dosing strategy.

Summary of Research Highlight

This team developed an evolutionary model of glioblastoma stem and differentiated cells. The parameters of the mathematical model were informed with data of a mouse model of glioblastoma. Model-based predictions regarding the optimum dosing schedule were obtained. The predictions were validated in the mouse model and led to almost double the survival of the mouse cohort.

Implications for Cancer Research

If the survival curve for GBM can be shifted using no more than a change in schedule for delivery of the same overall dose of radiation that patients are getting now, this would be one of the biggest advances in GBM therapy over the last several decades.

★ Optimization of Radiation Dosing for Brain Cancer with Evolutionary Cancer Modeling

Eric C. Holland and Timothy Chan, Memorial Sloan-Kettering Cancer Center; Franziska Michor, Dana-Farber Cancer Institute; Kevin Leder, University of Minnesota

Project Objectives and Significance

This work used mathematical models to calculate optimal dosing schedules for radiation therapy in mouse models of glioma with the ultimate goal of prolonging survival in human patients. The investigators measured and calculated many parameter values for tumor cells in gliomas with respect to stem cell characteristics, numbers, relative response to therapy, and interconvertability with the bulk of the tumor. These parameter values were used to calibrate the mathematical model and predict optimal dosing schedules for 10 Gray (Gy) of radiation treatment. These predicted optimal dosing schedules were implemented in genetically engineered mouse models of gliomas to test their effect compared to standard dosing schedule of 2 Gy per day. The optimized schedule proved significantly more effective than standard therapy.

Background

Patients suffering from glioblastoma multiforme (GBM), the most common and malignant primary tumor of the brain, often have very poor outcomes. The standard of care—surgery, when possible, followed by radiation and chemotherapy—has not changed substantially over the past 50 years and neither has the overall survival rate for this disease. Over the years, oncologists have undertaken several attempts to update radiation therapy for these tumors, and dose escalation studies have demonstrated that survival improvements are seen up to an overall dose of 60 Gy. Beyond this point, there are little if any improvements in survival at the cost of increased toxicity. Typically, the dosing schedule is 2 Gy per day, five days per week for six weeks. Alternative schedules have been attempted, including hypo-fractionated dosing of 3-6 Gy per session or hyper-fractionated dosing of 1 Gy fractions twice per day. Neither of these strategies has shown improvements over the standard 2 Gy in one dose per day.

Accomplishments and Scientific Advancements

Drs. Holland, Michor, and Leder designed a mathematical model of proneural GBM stem and progenitor cells to investigate the effects of radiotherapy on cell numbers. The model considers two distinct subpopulations of cells: GBM stem-like cells and GBM differentiated cells. Stem-like cells reproduce symmetrically to give rise to two daughter stem-like cells or asymmetrically to produce differentiated cells. In addition to differentiating cell divisions of stem cells, differentiated cells may also de-differentiate to become stem-like cells. Stem-like cells are largely resistant to radiation therapy, while differentiated cells respond to radiation therapy via cell cycle arrest and apoptosis. The cell population response to radiotherapy was modeled using the linear quadratic model, which is widely accepted in the radiation literature as a result of its close agreement with experimental results. Using this combined model, therapy was optimized over all

Green star = clinical implications

possible strategies that deliver 10 Gy in one week, identifying an administration schedule that was predicted to outperform standard treatment strategies. Using PDGF-induced mouse model the effects on survival of schedules predicted by the mathematical modeling to optimally deplete tumor cells were validated. The results showed that the optimized 10 Gy schedule was significantly better than standard 10 Gy dosing (2 Gy per day) (Figure 3.5), and in fact almost as effective as 20 Gy delivered on the standard schedule. These data support the functional importance of the dynamic effects of stem-like characteristics to therapy and suggest that, at least in proneural GBM, the standard radiation schedule used may not be optimal. This team is now planning to implement this experimentally validated and optimized protocol as a clinical trial at MSKCC.

Future Plans

Given the data obtained in this project, the clinical radiation oncologists at MSKCC are interested in undertaking a clinical trial comparing standard dosing therapy to the optimized schedule in humans. This trial should be conducted within the next two years.

Publication

Leder, K., Holland, E.C. & Michor, F. The therapeutic implications of plasticity of the cancer stem cell phenotype. *PLoS One* **5**, e14366 (2010).

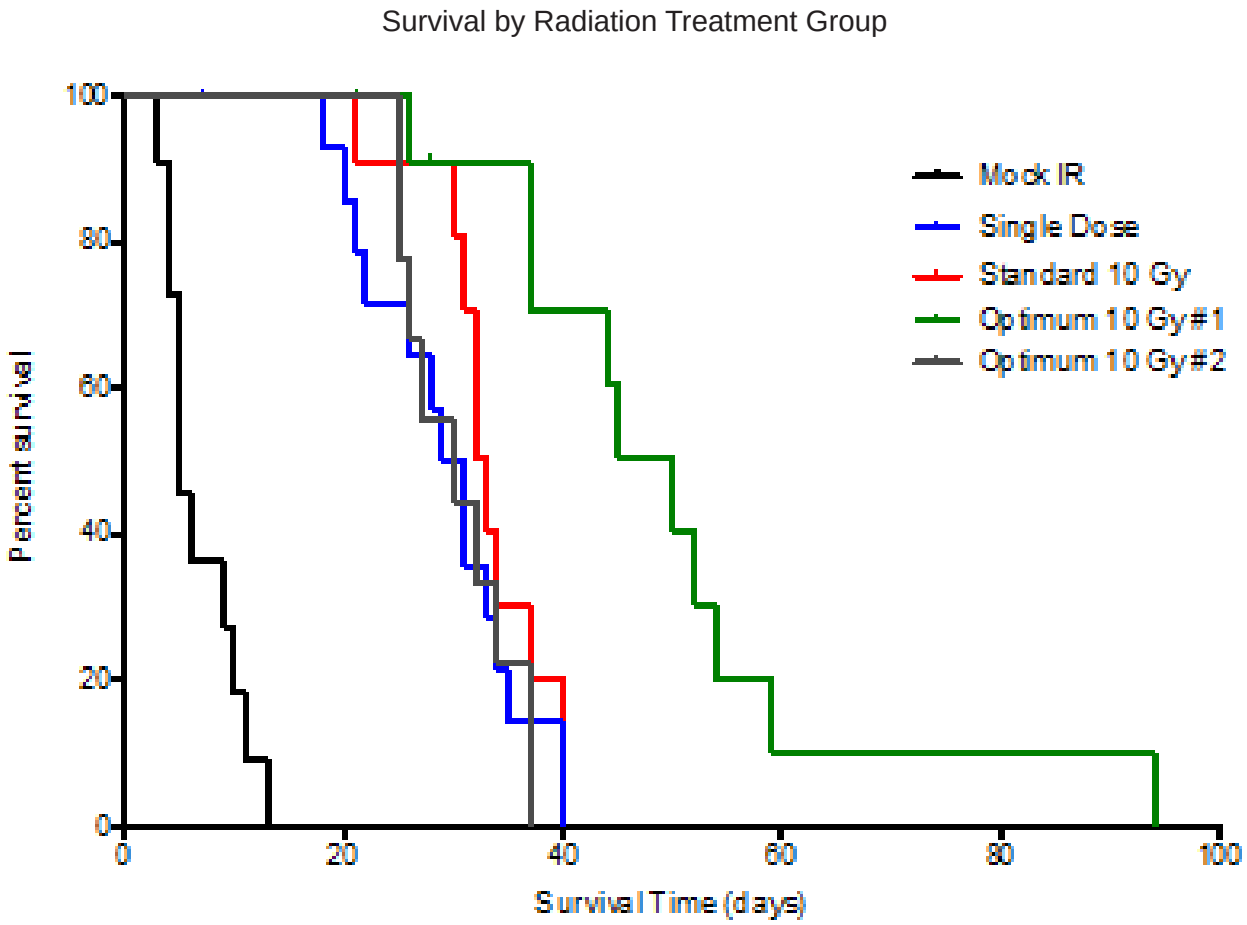
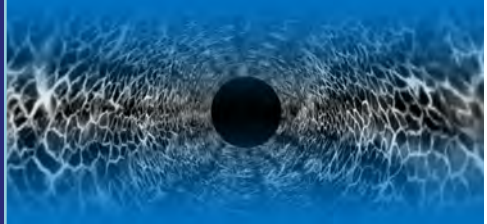


Figure 3.5. Survival of glioma-bearing mice following treatment of 10 Gy radiation in various fractions. The optimized schedule in green is nearly twice as effective as standard 10 Gy radiation divided into 2gy fractions, all at once, or other schedules.

"The application of physics principles and technologies to cancer allows scientists to gain knowledge about the potential impact of the biophysical components of the system, including diffusion, elasticity, topology, geometry, forces, and inertia, and to quantify the spatial-temporal dynamics of the system at all length scales."



3.2.2 Physics Principles and Technologies in Cancer

During the emergence and progression of cancer, cells and tissues transition through a series of defined changes to their behaviors and phenotypes that are often mediated or accompanied by altered physical properties at the subcellular, cellular, and tissue scale. Traditional biological approaches excel at identifying genes, proteins, and pathways that define cell phenotypes and cell function. However, these techniques are limited in their ability to understand spatial-temporal dynamics and identify biophysical components in the system that are not encoded in a single gene or pathway. The application of physics principles and technologies to cancer allows scientists to gain knowledge about the potential impact of the biophysical components of the system, including diffusion, elasticity, topology, geometry, forces, and inertia, and to quantify the spatial-temporal dynamics of the system at all length scales.

This section highlights work from the PS-OC Program investigating the role of physical properties in the development and progression of cancer, as well as the potential to ultimately exploit these properties and phenomena to detect and possibly treat cancer. Within the UCB PS-OC, two projects have examined the role of collective motion and force on formation of mammary acini architecture and progression to malignant phenotypes. Drs. Bissell and Tanner at Lawrence Berkeley National Laboratory examined the role of angular motion and inertia on the resulting architecture of glandular tissues. Using advanced optics, angular motion was found to be critical for the establishment of spherical architecture and not simply a consequence of multicellular aggregates. In a separate but related project, a collaboration between Drs. Liphardt and Weaver (PI and SI of the UCB PS-OC) identified long-range

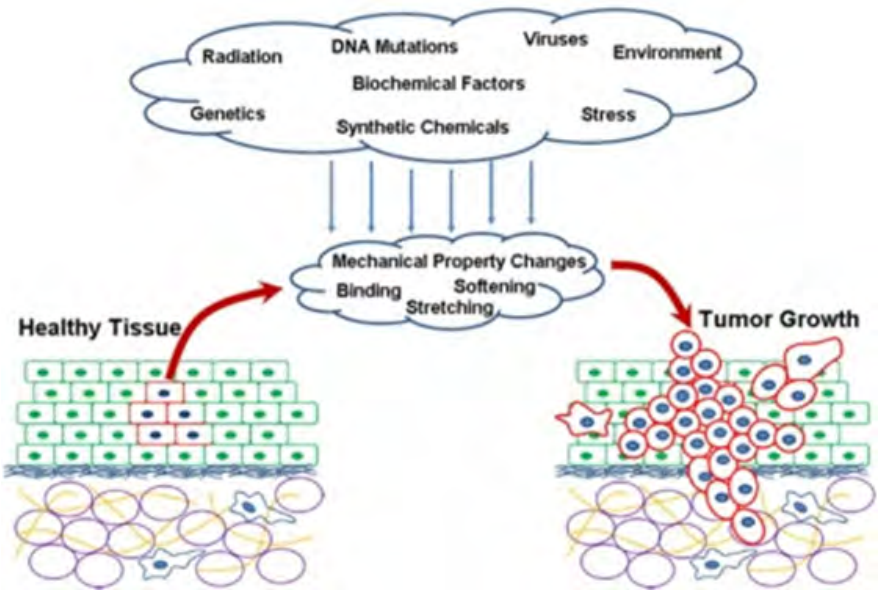


Figure 3.6. An example of the role of physics in cancer progressions. External factors drive changes in the mechanical and physical properties of cells, the extracellular matrix, and cell interactions that may drive cancer progression.

mechanical coupling between groups of mammary acini, suggesting that the likelihood for any one acinus to become disorganized is not only influenced by its own (local) genetic and microenvironmental defects, but by all the other acini around it. At the Cornell PS-OC, Dr. Reinhart-King discovered a potential mechanical biomarker for cancer—cellular traction force. Traction force, the stress and strain the cell places on its surrounding environment, increases as cells progress from normal to cancerous phenotypes in multiple cancer types. Questions remain on the downstream implication of this change in mechanical behavior of the cells with metastatic potential, and these questions are being investigated by the PS-OC. Finally, Dr. McCormick (UCSF), from the UCB PS-OC, is using super-resolution microscopy to address an outstanding question in cancer about the dynamics of Raf and Ras in time and space. The results confirm that Raf forms dimers under various activating conditions and that Ras also functions as a dimer in activating Raf/MAPK signaling. The novel insights from this study necessitate the revision of existing models on Ras/Raf signaling and may lead to novel strategies in targeting this pathway for cancer therapeutics.

Coherent Angular Motion in the Establishment of Multicellular Architectures in Glandular Tissues

Mina Bissell and Kandice Tanner, Lawrence Berkeley National Laboratory

Project Objectives and Significance

Cancer and tumor progression has been traditionally viewed as a dysfunction of signaling pathways, and a large number of studies have examined the genomic and proteomic response of cells to chemical molecules and protein-sized growth factors. While it is well established that cells respond to their chemical microenvironment, one understudied area is the response of cells to mechanical signals. Moreover, it has been recognized but not addressed that such changes in mechanical forces will result in biochemical feedback at different scales. It is therefore crucial to study the mechano-chemical feedback both at the cell as well as at the tissue and organ levels.

Background

Normal human epithelial cells in breast and other glandular tissue form either acini (spheres) or ducts (tubes). Dr. Bissel and colleagues were among the first to pioneer in vitro primary organotypic and three-dimensional cell culture for mouse and human mammary epithelial organs that recapitulate in vivo phenomena. The cell and tissue polarity that comes with the formation of acini is essential for tissue homeostasis. Loss of this polarity is one of the earliest signs of malignancy. To understand how the polarity and tissue architecture are lost in malignant tissues, it is first necessary to understand how normal cells are able to form the structures. To address the question of how single mammary cells can re-establish a polarized acinus in three-dimensional laminin-rich extracellular matrix (lrECM), human mammary epithelial cells (HMECs) were monitored with high-resolution real-time imaging to follow the establishment of these acini and compared to tumor formation for similar culture conditions of malignant cells. The investigators hypothesized that adult cells undergo a specific morphogenetic program to form and maintain quiescent acini and that this program is corrupted during malignant transformation.

Accomplishments and Scientific Advancements

The investigators identified a novel morphogenic behavior by which HMECs undergo multiple rotations in three-dimensional lrECM to establish an acinus. This movement, a spinning coherent angular motion (CAMo) with a self-generated centripetal force, proved to be required for spherical structures to develop. CAMo is conserved from primary human cells to established breast cell lines where the final realized geometry is a sphere. Cancer cells do not display CAMo and do not generate sustained centripetal forces, but instead undergo random uncoordinated motility (Figure 3.7). Additionally, cells that have long been removed from the instructive structural cues of the in vivo environment are able to re-enter a morphogenic program usually employed during embryonic development. Using sustained pharmacological inhibition of Myosin II, RhoA/Rho kinase (ROCK), and myosin light chain kinase (MLCK), activity of myosin light chain 2 (MLC2) was modulated in non-malignant cells, disrupting the generation of centripetal forces and resulting in establishment of non-polar structures, reinforcing the bidirectional relationship between physical and biochemical control of malignancy.

Physical Sciences Perspective

Advanced high-resolution imaging approaches were utilized to quantify and distinguish the different motilities involved during the establishment of multicellular structures. Specifically identifying cell generated centripetal forces as essential to establishment of spherical geometry.

Summary of Research Highlight

This work uncovered a novel type of cell motility, CAMo, in which single cells undergo multiple rotations and cohesively maintain that rotational motion as they divide and assemble into acini. CAMo is critical for the establishment of spherical architecture and not simply a consequence of multicellular aggregates. If CAMo is disrupted, the final geometry is not a sphere.

Implications for Cancer Research

These data suggest that the mechanisms of cell motility must be considered in addition to aberrations in the balance between proliferation and apoptosis as a regulator of tissue architecture.

These results show that myosin-regulated centripetal force can be added to the repertoire of known mechanisms by which cells can navigate their three-dimensional microenvironment. This movement highlights an often-ignored fact that cells are not simply passive responders to external mechanical perturbations but that they generate contractile forces to locally remodel their microenvironment to provide the structural cues to dictate the desired multi-cellular assembly.

Future Plans

This team aims to control a cell's ability to generate centripetal forces or the appropriate forces needed to establish specific geometries, such as tubes versus spheres. It also intends to quantify the relative importance of mechanical stresses for transition between malignant and reverted phenotypes by applying force gradients in their culture models.

Publications

Tanner, K., Mori, H., Mroue, R., Bruni-Cardoso, A. & Bissell, M.J. Coherent angular motion in the establishment of multicellular architecture of glandular tissues. *Proc Natl Acad Sci U S A* **109**, 1973-1978 (2012).

Press release:
http://newscenter.lbl.gov/news-releases/2012/01/26/camo-discovery/

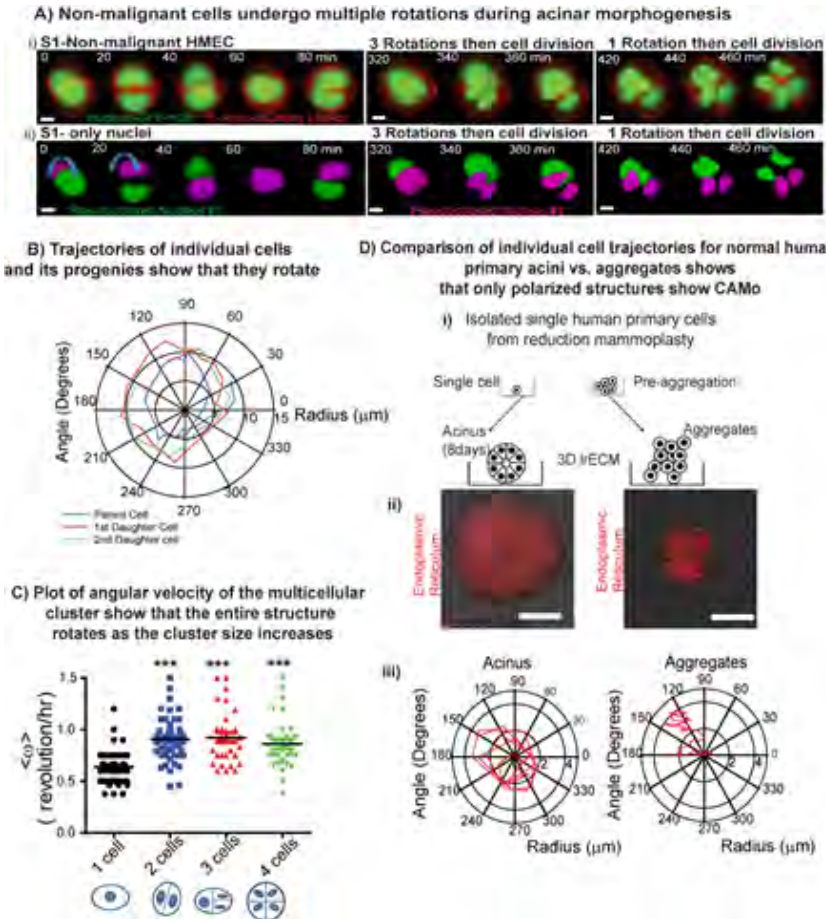


Figure 3.7. (A) Non-malignant cell lines undergo multiple rotations during mammary acinar morphogenesis. (i) Timed micrographs of S1 cells show the ordered development of multicellular aggregates as revealed by nuclear position and F-actin. Cells undergo multiple rotations before mitosis. (ii) Same micrographs as in i but showing only nuclei where one is false-colored purple. Blue arrows indicate the direction of rotation. (B) Angular rotation of the parent cell and its progeny by tracking the individual nuclei during acinar morphogenesis. (C) Average angular velocity as a function of increasing number of cells comprising multicellular structure. Using Mann–Whitney U test where $P < 0.0001$. (D, i) Schematic of experimental design. (ii) Micrographs showing acinus (8 d) (Left). (Right) The aggregates (6 h). Red represents the endoplasmic reticulum. (Scale bars, 50 μm.) (iii) Graphs comparing acinus vs. aggregates for a period of 24 h.

Cellular Traction Stresses Increase with Increasing Metastatic Potential

Cynthia Reinhart-King, Cornell University

Project Objectives and Significance

The objective of this project is to investigate traction force generation as a potential biophysical marker of metastatic ability. Because cells use physical force to both remodel and move through matrix, it was hypothesized that cells with higher metastatic activity exert greater traction force than do cells with lower metastatic activity. To test this hypothesis, traction force microscopy (TFM) was used to quantify contractile forces of highly metastatic breast, prostate, and lung cancer cell lines and these forces were compared to those exerted by non-tumorigenic epithelial cell lines. The resulting data indicate that cellular force increases with metastatic potential. Importantly, these data indicate that cellular force is a mechanical biomarker of metastasis that may be used to predict and prevent metastasis.

Background

During metastatic progression, phenotypic changes in cancer cells result in altered adhesion and migration behavior, allowing cells to escape from the tumor mass into surrounding tissue. To achieve this invasion, tumor cells must generate force to reorganize the basement membrane, invade into surrounding stroma, migrate along ECM fibers, and transmigrate through the endothelial cell barrier to enter the circulatory or lymphatic system. It is not clear how forces differ between non-metastatic and metastatic cells. During cancer progression, tumor cells are exposed to the continually changing physical properties of the tumor microenvironment, many of which could directly affect cellular force generation. For example, metastasizing cancer cells are exposed to both the increased stiffness of the stroma surrounding most solid tumors, as well as more compliant adipose tissue during invasion. During cancer progression, collagen metabolism is dysregulated, with elevated expression, increased deposition, and an increase in crosslinking that also contributes to the overall stiffening of the surrounding tumor microenvironment.

Accomplishments and Scientific Advancements

These studies were the first to compare the traction stresses of paired metastatic and non-metastatic cells from several human cancer models. In a series of experiments, three pairs of cell lines derived from metastatic and non-metastatic breast, prostate, and lung cancer cells were analyzed using TFM. Cells were seeded onto polyacrylamide (PA) gels whose mechanical and chemical properties could be tuned independently. By varying the ratio of bis-acrylamide (crosslinker) to acrylamide (monomer), PA gels were generated with a Young's Modulus (E) between 1 and 10 kPa, which is within a physiologically relevant stiffness range. Additionally, by varying the concentration of collagen with which the gels were incubated, the type I collagen density of the PA gels was tuned to between 0.0001 and 0.1 mg/mL. Experiments conducted using these gels indicate that metastatic breast, prostate, and lung cancer cells exhibit significantly stronger traction stresses than the non-metastatic cells across all matrix properties studied (Figure 3.8). Additionally, a significant increase in net traction force was measured with increasing substrate stiffness and increasing collagen density

Physical Sciences Perspective

Advanced imaging and biophysical measurement approaches were used to investigate the role that tractions stresses play in the metastatic process. The results suggest that cellular contractile force is a mechanical biomarker of metastatic potential.

Summary of Research Highlight

Cells use traction stresses to migrate and navigate through the extracellular matrix. This work deomonstrates that traction stresses increase with metastatic potential and that increased collagen density and increased matrix stiffness mimicking the physical properties found in the tumor stroma further increase tractions stresses. These data suggest that traction stresses may be a mechanical biomarker of metastasis.

Implications for Cancer Research

This work demonstrates that traction stresses may be a mechanical biomarker of metastasis and that identifying a mechanism to therapeutically target cellular force may be one promising avenue for inhibiting metastasis.

within all six cell lines, suggesting that both the elevated stiffness and the increased collagen content within the tumor microenvironment can drive force generation of cancer cells.

The data from these experiments also showed that cell spreading for these cell lines has a direct relationship with collagen density, but a biphasic relationship with substrate stiffness, indicating that cell area alone does not control the magnitude of traction stress generation. Together, these data suggest that contractile force may play an important role in metastatic invasion and that the physical properties of the stromal environment surrounding the tumor cells may regulate cellular force generation. These findings increase our understanding of the physical mechanisms of metastasis and the role that the extracellular microenvironment plays in metastatic progression. Additionally, the data suggest that identifying a mechanism to therapeutically target cellular force may be one promising avenue for inhibiting metastasis.

Future Plans

The next step of this project will be to investigate the force profiles of primary cells and compare these results to patient outcome. The results and knowledge gained using this two-dimensional system will be applied to study cellular force and migration in a more physiologically-relevant three-dimensional environment.

Publication

Kraning-Rush, C.M., Califano, J.P. & Reinhart-King, C.A. Cellular traction stresses increase with increasing metastatic potential. *PLoS One* **7**, 28 (2012).

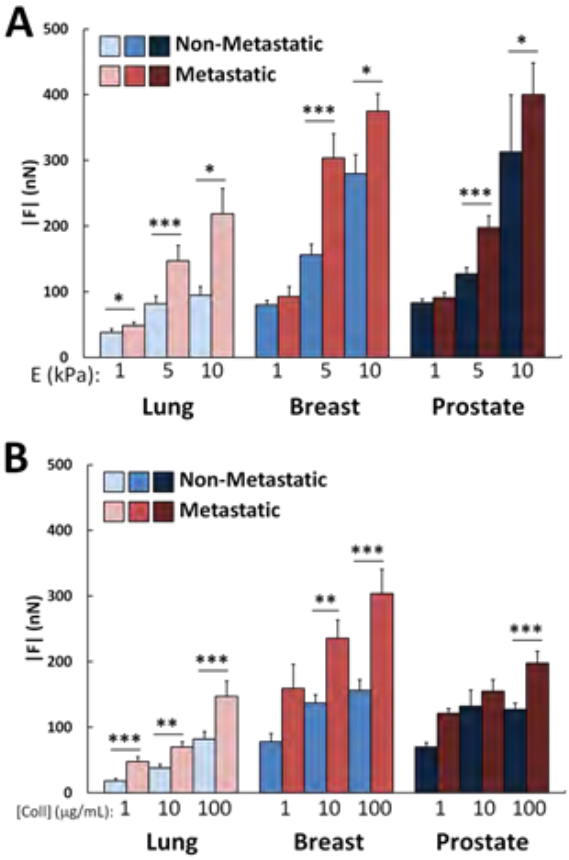


Figure 3.8. Traction Stresses Increase with Metastatic Potential. By independently varying stiffness and collagen density of polyacrylamide substrates, the effects of matrix mechanics and chemistry on cancer cell tractions were determined. These data indicate that increased stiffness (A) and collagen density (B) promote increased force. Metastatic cells generate higher forces than non-metastatic cells across a broad range of stiffness and collagen density. Mean + SEM;* indicates $p < 0.05$; ** indicates $p < 0.01$; *** indicates $p < 0.001$.

Single-Molecule Super-Resolution Imaging Reveals Dimerization-Dependent Ras/Raf Signaling

Frank McCormick, Tanja Tamguney, Eric Collisson, and Cameron Pitt, University of California, San Francisco; Xiaolin Nan, University of California, Berkeley

Project Objectives and Significance

Ras/Raf signaling plays a central role in cell physiology and is frequently implicated in human cancer. Efforts to target components of this pathway for treating cancers driven by oncogenic Ras have often failed, calling into question current models of the spatial-temporal regulation of Ras/Raf signaling. This team of investigators aimed to uncover the detailed molecular mechanisms of signal transduction along the Ras/Raf pathway with novel single-molecule super-resolution imaging approaches. The results provide direct evidence for Raf dimerization and reveal previously unknown mechanisms in cellular regulation of Ras activity, also through protein dimerization. These discoveries may lead to novel strategies in targeting Ras/Raf for cancer therapy.

Background

Ras and Raf are among the first identified oncogenes and have been studied extensively with biochemical, genetic, and conventional imaging methods, all of which have helped define the Ras/Raf/MAPK signaling pathway. Specifically, active, GTP-loaded Ras recruits Raf to the cell membrane, where the latter is activated and switches on downstream MAPK signaling. However, given the unexpected failure of small molecule inhibitors of Raf (RAFi) to treat tumors driven by oncogenic mutations in Ras, this simple model should now be considered inadequate. A key event in causing the loss of RAFi efficacy in these tumors is Raf dimerization or oligomerization upon drug treatment. In addition, some studies suggest that Ras also organizes into higher order structures for effective signaling on the cell membrane. These data allude to novel, spatial-clustering mediated regulation mechanisms of Ras/Raf signaling, but do not provide critical details to refine the current model. The recent advent of single-molecule super-resolution imaging techniques, such as photoactivated localization microscopy (PALM), has provided powerful tools to retrieve the missing molecular details of Ras and Raf during signaling. PALM offers simultaneous molecular enumeration and nanometer localization of proteins in intact cells and allows direct resolution of protein oligomers in intact cells.

Accomplishments and Scientific Advancements

A broad set of tools, including biochemical and super-resolution imaging and image analysis, were developed to characterize protein clustering or oligomerization and define its relationship with signaling. Using these tools, artificial protein dimers were imaged directly with PALM (Figure 3.9), and Ras/Raf protein organization was studied in detail under various signaling conditions. The results provided new insights into Ras and Raf signaling, including direct evidence for Raf dimerization under activating conditions that involve active (mutant) Ras, small molecule Raf kinase inhibitors (e.g., GDC-0879 and PLX4032), truncation of N-terminal regulator domains (e.g., catalytic domain of C-Raf), and artificial membrane localization (e.g., c-Raf-CAAX). A detailed analysis of cluster distributions under these conditions defined two distinct mechanisms that drive

Physical Sciences Perspective

The studies presented here demonstrate the power of advanced measurement tools in retrieving critical, quantitative information from complex biological systems. Emerging tools have brought measurements down to single molecule levels in intact cells.

Summary of Research Highlight

Single-molecule super-resolution imaging has been used to directly resolve protein complexes in Ras/Raf signaling processes. The results confirm that Raf forms dimers under various activating conditions and that Ras also functions as a dimer in activating Raf/MAPK signaling, changing our understanding of the Ras/Raf signaling pathway.

Implications for Cancer Research

The novel insights from this study necessitate the revision of existing models of Ras/Raf signaling and may lead to novel strategies in targeting this pathway for cancer therapeutics

Raf dimerization: binding of Raf to active Ras (that clusters by itself) and binding between Raf kinase domains. It was also discovered that Ras functions as a dimer in activating Raf/MAPK. Use of a regulated expression system demonstrated that active mutant Ras forms dimers and a small fraction of larger clusters and activates MAPK at expression levels slightly higher than endogenous wild-type Ras. In contrast, at expression levels much lower than endogenous Ras, mutant Ras is monomeric and fails to activate MAPK, unless artificially dimerized. Oligomerization is driven, at least in part, by the C-terminal membrane-targeting motif of Ras, referred to as the CAAX box. Together, these data strongly suggest that dimers of active, GTP-loaded Ras are the functional unit that activates Raf/MAPK signaling. These discoveries suggest a unified dimer model for Ras/Raf signaling, in which formation of Ras dimers facilitates Raf dimerization that in turn leads to Raf/MAPK activation.

Future Plans

Future work will focus on Ras dimerization and its biological implications. First, it has been known that wild-type Ras interferes with tumorigenesis driven by mutant Ras. This could take place by heterodimerization between the two. These investigators are building in vitro models to test this hypothesis. The dynamic assembly of Ras dimers upon natural or artificial stimuli will be analyzed and are the experimental system adapted to image living cells.

Publications

Nan, X., E.A. Collisson, S.Y.L. Kuo, J. Huang, T.M. Tamguney, J. Liphardt, F. McCormick, J.W. Gray, and S. Chu. Single-molecule super-resolution imaging provides new insights into Raf dimerization and signaling. *PNAS*, to be submitted

Nan, X., T.M. Tamguney, E.A. CollissonA, S.Y.L. Kuo, J. Liphardt, J.W. Gray, F. McCormick, and S. Chu. Dimers of Ras-GTP drive Raf/MAPK activation. *Nature*, to be submitted

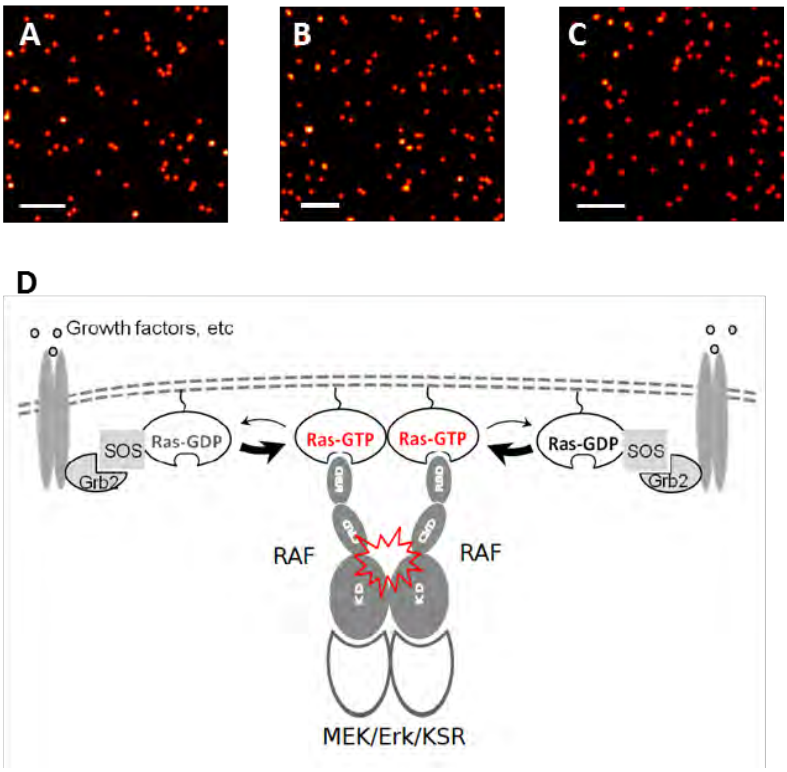


Figure 3.9. Single-molecule super-resolution imaging with PALM reveals dimerization-dependent activation of Ras and Raf. PALM images of an artificial PAmCherry1-PAmCherry1 dimer (A), PAmCherry1-c-Raf co-expressed with K-RasG12D (B), and PAmCherry1-K-RasG12D at an expression level that activates MAPK (C). (D) Proposed dimer model of Ras/Raf signaling.

Emergent Long Range Mechanical Cooperation Among Disorganizing Mammary Acini

Quanming Shi, Rajarshi Ghosh, Hanna Engelke, Jan Liphardt, Chris Rycroft, and James Sethian, University of California, Berkeley; Luke Cassereau and Valerie M. Weaver, University of California, San Francisco

Project Objectives and Significance

This work demonstrates that groups of Ras-transformed mammary acini can mechanically interact over long distances via abnormal collagen microanatomies and that interacting mammary acini can mutually accelerate their progression to a malignant phenotype. Therefore, the likelihood for any one acinus to disorganize is influenced not only by its own local genetic and microenvironmental defects, but by all the other acini around it. Transition to malignancy in this simplified model system is therefore a collective phenomenon, which has implications for detection, the statistics of cancer initiation and evolution, and intervention.

Background

Tissue mechanics influence the decisions made by single cells and multicellular structures. Matrix elasticity, for example, directs stem cell lineage specification, while transcription factors that control VEGF expression, and therefore angiogenesis, respond to both mechanical and chemical cues. Tissue mechanics are also important in cancer where inappropriate matrix crosslinking has been shown to drive tumor progression. It is important to consider that cells both respond to altered mechanics and modify their surroundings by producing collagen crosslinking enzymes (such as LOX) or by actively transporting collagen via actomyosin contractility. The ability of cells to modify the ECM around them and respond to such changes establishes the minimal theoretical conditions for unexpected cancer-relevant interactions and feedback mechanisms among groups of cells and multicellular structures. This work aims to find evidence for cooperativity in model systems and tissues as they disorganize toward a malignant phenotype.

Accomplishments and Scientific Advancements

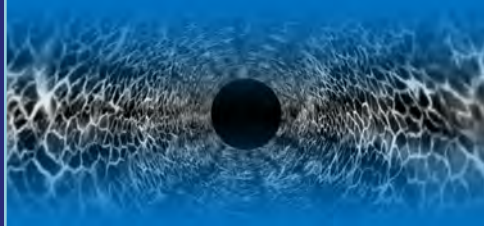
A novel three-dimensional mammary acini model system was developed to test whether acini can mechanically cooperate via a shared matrix. Acini with specific cancer-associated defects were grown from single cells in a standard three-dimensional basement membrane culture and then exposed to collagen 1. Under these conditions, the acini begin to radially import collagen and groups of acini begin to mechanically interact via lines of aligned collagen that form between them due to the acinar contractility and the nonlinearity of collagen mechanics (Figure 3.10A). This results in a planar network, in which the stiff collagen cables mechanically connect contractile acini along geodesics (Figure 3.10B). Disorganization of connected acini is more likely, rapid, and extensive than of isolated acini (Figure 3.10C), showing that groups of mammary acini can mechanically cooperate through their substrate, coordinating and accelerating their disorganization. These experimental observations were verified by the generation of a computational model and simulations that predicted the formation of multiple collagen cables, as observed in experiment. The analytical and modeling

Physical Sciences Perspective

This research utilized coordinated innovation in applied mathematics, precision optical measurement, polymer rheology, and cancer biology to demonstrate emergent behavior in groups of acini.

Summary of Research Highlight

By mechanically cooperating over long distances, groups of mammary acini can mutually accelerate their progression to a malignant phenotype. Therefore, the likelihood for any one acinus to disorganize is not only influenced by its own (local) genetic and microenvironmental defects, but by all the other acini around it.



results demonstrate that pairs or groups of acini work together to manipulate collagen in ways that isolated acini cannot, in terms of the magnitude of the strains generated within the collagen gel, the rate at which they are generated, and the specific details of the resultant collagen microarchitecture. Thus, the probability that any one acinus will transition to a malignant phenotype is determined not only by genetic abnormalities (such as a Ras mutation) and the integrity of its basement membrane, but also the contractility and location of neighboring acini (Figure 3.10D). In this model system, transition to a malignant phenotype is therefore a collective property of a group of cooperating acini. While this is a fundamental view of how tumors may start and what might influence transition to metastasis, it is fully consistent with

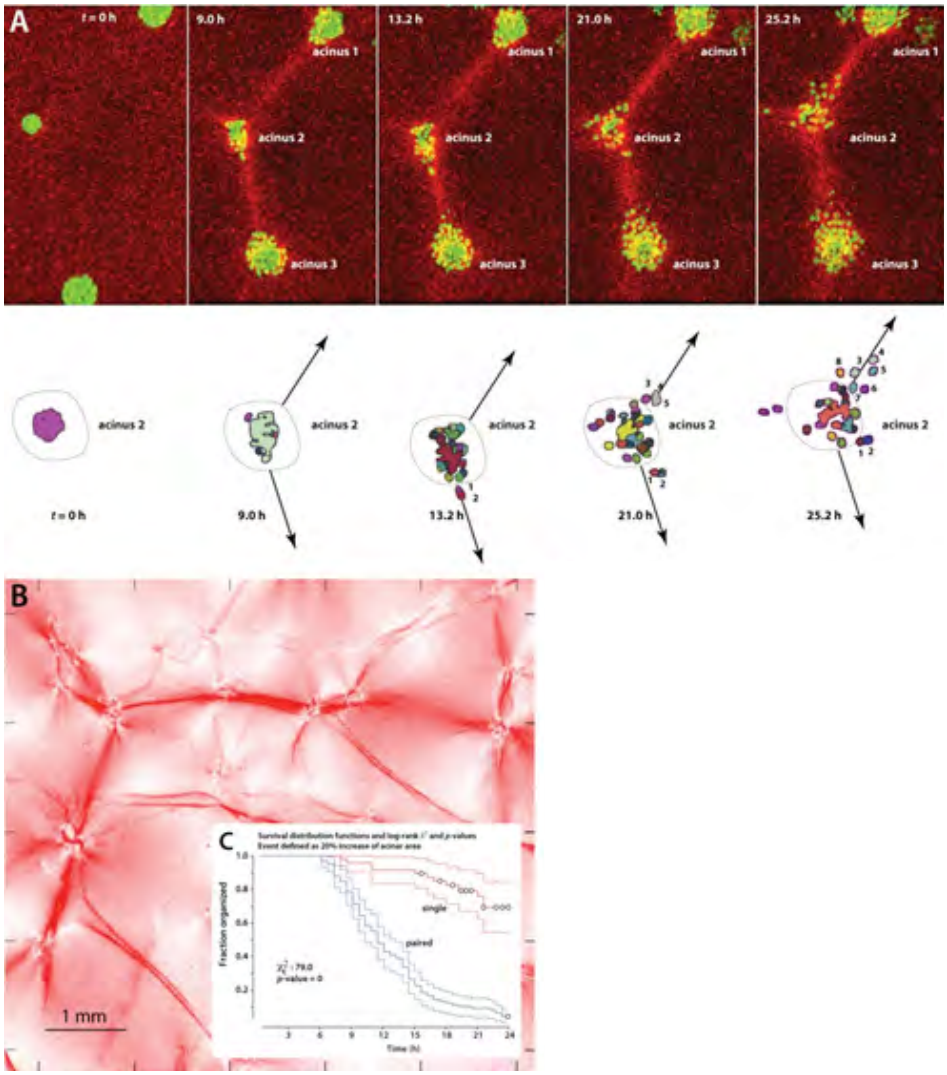
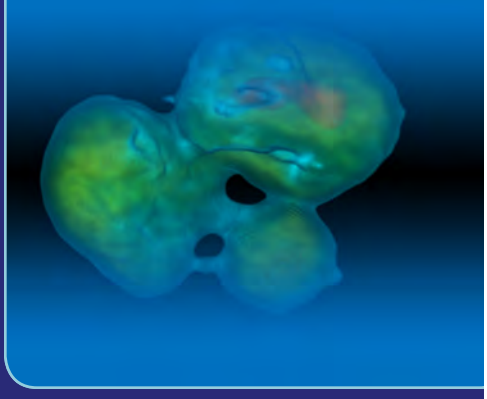


Figure 3.10. Mechanical cooperation among contractile acini via collagen cables. (A) Top row. Mechanical reorientation of direction and location of disorganizing edge. Collagen (red) and cell nuclei (yellow/green). Lower row. Segmentation analysis showing cells leaving the parent acinus 2 toward either acinus 1 or acinus 3, depending on which one is pulling more strongly. (B) Long range collagen network created by 18 contractile acini within a 1 cm2 region of the collagen gel. Signal = retardance, reflecting fiber alignment/concentration. (C) Survival analysis using Kaplan-Meier curves and the log-rank test show that interacting acini (blue) disorganize more rapidly than isolated acini (red).

Implications for Cancer Research

This work establishes a fundamentally new view of how tumors may start and what might influence transition to metastasis and provides further evidence that the tumor physical microenvironment is a critical determinant of cancer initiation and progression. These findings suggest that targeting the physical microenvironment may lead to novel treatment strategies.



other recent discoveries concerning cell and tissue mechanics in normal development and disease. Indeed, organized collagen fibers very similar to those observed in this model system have been detected in clinical samples at the tumor-stromal boundary and are associated with poor prognosis in women with breast cancer.

Future Plans

The key next steps will be to test whether similar processes occur in mouse tumor models and in the human breast during cancer initiation and progression. The first step is to collect detailed maps of collagen organization and mechanics in clinical samples and compare those maps to the microarchitectures and mechanics observed in the model system. Initial steps are under way as part of a PS-OC Pilot Project.

Publication

Shi, Q., R.P. Ghosh, H. Engelke, C.H. Rycroft, L. Cassereau, J. Sethian, V.M. Weaver, and J. Liphardt. Emergent long range mechanical cooperation among disorganizing mammary acini. *Nature Materials*, submitted (2012)

3.2.3 Information Transfer and Decoding

Current thinking in information coding and decoding in biological systems generally implies a (one-way) information flow from DNA—transcribed to RNA—translated to proteins. However, recent studies in developmental biology and epigenetics show that this information flow can be influenced by external physical forces while leaving the underlying DNA sequence unaltered. It is becoming increasingly clear that this complex information system has two-way communication and feedback loops that present another level of complexity, especially related to external environmental factors. Genomic alterations are a characteristic of many types of cancer and likely lead to changes in the flow and feedback of information.

Several PS-OCs are attempting to understand the physical mechanisms and micro-evolutionary processes driving formation and fixation of genomic alterations in cancer. Dr. Franziska Michor at the DFCI PS-OC and Dr. Leonid Mirny at the MIT PS-OC independently performed genome- wide analysis of DNA breakpoints using polymer physics, high-dimensional data analysis, bioinformatics, and statistical techniques. Dr. Mirny's research provides mechanistic insight into the occurrence of genomic alterations observed in cancer based on the three-dimensional chromatin architecture. Dr. Michor found that hypomethylation of CpGs, an epigenetic modification of DNA, located in G-quadruplex sequences could be an architectural driving factor in the alterations of the cancer cell genome. These discoveries provide insight into the impact of genome architecture on the accumulation of genomic alterations found in the cancerous phenotype.

Other PS-OC investigators have developed technologies to advance our understanding of cell heterogeneity at the chromosomal length scale and its impact on information transfer. Dr. Vadim Backman from the Northwestern PS-OC has developed a microscopic technology that can sense the spatial distribution of macromolecular density called Partial Wave Spectroscopy (PWS). This technology is capable of separating normal tissue, adenomas, and cancer of the colon and is currently being validated in other types of cancer. Research led by Dr. Harold Craighead at the Cornell PS-OC has developed a microfluidic platform to analyze genetic and epigenetic changes in single cells. Future work will address the epigenetic heterogeneity of cancer cell populations.

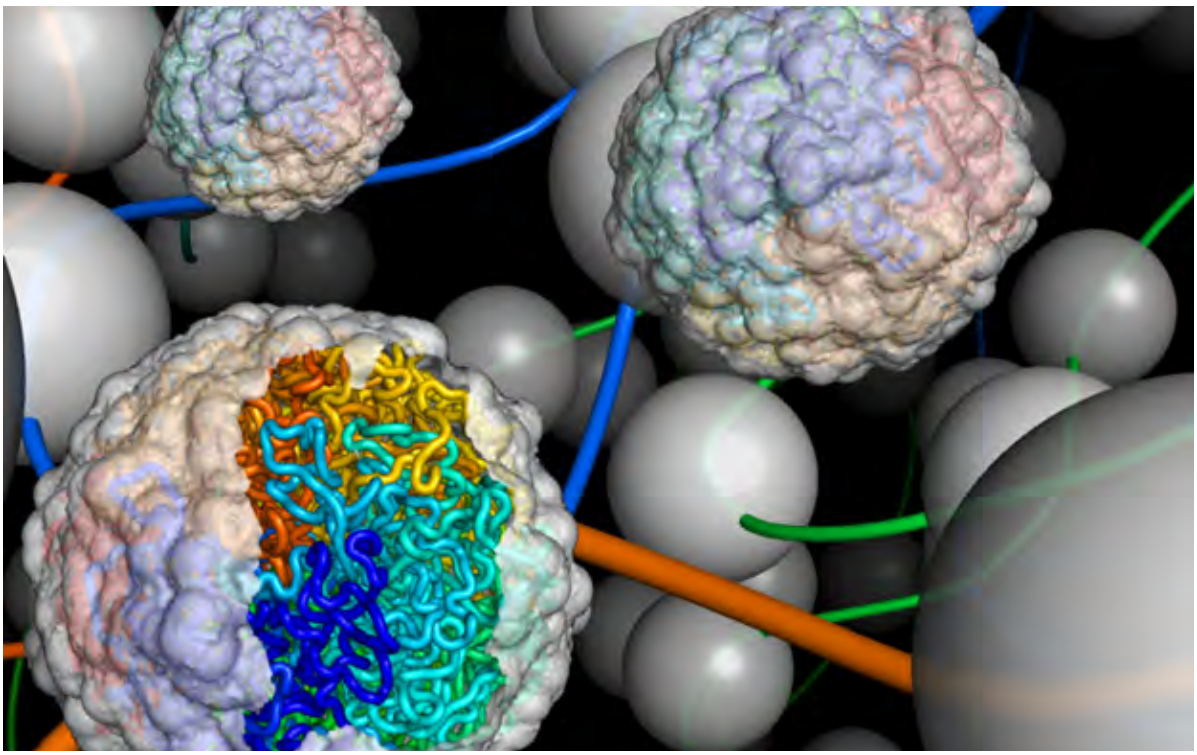


Figure 3.11. An artist depiction of the fractal globular structure of chromatin described by Leonid Mirny, a polymer physicist, and colleagues at the MIT PS-OC.

Selected Cell Epigenetic Analysis

Harold Craighead, Cornell University

Project Objectives and Significance

The object of this project is to develop methods for epigenetic analysis of a few selected cells. The intent is to overcome the problems of sample inhomogeneity with tissue samples and the loss of information that occurs when mixing genomic material from many cells. The goal is to develop technology that can rapidly analyze the material from individual cells and provide richer information by simultaneously detecting multiple epigenetic marks in the chromosomes of a selected cell.

Background

Research is revealing the epigenomic variations associated with the progression of cancer and other diseases. Detecting significant epigenetic modifications may provide a mechanism for early detection of disease and also provide the understanding needed to guide drug treatments. Techniques that can rapidly analyze selected cells could be used in diagnosis, cancer research, and for monitoring the effectiveness of therapy. By analyzing individual cells one can overcome the loss of information that occurs when mixing cells from inhomogeneous tissue samples such as tumors. In addition these technologies could permit analysis of a few rare cancer cells such a circulating tumor cells. In this approach, the investigator and his collaborators are utilizing new physical science-based approaches for manipulating and analyzing individual cells and molecules, utilizing micro and nanofluidic systems. These approaches are being explored for analyzing individual DNA and chromatin molecules using molecular isolation and optical analysis.

Accomplishments and Scientific Advancements

Dr. Craighead has demonstrated an approach for capturing a single cell or a few cells and extracting and purifying intact chromosomal DNA. The method uses a microstructured device and mechanical forces to immobilize the cell and extract and immobilize chromosomal DNA for purification and labeling (Figure 3.12). The immobilized DNA is also available for observation of DNA methylation labels or hybridization probes to reveal features of the individual isolated cell. The microstructured device also enables extraction and purification of DNA or intact chromatin for subsequent single molecule analysis. Using this device, label individual DNA or chromatin fragments with antibody-based or other fluorescently tagged probes were used to identify epigenetic marks of interest. These molecular fragments can be elongated and organized into ordered arrays on an optically transparent surface for optical analysis and image processing. This same approach can be used to reveal DNA methylation and have recently demonstrated the ability to elongate chromatin fragments and label histone modifications. The ordered and elongated molecules also provide the future possibility for super resolution optical imaging and simultaneously observing genetic and epigenetic labels on individual chromosomal fragments to obtain detailed gene-specific epigenetic information.

Physical Sciences Perspective

Using a physical sciences based understanding of chromatin mechanical behavior, a device was developed to isolate and elongate single chromatin fragments with microfluidics.

Summary of Research Highlight

A microstructured device was developed using mechanical forces to immobilize single cells and extract and immobilize chromosomal DNA for purification and labeling. Subsequent experiments have demonstrated the ability to elongate chromatin fragments and label histone modifications revealing DNA methylation on a single cell level.

Implications for Cancer Research

This technology has potential to rapidly analyze the material from individual cells and provide richer information by simultaneously detecting multiple epigenetic marks in the chromosomes of a selected cell to overcome problems of sample inhomogeneity with tissue samples and the loss of information that occurs when mixing genomic material from many cells.

Using flowing nanofluidic systems, the investigators have developed a method for identifying and quantifying epigenetic marks on single molecules using material collected from a single cell and to then sort and collect selected molecules of interest (Figure 3.13). The DNA of the selected molecules will be amplified for sequencing to reveal the genetic context of the identified combination of epigenetic marks of interest. Each of these analytical approaches can utilize current antibody-based labels for epigenetic marks but are adaptable to new aptamer probes that may emerge from other research efforts.

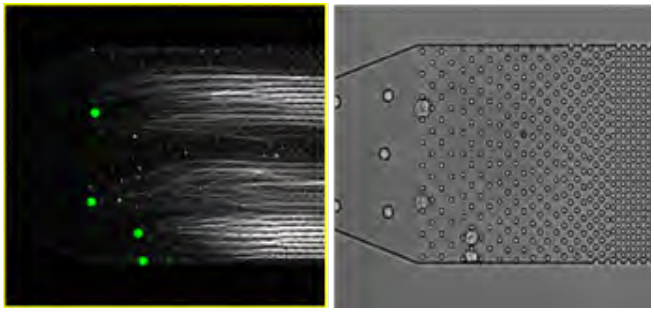


Figure 3.12. Images of cells and DNA capture in microfluidic pillar structure device. Strands of DNA extracted from four individual cells were stained with PicoGreen fluorescent dye and imaged using fluorescent microscopy. The green circles added in the left panel note the original location of the cells shown in the phase contrast image on the right panel from which the DNA has been extracted and immobilized. Multiply-folded strands elongated by hydrodynamic flow up to 27mm in length have been observed in some samples.

Future Plans

Future plans include integrating the single cell capture and chromosome extraction methods with the single molecule analysis. The investigators are in the process of analyzing the global DNA methylation state of single breast cancer cells and are beginning other cancer-specific analyses of selected cells.

Publications

Cipriany, B.R., et al. Single molecule epigenetic analysis in a nanofluidic channel. *Anal Chem* **82**, 2480-2487 (2010).

Cipriany, B.R., et al. Real-time analysis and selection of methylated DNA by fluorescence-activated single molecule sorting in a nanofluidic channel. *Proc Natl Acad Sci U S A* **109**, 8477-8482 (2012).

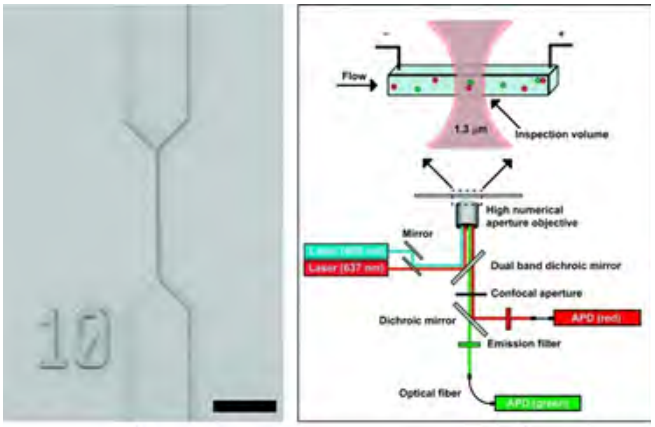


Figure 3.13. Experimental platform for single molecule analysis in a nanofluidic system. (A) A differential interference contrast optical micrograph of a typical nanofluidic channel used in single chromatin analysis at the nanoscale (SCAN). The narrow region, with a 500 nm wide and 250 nm deep cross section, was used during fluorescence detection. The investigators formed 432 of these channels on a single 100 mm diameter fused silica wafer. The scale bar is 10 µm. (B) Schematic diagram of a wafer mounted on a confocal fluorescence microscope. Two overlapped lasers illuminated a 1.3 µm length of the nanofluidic channel and formed an inspection volume of 0.16 fL. Collection of the dim, fluorescent signature for each molecule was achieved using a confocal aperture, which spatially restricted the optical collection to the inspection volume, and avalanche photodiodes (APDs), which provided single photon detection.

Physical Sciences Perspective

This team utilized high-dimensional data analysis, bioinformatics, and statistical techniques to investigate the location and length distribution of somatic copy number changes in cancer genomes and identified factors that contribute to these events.

Summary of Research Highlight

The investigators propose a model for the generation of somatic genetic aberrations in cancer, suggesting that data on spatial proximity of regions replicating at the same time as well as information on epigenetic modifications and DNA secondary structures can help shape the mutational landscapes of cancer genomes.

Implications for Cancer Research

These findings elucidate the roles of DNA replication timing and higher order genomic organization in shaping the mutational landscapes of cancer genomes, and suggest a model for the emergence of the characteristic genomic instability in cancer.

Towards a Mechanistic Framework of the Generation of Somatic Genomic Alterations in Cancer

Subhajyoti De and Franziska Michor, Dana-Farber Cancer Institute

Project Objectives and Significance

SCNAs are a hallmark of many cancer types, but the mechanistic basis underlying their genome-wide patterns remains incompletely understood. Dr. Michor and colleagues identified a mechanistic model for the generation of somatic genetic aberrations in cancer, suggesting that data on spatial proximity of regions replicating at the same time inside the nucleus as well as information on epigenetic modifications and DNA secondary structures can help shape the mutational landscapes of cancer genomes (Figure 3.13). Even though different cancer types represent very different diseases, the findings from this study are consistent across multiple cancer types, indicating a common mutagenic mechanism operating in those tissues.

Background

Cancer genomes display complex mutational landscapes including amplification, deletion, and rearrangement of genetic material. Many genomic alterations arise as a result of DNA damage or erroneous replication, while others occur because of replication-independent events such as exchange reactions between sister chromatids. Eukaryotic DNA replication is spatiotemporally segregated. Some regions are replicated early, while others are replicated late during S phase. The proposed fractal organization of the genome brings together distant genomic regions of similar replication timing to form replication factories, where DNA synthesis takes place in multiple DNA regions simultaneously. During replication, single-stranded double-stranded DNA ends can arise, and interaction between physically proximal segments increases the risk of genetic alterations through mechanisms such as microhomology-mediated break-induced repair. Hence, patterns of nuclear organization and co-localization of replicating DNA strands may contribute to a mechanistic explanation of the genome-wide frequency and size distribution of genomic alterations in cancer (Figure 3.14). Furthermore, epigenetic modifications and DNA secondary structures or other genomic features may contribute to the generation of breakpoints in cancer genomes.

Accomplishments and Scientific Advancements

De and Michor performed a genome-wide analysis of 663,446 DNA breakpoints associated with SCNAs from 2,792 cancer samples classified into 26 cancer types. Many SCNA breakpoints are spatially clustered in cancer genomes. A significant enrichment for G-quadruplex sequences (G4s) in the vicinity of SCNA breakpoints was observed and established that SCNAs show a strand bias consistent with G4-mediated structural alterations. Notably, abnormal hypomethylation near G4s-rich regions is a common signature for many SCNA breakpoint hotspots. From these findings, the investigators proposed a mechanistic hypothesis that abnormal hypomethylation in genomic regions enriched for G4s acts as a mutagenic factor driving tissue-specific mutational landscapes in cancer. This team has also integrated data on DNA replication timing, long-range interactions between genomic material, and 331,724 SCNAs from 2,792 cancer samples classified into 26 cancer types. Genomic regions of similar

replication timing were found to be clustered spatially in the nucleus, that the two boundaries of SCNAs tend to be found in such regions, and that regions replicated early and late display distinct patterns of frequencies of SCNA boundaries, SCNA size, and a preference for deletions over insertions. Long-range interaction and replication timing data alone was able to identify a significant proportion of SCNAs in an independent test dataset. The investigators have proposed a model for the generation of SCNAs in cancer, suggesting that data on spatial proximity of regions replicating at the same time can be used to predict the mutational landscapes of cancer genomes.

Future Plans

Future research will extend its analyses to include point mutations in cancer in order to investigate whether nuclear architecture and DNA replication timing affect local mutation rate in different regions. A mathematical model to predict the mutation landscape of cancer genomes integrating information about DNA replication timing, nuclear architecture, microhomology, and selection is currently being developed.

Publications

De, S. & Michor, F. DNA replication timing and long-range DNA interactions predict mutational landscapes of cancer genomes. *Nat Biotechnol* **29**, 1103-1108 (2011).

De, S. & Michor, F. DNA secondary structures and epigenetic determinants of cancer genome evolution. *Nat Struct Mol Biol* **18**, 950-955 (2011).

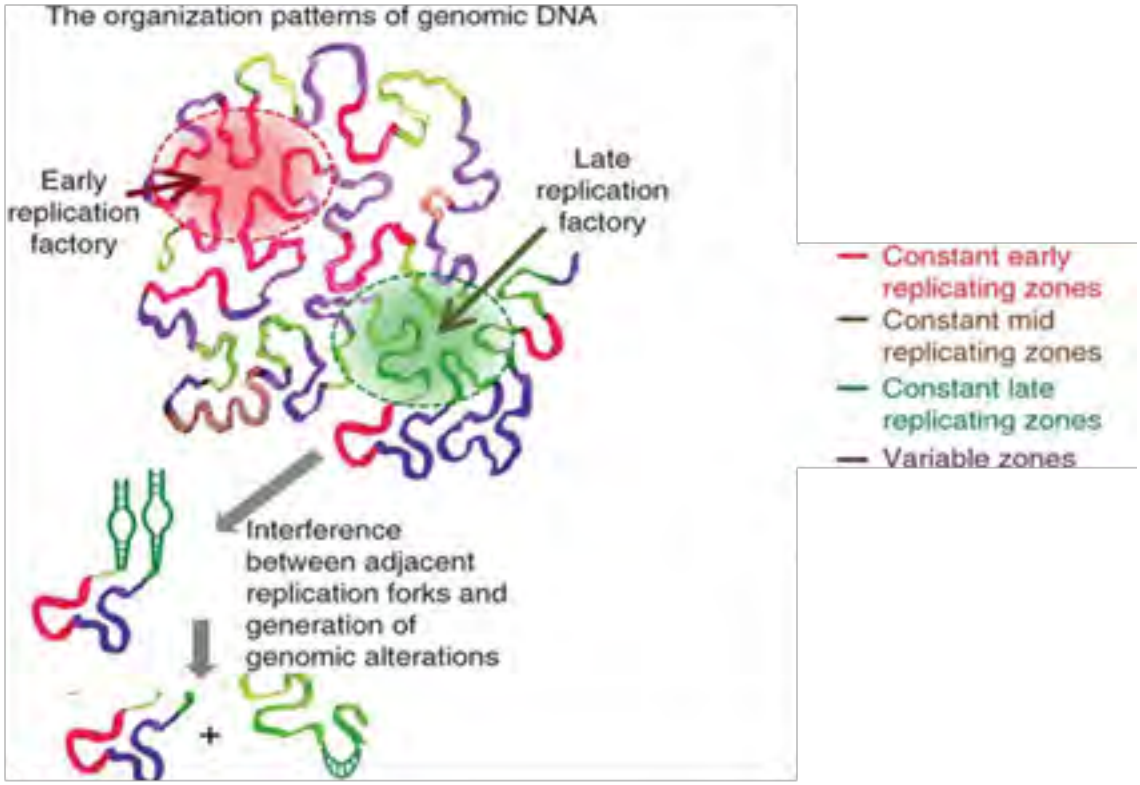


Figure 3.14. Nuclear organization and long-range interactions between distant replication timing zones can increase the risk of interference between adjacent replication forks, leading to genomic alterations.

★ Alterations of Chromatin Nanoarchitecture as a Universal Event in Early Carcinogenesis

Vadim Backman and Igal Szleifer, Northwestern University; Hemant Roy, NorthShore University HealthSystems

Project Objectives and Significance

The goal of this project is to elucidate some of the fundamental aspects of the role of the nanoscale organization of chromatin in genome regulation based on engineering and physical sciences principles. In addition, Backman, Szleifer, and Roy aim to understand how the alterations of chromatin nanoarchitecture play a role in the initiation of carcinogenesis and to translate their findings into a general, cost-effective, and highly accurate methodology for cancer screening.

Background

Most of what is known about genome regulation comes from the view of the cell as a molecular machine. Although powerful, this prevailing approach underestimates an important facet: the regulatory role of the nanoscale organization of the cell and, in particular, the nucleus on macromolecular interactions and genomic processes. It is becoming increasingly clear that nuclear nanoscale organization is inherently linked to biochemical and transport processes. Indeed, molecular processes do not happen in an empty space but in a highly complex and dense nanoenvironment, which has profound effects on many aspects of these processes. However, the ramifications of nanoarchitecture in regard to genomic events have been largely unexplored. This project aims at bridging this gap based on an engineering and physical sciences approach. From the technology perspective, the project is enabled by the development of a new type of optical microscopy technique to sense and image the statistical properties of intracellular nanoarchitecture, partial wave spectroscopic (PWS) microscopy (Figure 3.15). PWS enables sensing the spatial distribution of macromolecular density and, in particular, macromolecular condensation at the nanoscale with sensitivity down to 10 nanometers, far beyond what conventional microscopy reveals.

Accomplishments and Scientific Advancements

The investigators found that the condensation of nanoscale chromatin structure, as measured by PWS and quantified by parameter disorder strength, is a universal and initiating event in early carcinogenesis. The disorder strength quantifies the spatial variability of refractive index and, thus, the local concentration of intracellular material. The alteration is a hallmark of not only malignant cells but also an earlier stage, field carcinogenesis, the organ-wide fertile field from which focal neoplastic lesions originate due to further stochastic genomic events. Although at this early preneoplastic stage cells appear histologically normal, their chromatin nanoarchitecture is already altered in a manner similar to that of malignant cells. The investigators confirmed this finding using human data from 476 patients with 5 types of cancer: the identical alteration was found in tumors and histologically normal rectal colonocytes in patients

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Green star = clinical implications

Physical Sciences Perspective

PWS is a new optical microscopy technique with an unprecedented ability to sense cellular nanoarchitecture. Gene transcription can be regulated by chromatin nanoenvironment through physical mechanisms.

Summary of Research Highlight

Chromatin nanoarchitecture may affect gene expression. The disorder of nuclear nanoarchitecture is a universal and one of the initiating events in carcinogenesis.

Implications for Cancer Research

Field carcinogenesis is an early event in cancer progression, and understanding its mechanisms is crucial. With its ability to detect field carcinogenesis, PWS nanocytology may become a platform technology for population screening for a number of cancer types via nanocytology of cells obtained by brushing from easily accessible sites of affected organs.

harboring colonic adenomas, buccal cells in patients with lung cancer (n=175), periampullary cells in patients with pancreatic cancer (n=35), esophageal cells in patients with esophageal adenocarcinoma (n=50), and cells from the fallopian tubes, endometrium, and endocervix in patients harboring ovarian cancer (n=22).

Electron microscopy and molecular analyses confirmed in the animal models of colon carcinogenesis and in human tissue specimens that chromatin undergoes compaction in early and field carcinogenesis as part of the chromatin remodeling in an early step of transcription. The investigators demonstrated that this compaction is mediated partly by the overexpression of histone deacetylase HDAC2 and regulated by the SWI/SNF-1 chromatin remodeling complex. In turn, a change in the nuclear nanoenvironment may affect essentially all steps of gene expression. Molecular dynamics simulations showed that the access of chromatin to transcription factors and the free energy of DNA dehybridization in the pre-initiation of transcription are modulated by the nanoenvironment with a non-monotonic behavior. A change in nuclear disorder strength indicates chromatin remodeling and is an initial event that is required for a change in genomic homeostasis to occur.

Future Plans

Future research will focus on understanding the molecular mechanisms of the increased disorder of nuclear nanoarchitecture, physical mechanisms through which the nanoenvironment may affect gene expression and its role in early carcinogenesis. Nanoarchitecture is an important dimension of gene expression without which an understanding of genomic events would be incomplete.

Publications

Kim, J.S., Pradhan, P., Backman, V. & Szleifer, I. The influence of chromosome density variations on the increase in nuclear disorder strength in carcinogenesis. *Phys Biol* **8**, 015004 (2011).

Pradhan, P., et al. Quantification of nanoscale density fluctuations by electron microscopy: Probing cellular alterations in early carcinogenesis. *Phys Biol* **8**, 026012 (2011).

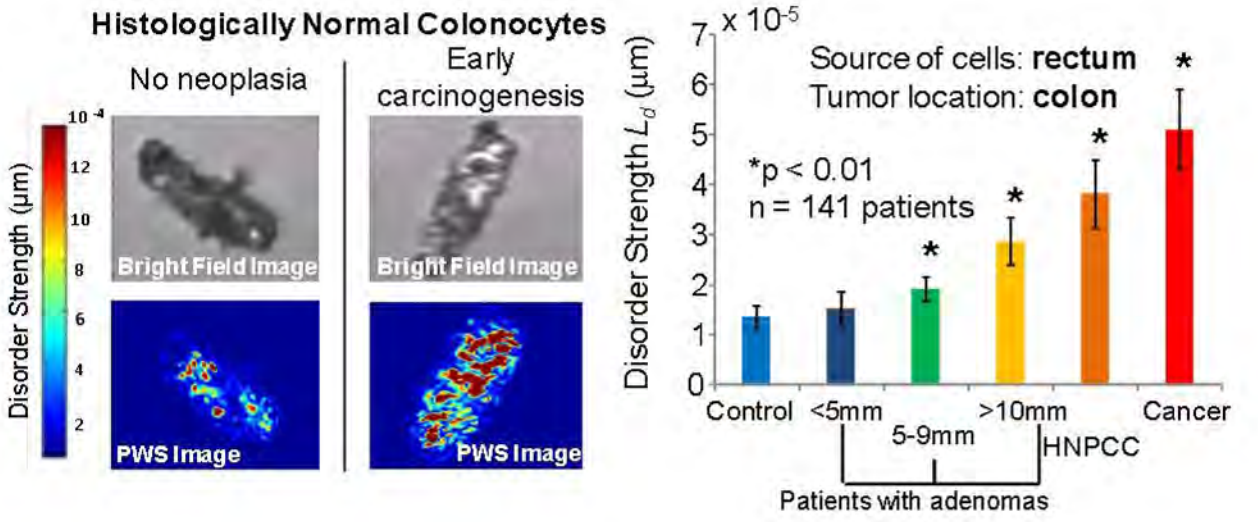


Figure 3.15: The project is supported by a new biophotonics technology, PWS, which affords an unprecedented ability to sense cellular nanoarchitecture. From the cancer biology perspective, PWS data have demonstrated that the disorder of nuclear nanoarchitecture is a universal and one of the earliest events in carcinogenesis. This change in the chromatin nanoenvironment may significantly affect gene transcription through a number of physical mechanisms including a change in DNA accessibility free-energy of dehybridization. From the societal and translational perspectives, ability to detect field carcinogenesis may become a platform technology for cost-effective, practical, and patient-friendly population screening for lung, colon, ovarian, and other types of cancer types via PWS nanocytology of cells obtained by brushing from easily accessible sites of affected organs.

High Order Chromatin Architecture Shapes the Landscape of Chromosomal Alterations in Cancer

Geoff Fudenberg, Harvard University, Leonid A. Mirny, Massachusetts Institute of Technology

Project Objectives and Significance

The objective of this project is to understand the physical mechanisms and micro-evolutionary processes driving formation and fixation of chromosomal alterations in cancer. Specifically, Drs. Fudenberg and Mirny are focusing on the role of three-dimensional genome architecture in the formation of copy-number alterations. This project leverages data from recent genome-wide characterizations of chromosomal alterations and Hi-C data on chromatin organization, demonstrating the utility of biophysical analysis of genomic datasets. Beyond providing insight into mutational mechanisms of somatic alterations, their analyses reveal the potential role of negative, or purifying, selection in cancer. Observations consistent with negative selection indicate that certain observed passenger somatic alterations may be deleterious to cancer cells, and in turn point toward new therapeutic strategies for cancer.

Background

Somatic copy-number alterations (SCNAs) are among the most common genomic alterations observed in cancer. During neoplastic progression, as in other evolutionary processes, two forces determine their accumulation: generation of new alterations and fixation of these alterations in the neoplastic population.

Generation of new alterations can depend on three-dimensional chromatin organization: Distant genomic loci brought spatially close by three-dimensional chromatin architecture are more likely to undergo structural alterations and become end points for SCNAs observed in cancer. Until now, the connection between chromosomal alterations and three-dimensional chromatin organization was established by observing a small set of loci using fluorescent in situ hybridization. However, recent high-throughput techniques are now available for creating genome-wide maps of chromosomal alterations and chromosomal contacts. Analysis of chromosomal contacts obtained using the Hi-C technique revealed that the sub-chromosomal organization of interphase chromosomes is well-described theoretically by the Fractal Globule model for polymers.

Accomplishments and Scientific Advancements

Drs. Fudenberg and Mirny, in collaboration with Drs. Matthew Meyerson and Gad Getz at the Broad Institute, analyzed 39,568 recently mapped SCNAs across 3,131 cancer specimens from 26 histological cancer types. To establish that these results were robust to positive selection acting on cancer-associated genes, collection of SCNAs was analyzed that do not span highly recurrent SCNA regions or known cancer-causing genes. Their genome-wide analysis of Hi-C measurements and cancer SCNAs found multiple connections between higher-order genome architecture and

Physical Sciences Perspective

While somatic copy-number alterations are known to play a central role in cancer progression, the range of mechanisms contributing to these genomic alterations remains unclear. Similarly, while three-dimensional chromatin structure has been recently characterized at the genomic scale, the functional implications of chromatin structure are currently limited. This research investigates the physically motivated connection between three-dimensional chromatin architecture and somatic copy-number alterations observed in cancer.

Summary of Research Highlight

Working in the context of the structure-to-function paradigm, this research provides mechanistic insight into the occurrence of genomic alterations observed in cancer and presents a functional consequence of three-dimensional chromatin architecture.

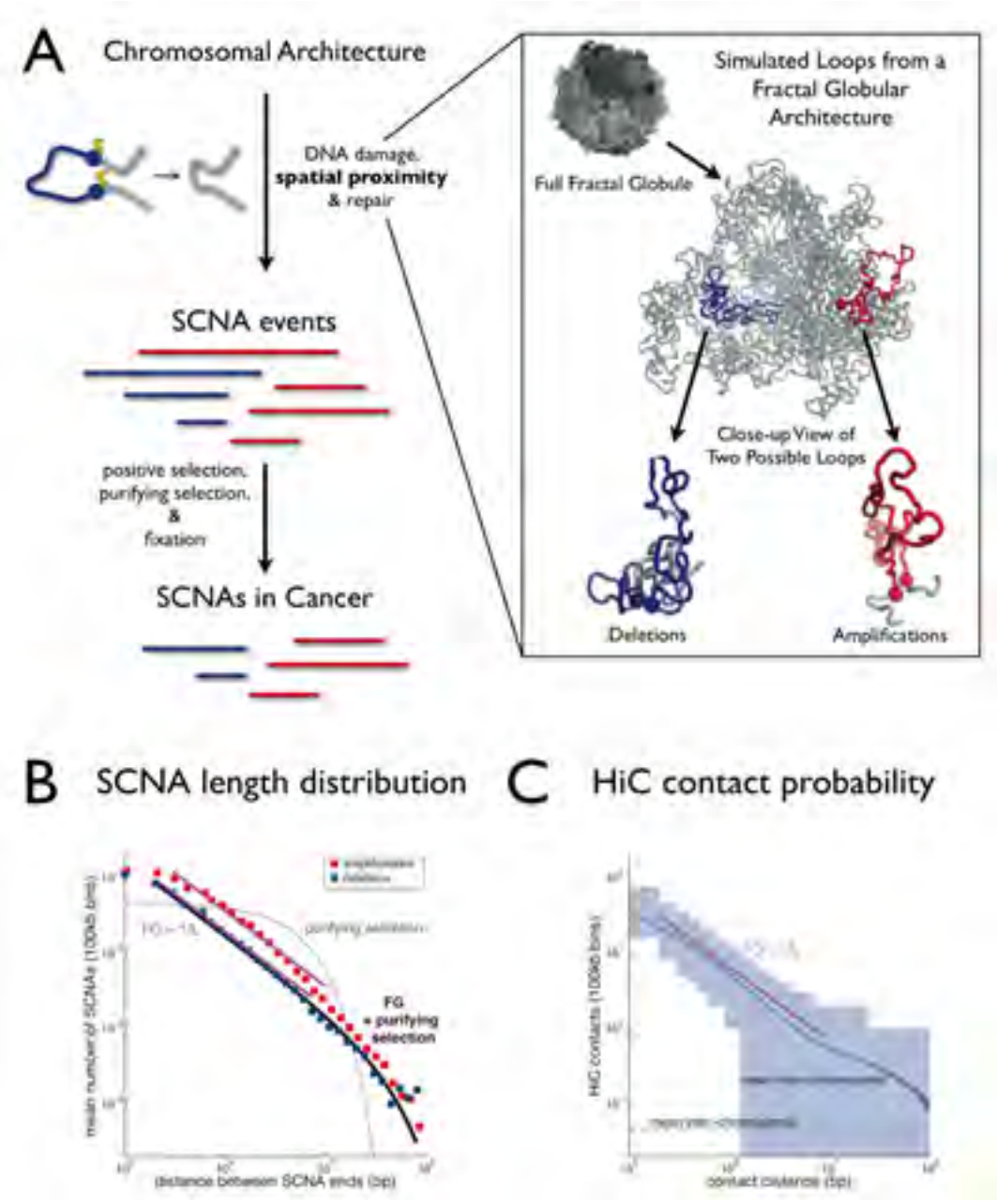


Figure 3.16. Three-dimensional proximity and selection as mechanisms for SCNA formation. A: (left) Model of how chromosomal architecture and selection can influence observed patterns of SCNAs. (right) inset illustrates looping in a simulated fractal globule. B: SCNA length distribution. C: The mean number (blue line, shaded: 5th to 95th percentiles) of contacts between two loci distance L apart on a chromosome according to Hi-C data.

Implications for Cancer Research

These results suggest that a comprehensive understanding of mutational and selective forces acting on the cancer genome is important for explaining the observed distribution of SCNAs.

alterations in cancer. Using a likelihood-based approach, the investigators found that (1) the probability of a three-dimensional contact between two loci based on the Fractal Globule model explains the length distribution of SCNAs better than a model of purifying selection alone; (2) there is a statistically significant connection between positions of SCNAs and three-dimensional chromosomal contacts observed in Hi-C; and (3) SCNA data reflect negative selection, suggesting that SCNAs affecting non-cancer promoting genes may lead to decreased fitness (Figure 3.16).

These results argue for the importance of three-dimensional chromatin organization in the formation of chromosomal alterations, which is parsimonious when considering the complicated mutation and selection landscape of SCNA. Along these lines, recent experimental studies of translocations suggest that physical proximity is among the key determinants of genomic rearrangements. These results also suggest that a comprehensive understanding of mutational and selective forces acting on the cancer genome is important for explaining the observed distribution of SCNAs.

Future Plans

Future studies shall address the importance of variability in three-dimensional genomic organization to the observed chromosomal rearrangements across cell types and cell states. High-throughput, whole-genome sequencing data will allow for both a high-resolution analysis of inter-chromosomal rearrangements and yield insight into the interplay between sequence features, chromatin modifications and three-dimensional genomic structure. Future theoretical work will develop more detailed models of how mutational and selective forces influence the observed set of somatic alterations in cancer.

Publications

Fudenberg, G., Getz, G., Meyerson, M. & Mirny, L.A. High order chromatin architecture shapes the landscape of chromosomal alterations in cancer. *Nat Biotechnol* **29**, 1109-1113 (2011).

Fudenberg, G. & Mirny, L.A. Higher-order chromatin structure: Bridging physics and biology. *Curr Opin Genet Dev* **22**, 115-124 (2012).

3.2.4 De-convoluting Cancer's Complexity

The more we have learned about cancer, the greater our appreciation is of its complexity. There are more than 100 types of human cancer, plus additional subtypes and heterogeneity between patient response. Tumors within a subtype can consist of genotypically distinct populations of cells. At a smaller length scale, there are dynamic interactions of cancer cells with their local environment and host cells that govern interplay, cross-talk, and feedback loops driving cell response. Plus, there is the overall complexity of the system with time as tumors accumulate genetic and epigenetic changes.

Together, the problems of diversity, interactions, and dynamics make understanding and treating cancer an extremely complex problem. Faced with these issues, biologists have operated largely from a reductionist framework, looking to explain the properties of cancer by studying single genes or proteins, connected by simple "pathway" diagrams. Although this approach has enabled great progress in our understanding of this disease, it fails to account for all the complexity of the system.

Physicists, mathematicians, computer scientists, and engineers bring a different set of tools to address problems of complexity. This section highlights three advances toward fulfilling this aim. At the USC PS-OC, a large multidisciplinary team has combined multiscale mathematical modeling with advanced intravital microscopy to accurately model lymphoma size and growth rates and has used the model to generate hypotheses that were confirmed experimentally. Work at the Scripps PS-OC used a comprehensive autopsy dataset to create computational simulations of metastasis based on Markov Chain modeling that unravel the complex dynamics and multidirectional nature of metastatic spread. Using control theory optimization, the MIT PS-OC published a theory on stem cell differentiation during intestinal crypt development. The Bang-Bang theory is a new hypothesis that could be applicable to stem cell differentiation in cancer. Lastly, the UCB PS-OC is building a computational cell model based on physical and mechanical principles that has the potential to provide a platform for developing and testing physical models of how cancer cells interact with each other and their microenvironment.

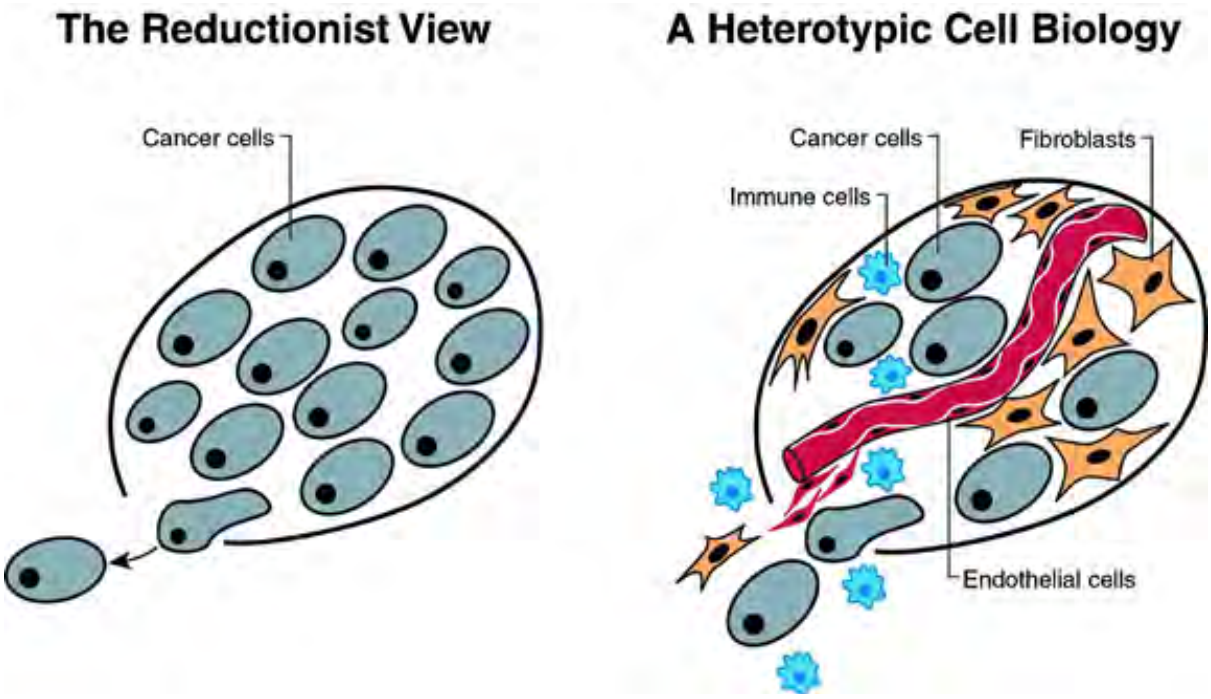


Figure 3.17. The complexity of cancer often forces scientists to take a reductionist approach to simplify the system. Physicists, mathematicians, computer scientists, and engineers bring a different set of tools to address problems of complexity, such as multiscale models, 3-D realistic models, and optimization theories.

Physical Sciences Perspective

Physical modeling of lymphoma enables predictions of tumor size that are borne out by experiments.

Summary of Research Highlights

- Tumor cells reproducibly efflux out of bone marrow and spleen and into lymph nodes to form rapidly growing tumors at the lymph node sites.
- The investigators have identified the molecular pathway by which the lymphoma cells "burst" out of spleen/bone marrow to remote sites.
- Physical modeling predicted that the tumor could not have solely grown in the lymph node given the immunohistochemistry cell-scale parameters, but instead must have been fed by other sites, thus bearing out the experimental findings.

Implications for Cancer Research

- Metastatic invasion in lymphomas may occur in discrete "bursts" or waves due to specific molecular pathways rather than on a cell-by-cell basis – potential implications for treatment exist.
- Physical modeling that predictively bridges the cell-to the tissue-scale can provide unique insights into lymphoma behavior.

Multiscale Complex Systems Transdisciplinary Analysis of Response to Therapy: Integrated Multi-Modality Imaging and Computer Simulations

Bryan R. Smith, Ken Ito, Masakatsu Kotsuma, and Sanjiv Sam Gambhir, Stanford University; Hermann Frieboes and Allison Roettgers, University of Louisville; Yao-li Chuang, Jennifer Pascal, and Vittorio Cristini, University of New Mexico

Project Objectives and Significance

The goal of this project is to understand the initiation, genesis, and development of drug-sensitive and drug-resistant lymphomas using a sophisticated combination of advanced cancer imaging techniques and mathematical simulations. The integration of the experimental and theoretical methods is anticipated to provide insights into the understanding of lymphoma growth, drug resistance, and ultimately to treatments. This would not be possible with either technique alone. By directly visualizing and simulating dynamic lymphoma growth and treatment, the team working on this project aims to predict lymphoma response to therapy given a limited number of input tumor parameters.

Background

The experimental arm of the project focuses on intravital imaging (IVM), bioluminescence imaging (BLI), and molecular and cellular cancer biology to uncover novel features of lymphoma dissemination and detect differences between the drug-sensitive and drug-resistant tumors. This group employs animal tumor models, including the $Arf_{-/-}$ and $p53_{-/-}$ drug-sensitive and drug-resistant models (each transfected with EGFP and luciferase for visualization with IVM and BLI) and has developed specialized surgical techniques for microscopic visualization in the lymph nodes over a period of weeks. Images of tumor growth, vascularization, drug dispersion, and size are acquired, and this group is now investigating tumor cell apoptosis and hypoxia. For tumor modeling, the team implemented computational and experimental approaches to link tumor growth and treatment response in a predictive manner from cell-scale to tumor tissue-scale behavior in order to gain insights into tumor etiology and progression over time. To this end, cell-scale calibration of the model, including rates of proliferation, apoptosis, and necrosis, hypoxic levels, and cell viability and vasculature density parameters, are employed based on immunohistochemistry (IHC) values and quantitation from measurements made in live subjects. These values are used to simulate patient-scale tumor growth and treatment response. Observations of lymphoma size, morphology, and vasculature from lymph node imaging in live mice then provide part of the tumor-scale information to validate the model simulations.

Accomplishments and Scientific Advancements

The tumor biocomputational model successfully predicts lymphoma size and growth rates without curve fitting. The investigators used IHC data to first perform a detailed cell-scale calibration of the model as described above, and then verify the tissue-scale tumor growth from IVM observations. Using this model, the researchers developed a fundamental hypothesis: lymphoma growth cannot be solely attributed to in situ proliferation, but rather requires an additional source of lymphoma cells external to the lymph node. Incorporating this hypothesis into the model allowed simulations

to match tumor sizes measured in the group's experimental protocols. Furthermore, direct experimental observations of lymphoma cell influx into lymph nodes corroborate the model-generated hypothesis.

IVM combined with BLI showed unexpectedly that in the lymphoma mouse model, lymphoma cells do not actually grow in the inguinal lymph node initially (Figure 3.18) as has been assumed. Rather, they proliferate in the spleen and bone marrow, then very rapidly (within 12 hours) "burst" or migrate into the inguinal lymph node around day 9-10 post-injection of lymphoma cells (Figure 3.18). Using a series of biological experiments and further imaging data, the investigators have since acquired data implicating Angiotensin II in the release of lymphoma cells from the spleen and their growth and proliferation in the inguinal lymph node. In particular, the team found that the NF-κB pathway is activated in part by the increasing tumor cell density in the spleen and bone marrow, the result of tumor proliferation in those sites. Increasing cell density triggers an increase in reactive oxygen species that leads to NF-κB activation and eventual cell efflux.

Future Plans

Collaborative work in underway with other research projects at the USC PS-OC to understand the presence of a variety of cell cycle, differentiation, and other cell markers in spleen, bone marrow, and lymph node using CyTOF at several time points before, during, and after efflux. This data will be used in simulations to further refine their models. Hypoxia and apoptosis measurements will be made with IVM to further calibrate and develop the mathematical tumor model simulation of drug response.

Publications

Ito, K., et al. Unexpected dissemination patterns in lymphoma progression revealed by serial imaging within a murine lymph node. *Cancer Res* **72**, 6111-6118 (2012).

Frieboes, H.B., B.R. Smith, Y.-L. Chuang, K. Ito, A. Roettgers, S.S. Gambhir, and V. Cristini. An Integrated Computational/Experimental Model of Lymphoma Growth. *Cancer Research*. In Review.

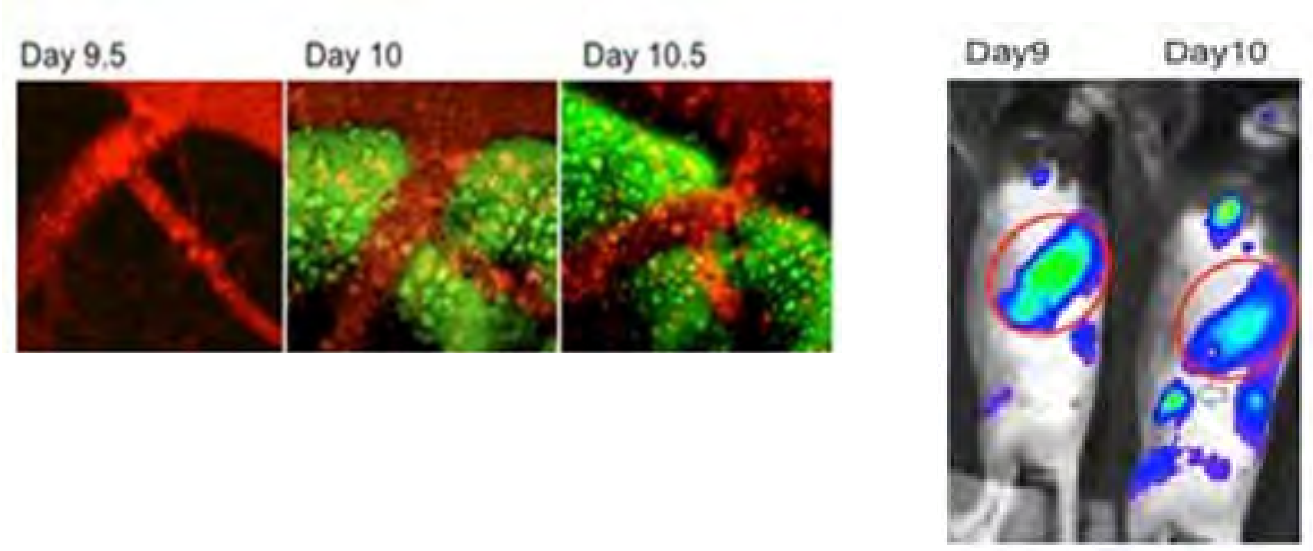


Figure 3.18. (Left) Lymph node window chamber images. Within 12 hours of injection lymphoma cells migrated into the inguinal lymph node. These are high spatial and temporal resolution obtained twice daily. Red: blood vessels (via dye) and adipocytes (by autofluorescence). Green: Arf-/- cells. Yellow: indicates merged green/red regions. (Right) Efflux of lymphoma cells from spleen and bone marrow to the circulation and into the peripheral lymph nodes in the middle stage.

Physical Sciences Perspective

The models use information extracted from autopsy datasets to construct transition matrices and directed graph (network)-based models of metastatic disease progression. The models are used to probabilistically quantify systems-level (whole-body) metastatic progression pathways and perform computer-based cancer metastasis simulations.

Summary of Research Highlight

This study provides a first theoretical and quantitative framework in support of the multi-directional nature of cancer spread and provides a model to carry out computational Monte Carlo simulations of disease progression.

Implications for Cancer Research

The model quantitatively supports known cancer progression pathways, predicts new multi-directional pathways, and provides the key `tipping point' motivation to focus on promising newly identified pathways, such as the lung to adrenal connection in primary lung cancer, as well as self-seeding and metastasis re-seeding pathways. The models allow clinicians to test hypotheses of cancer progression via computer simulation which can be used as a new exploratory tool.

★ Metastatic Pathway Diagrams for Cancer Progression via Markov Chain Models

Paul Newton and Jeremy Mason, University of Southern California; Peter Kuhn, The Scripps Research Institute; Kelly Bethel, Scripps Clinic; Lyudmila Bazhenova, University of California, San Diego Moores Cancer Center; Jorge Nieva, Billings Clinic

Project Objectives and Significance

The main objective of this project is to develop models that can represent the pathways and time scales in the "natural progression" of metastatic disease for use as a baseline model for the major tissue cancers. The designed approach was to (1) extract transition probabilities for metastatic tumor distributions and cancer progression from large autopsy datasets; (2) construct network diagrams of cancer pathways for lung, breast, liver, and prostate cancer, for which there are good datasets and strong oncologist input; and (3) run Markov chain/Monte Carlo simulations of cancer progression based on ensembles of random walkers on the cancer networks constructed from the datasets.

Background

The classic view of metastatic progression is that it is a unidirectional process initiated at the primary tumor site progressing to variably distant metastatic sites. In light of recent evidence highlighting the potential significance of self-seeding of primary tumors, this team has developed a probabilistic Markov chain model that drives Monte Carlo computer simulations of an ensemble of random walkers on the network associated with the Markov transition matrix. These simulations aim to quantify the multidirectional aspects of cancer progression in terms of the multistep metastatic pathways associated with tissue cancers, focusing first on lung, breast, and prostate cancer. The baseline models, which represent the natural history of the disease, are built on datasets for autopsies performed from 1914 to 1943, comprising 41 different primary cancers and 30 different metastatic sites of untreated cancer patients.

Accomplishments and Scientific Advancements

The model is used to quantify disease progression pathways and build a framework for computational simulation of cancer using Markov chain/Monte Carlo methods. Using the model, this group is quantifying three key elements of multidirectional metastatic spread: (1) primary tumor self-seeding; (2) re-seeding of the primary tumor from a metastatic site, or primary re-seeding; and (3) re-seeding of a metastatic tumor from the same metastatic site, or metastasis re-seeding. These multidirectional aspects of cancer progression are quantified by calculating the metastatic pathway probabilities associated with the most likely one-step and two-step progression paths in the directed network graph obtained from the Markov transition matrix. A ranked probabilistic list of both unidirectional and multidirectional paths is used to produce a

Green star = clinical implications

reduced pathway diagram from which researchers identified certain key metastatic sites as "spreaders" and others as "sponges." The most important spreader for lung cancer, measured both in terms of the probability ratio of two-step path out over in—the amplification factor—as well as the total number of paths into and out of the site, is adrenal, while the most important sponges quantified in terms of absorption factors are the regional lymph nodes (Figure 3.19). In contrast, for primary breast cancer the most important spreader is bone, with lymph nodes and liver acting as sponges. This study provides a first theoretical and quantitative framework in support of the multidirectional nature of cancer spread and can be used as a platform to quantitatively simulate disease progression for all of the major types of tissue cancers that are represented in the team's database.

Future Plans

The current set of models provides a baseline approach to the natural progression of metastatic cancer in ensemble populations of untreated cancer victims. This team now plans to develop Bayesian updating and particle filtering data assimilation methods to tailor and update the transition probabilities with targeted datasets using specific treatment protocols, targeted genetic subpopulations of patients, and targeted age-based subpopulations. In addition, the team plans to test various specific hypotheses about cancer progression and how it is altered with the removal of spreader sites.

Publication

Newton, P.K., J. Mason, K. Bethel, L. Bazhenova, J. Nieva, and P. Kuhn, *A stochastic Markov chain model to describe lung cancer growth and metastasis.* PLOS ONE, 2012. **7**(4):e34637

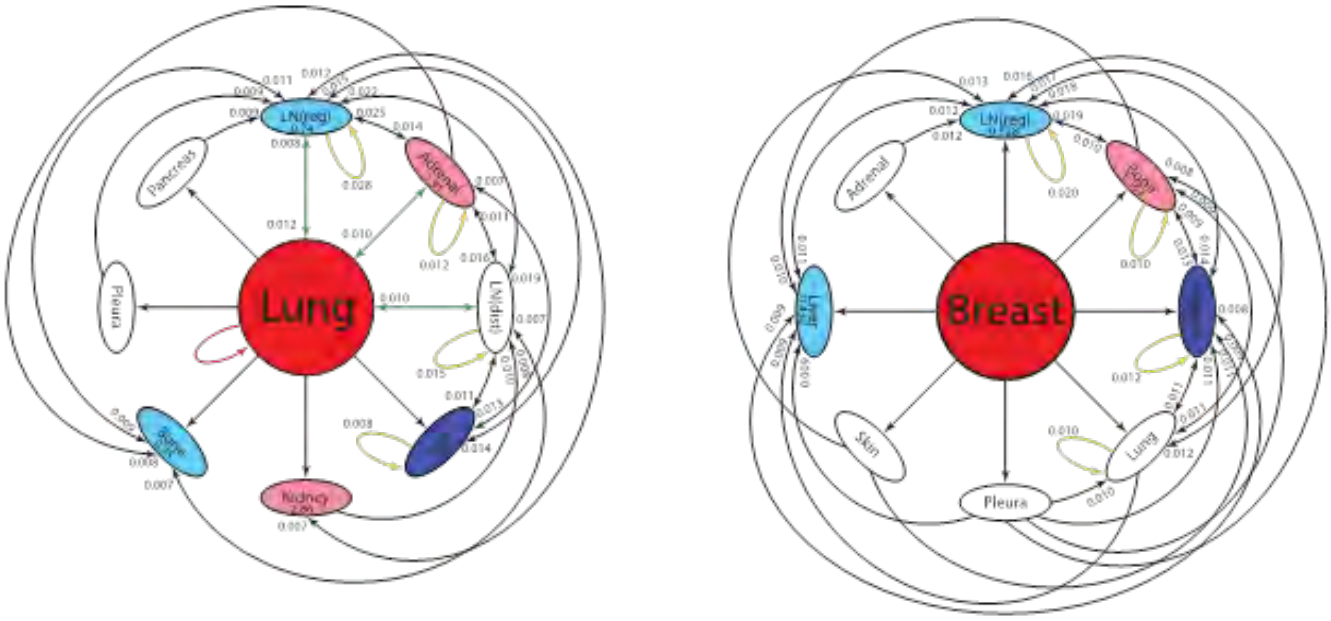


Figure 3.19. Reduced two-step pathway diagrams for lung and breast cancer. Numbered edges show two-step transition probabilities from primary tumor to metastatic sites, and trafficking among the sites highlighting the multidirectional nature of metastatic spread. Red coloring highlights "spreaders" (darker indicating higher amplification factor), and blue coloring highlights "sponges" (darker coloring indicating higher absorption factor). Diagrams are obtained from Markov model reduction using the top 30 two-step transitional pathways.

Physical Sciences Perspective

This project combines theoretical modeling (optimal control theory) with state-of-the-art mouse model experiments.

Summary of Research Highlight

Intestinal crypts display a developmental temporal order in which the establishment of stem cells precedes the expansion of non-stem cells. Optimal control theory shows that this strategy minimizes the time needed to create a mature crypt.

Implications for Cancer Research

Understanding how (cancer) stem cells control division pattern by modulating asymmetric versus symmetric divisions is important for understanding malignant tissue growth.

Optimality in the Development of Intestinal Crypts

Shalev Itzkovitz, Irene Blat, Tyler Jacks, and Alexander van Oudenaarden, Massachusetts Institute of Technology; Hans Clevers, Hubrecht Institute

Project Objectives and Significance

Intestinal crypts in mammals consist of long-lived stem cells and shorter-lived progenies. These two populations are maintained in specific proportions during adult life. The design principles governing the dynamics of these proportions during crypt morphogenesis were investigated. Using optimal control theory, the investigators show that a proliferation strategy known as a "bang-bang" control minimizes the time to obtain a mature crypt. This strategy consists of a surge of symmetric stem cell divisions, establishing the entire stem cell pool first, followed by a sharp transition to strictly asymmetric stem cell divisions, producing non-stem cells with a delay. These predictions were validated using lineage tracing and single-molecule fluorescence in situ hybridization of intestinal crypts in infant mice, uncovering small crypts that are entirely composed of Lgr5-labeled stem cells, which become a minority as crypts continue to grow. This approach can be used to uncover similar design principles in other developmental systems.

Background

The mouse small intestine is a classic model system for mammalian stem cell biology. Intestinal crypts in adults consist of a minority of long-lived stem cells that divide continuously to maintain their numbers while producing shorter-lived, differentiated non-stem cells. The availability of intestinal stem cell markers and mouse models that enable tracking the fates of individual stem cell divisions makes this an ideal system to study stem cell dynamics during the complex processes of tissue development, homeostasis, and repair. This project aimed to elucidate the design principles that govern the dynamic proportions of stem cells and non-stem cells in developing crypts. Intestinal crypts appear shortly after birth as small indentations and are rapidly expanded toward their mature size. The crypt expansion process is governed by the way in which stem cells divide. Symmetric stem cell divisions expand the stem cell pool, while asymmetric divisions create new non-stem cells. Both the dynamic crypt composition and the total time required to expand the crypt to its mature size depend on the way in which stem cells dynamically modulate these modes of divisions.

Accomplishments and Scientific Advancements

To address this question of which stem cell proliferation strategy will most quickly produce a mature crypt, the investigators devised a mathematical model of crypt growth in which stem cells dynamically allocate their division resources according to a stem cell control function. At any time along the developmental process, a fraction of the existing stem cells divides symmetrically to make more stem cells while a second fraction divides asymmetrically to generate new non-stem cells. To understand which control function leads to a mature crypt in the minimum amount of time, the researchers used the mathematical tools of optimal control theory and found that the mature crypt

would be attained in the minimal time if stem cells employ a division strategy known as a "bang-bang" control. In this strategy the crypt expansion process consists of two distinct phases. In the first phase, all stem cells should divide only symmetrically, which rapidly expands the mature crypt stem cell pool while delaying production of non-stem cells. In the second phase, all stem cells should sharply transition to asymmetric divisions, maintaining stem cell numbers while generating the non-stem cells with a delay (Figure 3.20). The investigators then tested these predictions using single molecule fluorescence in-situ hybridization of the Lgr5 stem cell marker in infant mice to count the numbers of stem cells in crypts of increasing sizes at successive stages along the crypt developmental process. These experiments showed that small crypts are at first entirely made up of stem cells, and that once the crypts reach a critical size they continue to grow by adding Lgr5-negative non-stem cells. The investigators also tracked the divisions of individual Lgr5 stem cells using short-term lineage tracing and found that a predominance of stem cell symmetric divisions precedes a switch to asymmetric divisions at the single-cell level, confirming the theoretical predictions.

Future Plans

This approach, combining theoretical models with sensitive detection of individual cells in intact tissues, can be used to analyze complex processes of development, homeostasis, and repair in other tissues. The investigators plan to extend these approaches to explore cancer stem cell biology in mouse models for colon cancer.

Publications

Itzkovitz, S., Blat, I.C., Jacks, T., Clevers, H. & van Oudenaarden, A. Optimality in the development of intestinal crypts. *Cell* **148**, 608-619 (2012).

Itzkovitz, S., et al. Single-molecule transcript counting of stem-cell markers in the mouse intestine. *Nat Cell Biol* **14**, 106-114 (2011).

Itzkovitz, S. & van Oudenaarden, A. Validating transcripts with probes and imaging technology. *Nat Methods* **8**, S12-19 (2011).

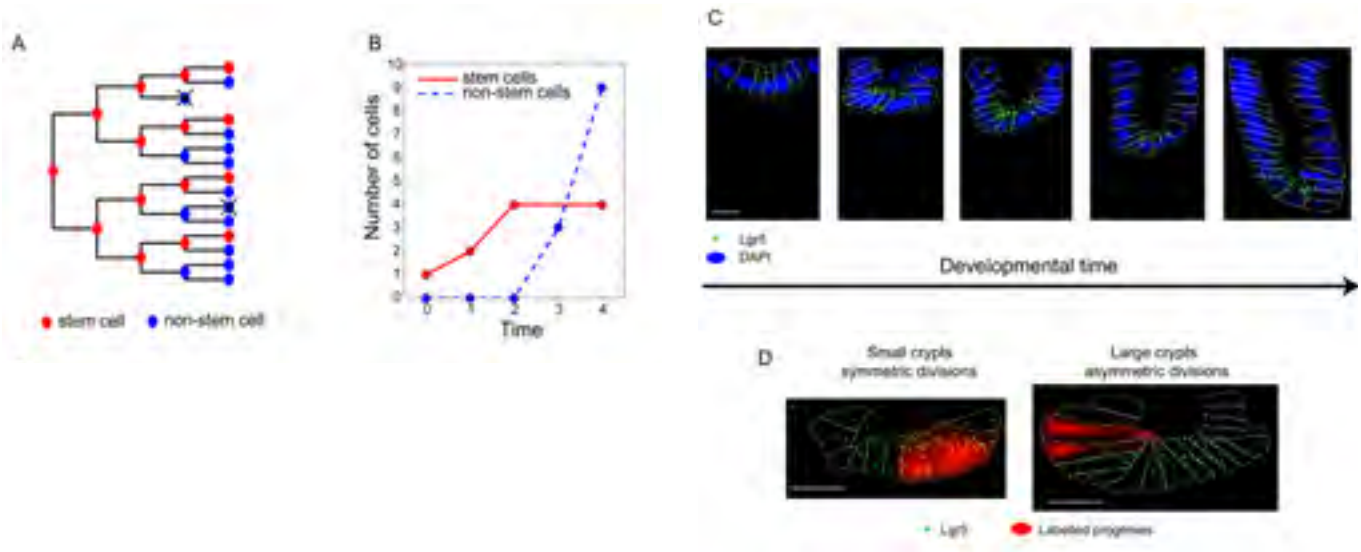


Figure 3.20. Bang-bang control of intestinal crypt development. (A) Expanding crypts minimize the time to achieve a mature crypt by first creating stem cells (red) through symmetric divisions and only later switching to asymmetric divisions, generating non-stem cells (blue) with a delay. (B) Dynamics of stem cells and non-stem cells predicted from the bang-bang optimal control. (C) Fluorescence detection of Lgr5 transcripts (green) in developing intestinal crypts. (D) Short-term lineage tracing of progenies of Lgr5 stem cells (labeled in red) reveal a switch from symmetric stem cell divisions to asymmetric divisions.

Predictive Computation of Cell-Tissue Mechanics from First Principles

Chris Rycroft, Robert Saye, Daniela Ushizima, and James Sethian, University of California, Berkeley

Project Objectives and Significance

James Sethian and colleagues at the UCB PS-OC are building computational models to understand the mechanics of cell and cell/tissue interactions. Computational cell models based on physical and mechanical principles provide a platform for developing and testing physical models, often institutions that would be experimentally infeasible. However, mechanics-based modeling of cells has been challenging, partly because of the difficulty of modeling the mechanics of biological systems in a way that incorporates a complex interplay of mechanics and underlying signaling methodologies.

Background

Recent breakthroughs in computational fluid mechanics, computational elasticity, numerical methodologies for tracking multiple junction interfaces, complex grid/meshing discretization schemes, and high-performance computing environments have opened the door to modeling some of the most critical yet subtle cell-tissue dynamics. This team built a new predictive computational environment by applying state-of-the-art computational methodologies for projection methods for incompressible fluid flow, referenced-based mixed Eulerian-Lagrangian techniques for elasticity, and advanced interface techniques to track multiple interacting bodies under complex physics. This virtual laboratory can be used to test the stability and range of the cell-tissue mechanical landscape. It is anchored in first principles mechanics, and tailored for highly accurate and robust modeling of cell-tissue interactions acting under a host of physical forces, including individual cell motions, interactions and organization of cell clusters, and interplay with microenvironments.

Accomplishments and Scientific Advancements

This team has been modeling the mechanical properties of acini in the MCF10 breast tumor progression series and has developed a three-dimensional simulation of the acinus as a linear viscoelastic solid immersed in an incompressible fluid, which can be squashed in a similar manner to the AFM experiments. Experimental results quantitatively match simulations, which together provide a new understanding about how the different geometries of the acini affect the measured stiffness. This group has also modeled the motion of acini placed on a liquid-collagen interface, creating a non-linear elasticity model in which collagen is stronger in tension than in compression.

An important step in breast cancer progression may occur when malignant cells within an acinus escape the confines of the surrounding basement membrane, allowing them to invade the surrounding tissue. This process is difficult to measure experimentally, but the team's simulation methods allow it to capture aspects of this mechanism (Figure 3.21). The investigators are also building a complete Navier-Stokes

Physical Sciences Perspective

Investigators developed a computational simulation accounting for the elastic and compressive forces in a tumor using physical sciences principles such as computational fluid mechanics.

Summary of Research Highlight

A 3-D simulation of MCF-10 breast tumor progression was developed describing the tumor as a linear viscoelastic solid immersed in an incompressible fluid. Preliminary simulations have quantified how the different geometries of the acini affect the measured stiffness.

Implications for Cancer Research

A physics-based computational simulation of a mammary acini provides a platform for developing and testing physical perturbations that would be experimentally infeasible and will provide a more comprehensive understanding of cancer progression.

solution to analyze the dynamics of a cell cluster in a three-dimensional viscous incompressible shearing flow in which the cell-cell adhesive forces are varied to analyze cluster breakup.

Future Plans

This team expects to couple an entire suite of models, including elasticity, hydrodynamic, geometric constraint, and adhesive force models, to provide an accurate and robust computational methodology for cell-cell modeling in complex mechanical environments.

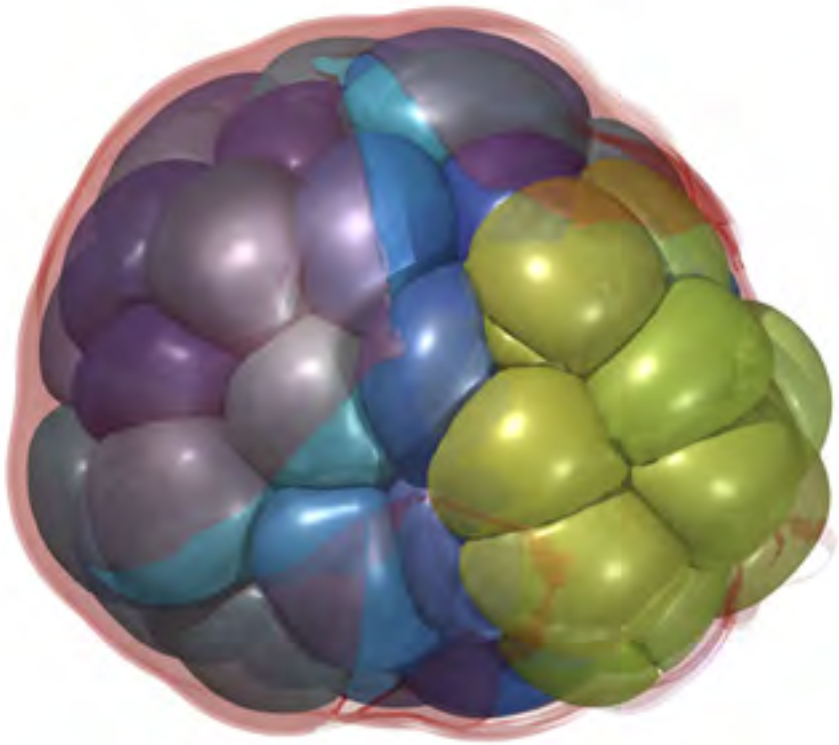
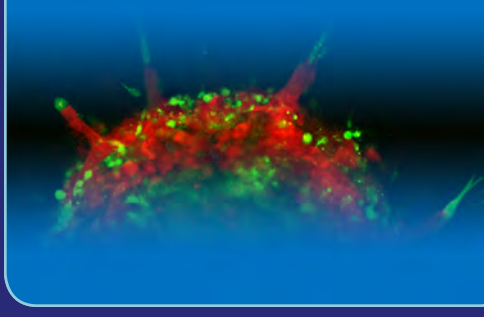


Figure 3.21. Three-dimensional rupture of basement membrane (pink) resulting from clonal expansion of a single rapidly dividing cell (green/yellow) in normal cell cluster (blue/green). The simulation combines incompressible fluid mechanics, elasticity solvers, multiple interface simulations, and geometric constraint growth laws. This is not an illustration or cartoon, but a rigorous calculation from first principles and the basic laws of physics, mechanics, and fluids.

"In total, the papers were published in 176 journals, including the prestigious journal *Nature, Cell, Science,* and *PNAS*."



3.3. Reported Outputs of the PS-OC Network

Along with the scientific highlights described above, additional reported outputs reflect the progress of the PS-OC Network. The work supported by the PS-OC Network has generated hundreds of publications over the first three years of the Program. Many of these manuscripts have been published in leading journals and bibliometric analysis. PS-OC Network research has also led to a number of patents for technological advances and inventions. The innovations developed by the PS-OC Network range from advanced microscopy techniques, to image analysis algorithms, to devices for capturing and measuring circulating tumor cells. Additionally, technology and approaches cultivated by the PS-OC Network have informed development of exploratory objectives in clinical trials. Emerging clinical applications from the Network include testing devices for diagnostic and prognostic power and using mathematical models to inform chemotherapeutic and radiation dosing schemes. The progress made by PS-OC investigators has been leveraged by the Network into millions of dollars of additional funding to support physical sciences in oncology research. Additional funding to support applications of a physical science perspective to cancer research has come from the NIH and other federal funding agencies, universities, private foundations, and corporations. These outputs of the PS-OC Network are detailed below.

3.3.1 Publications

As the NCI PS-OC Program completes the third year of funding, its innovative research has been communicated through a rapidly growing number of publications. To date, 538 publications have been identified from the progress reports. Because of the lag in updating the NIH databases, the number retrieved from these sources is lower and stands at about 400 (Figure 3.18). In total, the papers were published in 210 journals, including the prestigious journal *Nature* (11), *Cell* (6), *Science* (5), and *PNAS* (28). Although it is still early to assess the impact of the research supported by the PS-OC Program, two widely used metrics were used to evaluate the value of scientific output. Both the average impact factor and the average number of citations in one year were slightly higher for PS-OC-funded research compared to the work published by the investigators in the three years prior to the PS-OC Program. The average impact factor increased from 7.29 to 9.18, while the average number of first year citations increased from 5.82 to 6.54 (please see more details in PS-OC Metrics Update). The following list highlights six key publications from each of the four PS-OC themes.

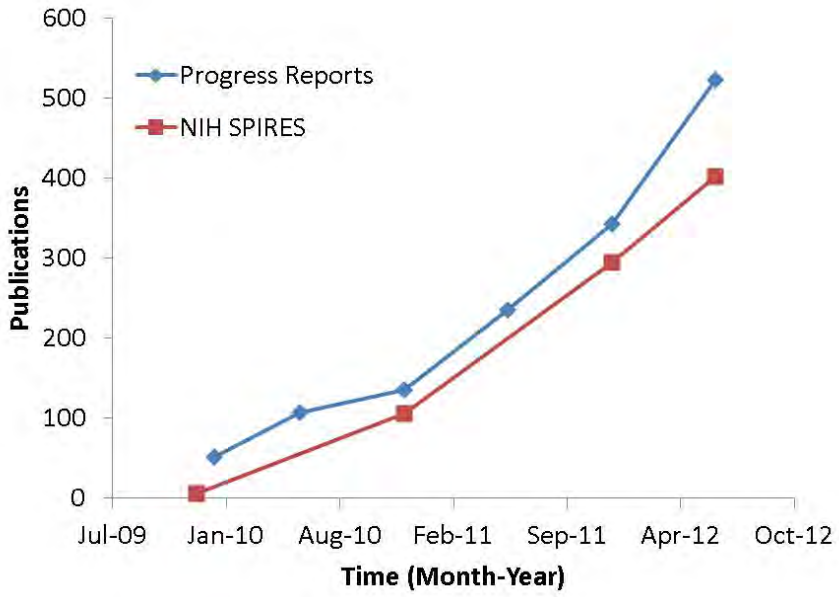


Figure 3.22. The number of PS-OC Network publications. Data from the NIH progress reports submitted by the PS-OCs are shown in blue. The number of publications reported from NIH SPIRES is shown in red. The total number of publications reported by NIH SPIRES is lower than what is reported in the progress reports due to the lag time associated with updating the NIH SPIRES database.

Selected Publications

Physics of Cancer

Fraley, S.I., et al. A distinctive role for focal adhesion proteins in three-dimensional cell motility. *Nat Cell Biol* **12**, 598-U169 (2010).

Wong, C.C.L., et al. Hypoxia-inducible factor 1 is a master regulator of breast cancer metastatic niche formation. *Proc Natl Acad Sci U S A* **108**, 16369-16374 (2011).

Adriani, G., et al. The preferential targeting of the diseased microvasculature by disk-like particles. *Biomaterials* **33**, 5504-5513 (2012).

Lee, M.H., et al. Mismatch in mechanical and adhesive properties induces pulsating cancer cell migration in epithelial monolayer. *Biophys J* **102**, 2731-2741 (2012).

Liu, L., et al. Probing the invasiveness of prostate cancer cells in a 3d microfabricated landscape. *Proc Natl Acad Sci U S A* **108**, 6853-6856 (2011).

De-Convoluting Complexity

Mukherji, S., et al. Microrna s can generate thresholds in target gene expression. *Nat Genet* **43**, 854-859 (2011).

Patel, J.P., et al. Prognostic relevance of integrated genetic profiling in acute myeloid leukemia. *N Engl J Med* **366**, 1079-1089 (2012).

Stroock, A.D. & Fischbach, C. Microfluidic culture models of tumor angiogenesis. *Tissue Engineering Part A* **16**, 2143-2146 (2010).

van de Ven, A.L., et al. Integrated intravital microscopy and mathematical modeling to optimize nanotherapeutics delivery to tumors. *AIP Adv* **2**, 11208 (2012).

Swanson, K.R., et al. Quantifying the role of angiogenesis in malignant progression of gliomas: In silico modeling integrates imaging and histology. *Cancer Res* **71**, 7366-7375 (2011).

PS-OC Publications – Quick Facts

Total Peer-Reviewed Publications:
572

Average Impact Factor:
9.6 (7.12 baseline)

Average 1 year citations:
6.87 (5.61 baseline)

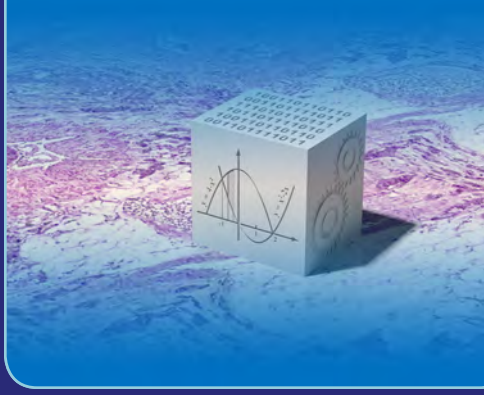
Unique Journals:
176

Nature Publications:
12

Cell Publications:
7

Science Publications:
4

PNAS Publications:
33



Information Theory and Information Transfer

Brogaard, K., Xi, L., Wang, J.P. & Widom, J. A map of nucleosome positions in yeast at base-pair resolution. *Nature* **486**, 496-501 (2012).

Damania, D., et al. Role of cytoskeleton in controlling the disorder strength of cellular nanoscale architecture. *Biophys J* **99**, 989-996 (2010).

Inobe, T., Fishbain, S., Prakash, S. & Matouschek, A. Defining the geometry of the two-component proteasome degron. *Nat Chem Biol* **7**, 161-167 (2011).

Evolution and Evolutionary Theory

Haeno, H., et al. Computational modeling of pancreatic cancer reveals kinetics of metastasis suggesting optimum treatment strategies. *Cell* **148**, 362-375 (2012).

Attolini, C.S., et al. A mathematical framework to determine the temporal sequence of somatic genetic events in cancer. *Proc Natl Acad Sci U S A* **107**, 17604-17609 (2010).

Cheng, Y.K., et al. A mathematical methodology for determining the temporal order of pathway alterations arising during gliomagenesis. *PLoS Comput Biol* **8**, 5 (2012).

Table 3.1. A list of journals with the highest number of PS-OC reported publications.

Journal	Number of Publications	Journal Impact Factor (2009-2012)
PLoS One	36	4.4
Proceedings of the National Academy of Sciences of the United States af America	28	9.8
Physical Biology	20	3.1
Blood	16	10.6
Cancer Research	14	8.2
Nature	11	36.1
Nucleic Acids Research	10	7.8
Biophysical Journal	8	4.2
Clinical Cancer Research	8	7.3
Journal of Theoretical Biology	8	2.4
PLoS Computational Biology	8	5.8
Biomaterials	7	7.9
Molecular Pharmaceutics	7	5.4
Cancer Cell	6	26.9
Cell	6	32.4
Nanotechnology	6	3.6
Nature Biotechnology	6	31.1

3.3.2 Patents

Although the PS-OC Program was not designed with the goal of stimulating translational research, it has been encouraging to see that the work support has led to technological breakthroughs that have resulted in 23 reported patent disclosures. Of the 12 Centers, 8 have reported at least 1 disclosure. The inventions cover a broad range of functions and illustrate the broad impact that incorporating a physical sciences perspective has had in terms of clinical and biological relevance. Applications include novel imaging technologies, contrast and therapeutic agents, devices for capturing circulating tumor cells, image analysis and processing algorithms, and techniques for modulating the tumor microenvironment.

Examples of patents disclosures from the PS-OC Network include:

Gillies R., Morse D., et al. (2011). Method for Reducing Intratumoral pHe and Acid-Mediated Invasion. *H. Lee Moffitt Cancer Ct and Res Inst Inc.*

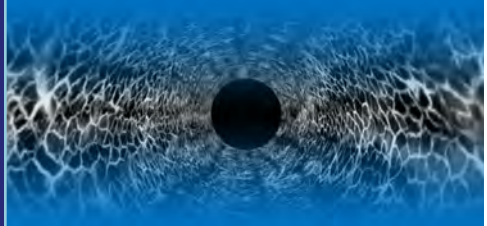
Dravid V, Sharma S, Tomita T, Viola K, Klein W. Magnetic Nanostructures as Theranostic Agents. *Northwestern University.*

Decuzzi P., et al. MRI Contrast Agents in Nanoporous Particles. *The University of Texas Health Science Center.*

3.3.3 PS-OC Related Clinical Trials

The PS-OCs have accelerated faster than anticipated in applying their physical sciences perspective to inform development of exploratory objectives in clinical trials. Many PS-OCs are conducting prospective clinical trials, retrospective trials, or studies using clinical samples. The clinical questions the Centers are asking cover a range of issues that include using sodium bicarbonate to improve survival and relieve cancer-associated pain; mathematical modeling to provide guidance in drug dosing and dosing schedules to prevent or delay drug resistance; testing the reliability, correlation with disease stages, and predictability of circulating tumor cells (CTCs), and profiling tumor tissue mechanics in palpable

"The total amount of leveraged funding to date is more than $100 million (accumulative amount for the next five years), which includes awards from various sources including federal and state agencies, private foundations, and industrial partners."



versus non-palpable breast tumors. Other clinical questions are being explored as well, such as simultaneous mappings of multiple mutations in tissue slices by microfluidic PCR matrix, identifying genetic predictors of outcome that improves risk stratification in AML, and mapping the landscape of tumor heterogeneity by quantitative single-cell assays.

Current clinical trial design for drug dosing and dosing schedules is based on trial and error to achieve safety and efficacy. Current drug or radiation therapies are often effective in shrinking or killing the tumor, but with few exceptions the most challenging issue in cancer therapy is that most tumors recur in a more aggressive and drug- and radiation-resistant state. A current effort by the DFCI PS-OC is to use evolutionary mathematical modeling to design clinical trials for glioblastoma multiforme and non-small cell lung cancer. These models are expected to guide clinicians in designing optimized treatment schedules to eradicate the tumor cell population and to prevent or delay the onset of drug resistance.

Another physical sciences perspective that is being tested for clinical utility is the use of the so-called liquid biopsy technologies based on isolating CTCs from cancer patients. The Scripps PS-OC and the Cornell PS-OC are running clinical trials using CTCs as a noninvasive way to biopsy tumors and avoid the ethical and technical issues encountered with traditional tumor biopsy methods. Currently, the Scripps PS-OC is conducting a phase I study to determine if its CTC technology, which does not include enrichment, can predict recurrence in patients with resectable breast cancer. The Scripps PS-OC is also using CTC technology to evaluate the safety and efficacy of two cancer drugs in subjects with hepatocellular carcinoma and to change tumor-staging modalities in liver cancer patients receiving liver transplants. The Cornell PS-OC is running a phase II clinical trial to personalize drug treatment in prostate cancer patients based on tubulin bundling measurements of CTCs captured using a Geometrically Enhanced Differential Immunocapture (GEDI) microchip.

3.3.4 Leveraged Funds

The PS-OC Program has generated an amount of leveraged funding by all 12 Centers over the past 3 years. The total amount of leveraged funding to date is more than $100 million (accumulative amount for the next five years), which includes awards from various sources including federal and state agencies, private foundations, and industrial partners. Major funding awarded to PS-OC investigators are listed below.

Biomedical Advanced Research and Development Authority (BARDA)

- S. Wang *(USC PS-OC), Stanford Center for Magnetic Nanotechnology,* $38M, *2009-2014*

National Institutes of Health

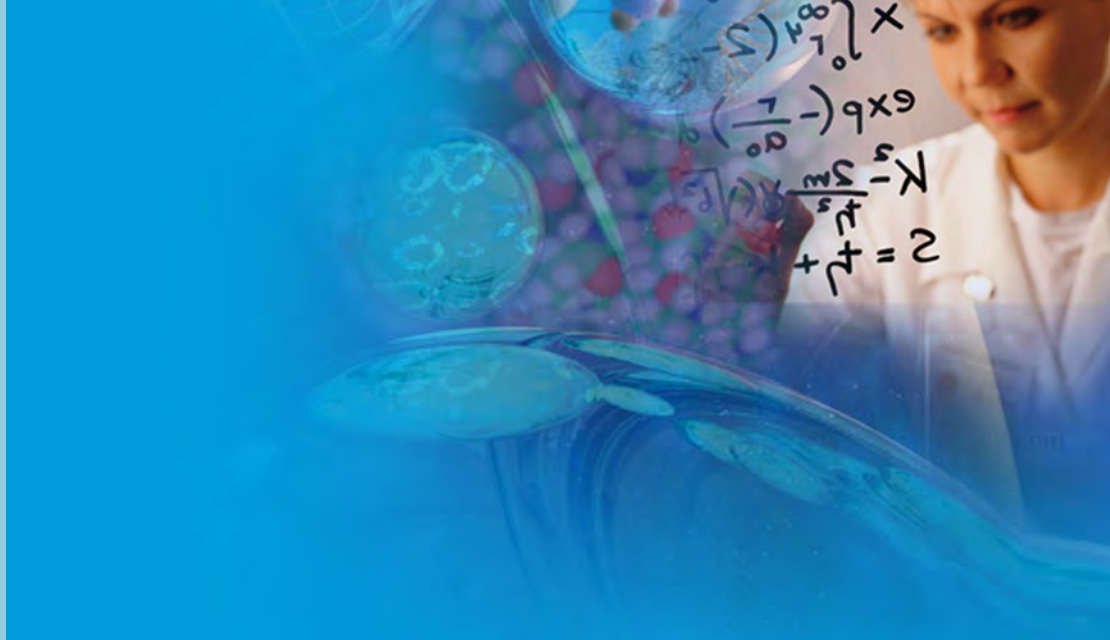
- D. Wirtz *(JHU PS-OC), Single-cell Phenotyping for Therapeutic Stratification in Pancreatic Cancer,* $3.8M, *2012-2017*
- V. Backman *(NU PS-OC), Nanoscale/Molecular Analysis of Fecal Colonocytes for Colorectal Cancer Screening,* $2.8M, *2012-2017*
- M. Shuler, J. Hickman *(Cornell PS-OC), Microphysiological Systems and Low Cost Microfluidic Platform with Analytics,* $2.2M, *2012-2014*
- S. Gerecht *(JHU PS-OC), Engineered Stem Cell Microenvironments for Controlled Vasculogenesis,* $2M, *2011-2016*
- T. Ugarova, R. Ros *(ASU PS-OC), Molecular Basis for Nonadhesive Properties of Fibrinogen Matrices,* $1.7M, *2011-2014*
- K. Smalley *(Moffitt PS-OC), Microenvironment Mediated Drug Resistance in Melanoma,* $1.7M, *2011-2016*
- D. Meldrum *(ASU PS-OC), Live-cell Microarray for High-throughput Observation of Metabolic Signatures,* $1.4M, *2011-2013*
- E. Holland, F. Michor *(DFCI PS-OC), Physical Science Oncology Center Training Program,* $0.58M, *2012-2017*
- C. Reinhart-King *(Cornell PS-OC), Chemotactic Properties of Individual Cells that Contribute to Metastatic Migration,* $0.43M, *2012-2017*

Leukemia & Lymphoma Society

- J. Licht *(NU PS-OC), Chromatin Mechanisms and Epigenetic Targeting in Hematological Malignancies,* $6.1M, *2012-2017*

James S. McDonnell Foundation

- K. Swanson, P. Canoll, A. Anderson *(Moffitt PS-OC), Brain Oncology Network of Knowledge,* $1.6M, *2011-2014*



4. PS-OC Pilot Projects

4.1. PS-OC Center Pilot Projects

Each PS-OC dedicates approximately 5 percent of its total costs to fund Center Pilot Projects. These are projects that each Center administers, with assistance from OPSO program officials, to support new projects within its Center. The Centers are free to develop the Center Pilot Project Program to meet their specific needs (Figure 4.1). PS-OCs have used this mechanism to fund a variety of projects that include the following:

- High-risk, high-reward projects that have the potential to lead to major breakthroughs in cancer research
- Projects that could replace those that are underperforming or completed in a potential renewal proposal
- Projects that would meet a specific short- or long-term need within the PS-OC
- Independent projects that emerge from the findings of ongoing work

This section highlights four Center Pilot Projects that illustrate the impact that this mechanism has had in facilitating cutting edge science. At the DFCI PS-OC, researchers combined comprehensive genetic profiling of acute myeloid leukemia (AML) samples with sophisticated mathematical and computational analysis to generate a novel prognostic schema. This study examined samples from a clinical trial and analyzed all genes that were mutated in more than 5 percent of AML patients. This analysis enabled the investigators to predict response to chemotherapy and stratify patients into favorable, intermediate, and adverse response groups. Furthermore, the algorithm succeeded in stratifying patients enrolled in an independent clinical trial, confirming the predictive power and clinical relevance of this work. In a JHU PS-OC Pilot Project, investigators used advanced engineering techniques to demonstrate that the loss of obscurin gene function enhances the ability of breast cancer cells to migrate through confined spaces. This work highlights an important function of this poorly characterized gene, and the preliminary results generated through this Pilot Project enabled the investigators to apply for a Department of Defense Impact Award grant.

At the Moffitt Cancer Center PS-OC, a Pilot Project has successfully employed mathematical and computational approaches to investigate the somatic evolution of breast cancer cells through multiparametric analysis of cells in histology sections. Automated image and pattern recognition techniques enable the researchers to quantify the heterogeneity in physical properties in the tumor cell population and may reveal the evolutionary dynamics of tumor progression and help pathologists more accurately interpret histological data. Finally, a Scripps PS-OC Pilot Project is evaluating whether circulating tumor cells (CTCs) are a prognostic measure of how patients will perform after liver transplants. In this study, CTCs were collected before and after a liver transplant procedure, along with primary tissue from the tumors. The collected samples will be analyzed retrospectively to determine whether CTC numbers and characteristics are correlated with clinical outcomes. The current protocol for determining whether patients meet the criteria for liver transplants are severely limited. Thus, the potential for this research to more accurately identify patients who will benefit from transplants could have a dramatic impact on patient care.

Figure 4.1. A map depicting the number of Pilot Projects at each PS-OC. The size of the circle for each Center indicates the number of Pilot Projects. The number of projects varies between 1 and 11, depending on how the PS-OC has conducted its individual Pilot Project Programs.

★ Prognostic Relevance of Integrated Genetic Profiling in Acute Myeloid Leukemia

Ross Levine and Mithat Gonen, Memorial Sloan-Kettering Cancer Center

Project Objectives and Significance

This study shows that integrated genetic profiling of a large, clinically annotated cohort of AML patients, combined with statistical/mathematical analysis, results in the development of novel prognostic algorithms with immediate clinical utility in AML. These analyses also provide new insights into leukemia biology and AML pathogenesis.

Background

Acute myeloid leukemia (AML) is heterogeneous with respect to biology, presentation, and clinical outcome, but research has shown that only relatively small numbers of cytogenetic and molecular lesions have sufficient relevance to influence clinical practice. Although progress has been made in defining prognostic markers for AML, a significant proportion of patients lack a specific abnormality of prognostic significance. Additionally, there is significant heterogeneity in outcome for individual patients in each risk group. The prognostic value of recently identified somatic mutations has not been systematically evaluated in AML patients treated in a phase III clinical trial. Moreover, no group has used statistical and mathematical modeling to identify mutational complex genotypes that inform AML biology, accurately predict prognosis, and elucidate mechanisms of leukemia etiology. In addition, no clinical trial has been conducted to determine whether mutational profiling of a larger set of genes, including these novel disease alleles, improves prognostication in AML. Drs. Levin and Gonen hypothesized that integrated mutational analysis of all known molecular alterations occurring in greater than 5 percent of AML patients identifies novel molecular markers of outcome in AML and defines molecular subsets of patients who benefit from dose-intensified induction chemotherapy.

Accomplishments and Scientific Advancements

In this Pilot Project, the investigators performed mutational analysis of 18 genes in 398 patients younger than 60 with AML (Figure 4.2). Patients were randomized to receive induction therapy including high-dose or standard dose daunorubicin. Based on their analysis, the investigators developed a prognostic schema integrating their findings from comprehensive mutational analysis with cytogenetic data into three risk groups with favorable, intermediate, and adverse risk of recurrence. The mutational prognostic schema predicted outcome independent of age, white blood cell count, induction dose, and transplantation status in a multivariate analysis and held true regardless of post-remission therapy. These mutational predictors involved complex genotypes, suggesting combinations of mutations mark prognostically relevant groups and segregate AML into distinct biologically significant subsets. Given the number of variables on this prognostic classification, the investigators tested the reproducibility of this predictor in an independent cohort of 104 patients from the ECOG E1900 trial and confirmed the reproducibility of the prognostic schema to predict outcome in AML.

Green star = clinical implications

Physical Sciences Perspective

This study shows that integrated genetic profiling of a large and clinically annotated cohort of AML patients, combined with statistical/mathematical analysis, results in the development of novel prognostic algorithms with immediate clinical utility in AML, informs biologic decisions and leads to novel insights into AML pathogenesis. Moreover, it shows how biologists, computational experts, and mathematicians can work together to develop novel clinically relevant insights through the analysis of high content genomic data.

Summary of Research Highlight

These data provide important clinical implications of genetic alterations in AML by delineating mutations that predict outcome in AML and improve AML risk stratification.

What Makes It Innovative?

This study demonstrates the utility of mutational profiling to improve prognostic and therapeutic decisions in AML and how computational analysis of genomic data can be used to make novel clinically relevant insights.

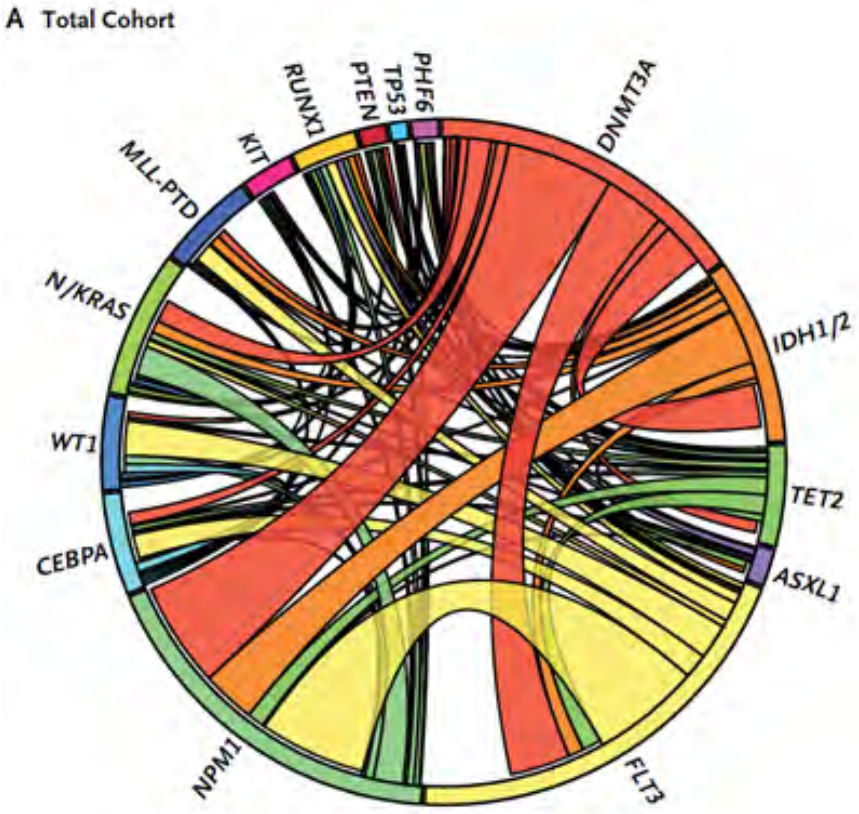
Integrating mutational data with dose-intensity revealed that high-dose daunorubicin improved survival in patients with DNMT3A/NPM1 mutations or MLL translocations relative to treatment with standard dose daunorubicin, but not in patients wild-type for these alterations. These data provide important clinical implications of genetic alterations in AML by delineating mutation combination genotypes that predict outcome in AML and improve AML risk stratification.

Future Plans

Current and future studies are aimed at addressing whether similar prognostic approaches can be used to predict outcomes in other myeloid malignancies, including elderly AML, myeloproliferative neoplasms, and myelodysplastic syndrome. In addition, these investigators are now using next-generation sequencing to determine if genetic heterogeneity affects outcome in AML.

Publication

Gonen, M., et al. Cd25 expression status improves prognostic risk classification in aml independent of established biomarkers: Ecog phase 3 trial, e1900. *Blood* **120**, 2297-2306 (2012).



Gene	Overall Frequency (%)
FLT3 (ITD, TKD)	37 (30, 7)
NPM1	29
DNMT3A	23
NRAS	10
CEBPA	9
TET2	8
WT1	8
IDH2	8
IDH1	7
KIT	6
RUNX1	5
MLL-PTD	5
ASXL1	3
PHF6	3
KRAS	2
PTEN	2
TP53	2
HRAS	0
EZH2	0

Figure 4.2. Mutational Complexity of AML: Circos diagram depicting relative frequency and pairwise co-occurrence of mutations in de novo AML patients enrolled in the ECOG protocol E1900 (Panel A). The arc length corresponds to the frequency mutations in the first gene and the ribbon width the percentage of patients that also have a mutation in the second gene. Pairwise co-occurrence of mutations is denoted only once, beginning with the first gene in the clockwise direction. Since only pairwise mutations are encoded for clarity, the arc length was adjusted to maintain the relative size of the arc and the correct proportion of patients with a single mutant allele is represented by the empty space within each mutational subset.

Elucidation of the Roles of Giant Obscurins in Breast Cancer Development and Progression

Aikaterini Kontrogianni-Konstantopoulos, University of Maryland School of Medicine, and Konstantinos Konstantopoulos, Johns Hopkins University

Project Objectives and Significance

The obscurin gene (OBSCN) is frequently mutated in breast cancer, yet its function in tumor development and metastasis remains unclear. In this Pilot Project, the investigators employed a multidisciplinary approach involving innovative biophysical, imaging, and cell and molecular biology tools to demonstrate that the loss of giant obscurins confers a survival and growth advantage, induces epithelial to mesenchymal transition, and increases the migratory and invasive potential of breast epithelial cells.

Background

Obscurins comprise a family of cytoskeletal and membrane regulators encoded by the single *OBSCN* gene, which undergoes extensive splicing to give rise to at least four isoforms. The prototypical obscurin, obscurin A, is an approximately 720kDa protein containing multiple adhesion and signaling motifs arranged in tandem. The OBSCN gene gives rise to another large isoform, obscurin B, an approximately 870kDa protein containing two putative serine/threonine kinase domains. Sequencing analysis of 13,023 genes in breast and colorectal cancers identified 189 candidate genes that were highly mutated in these cancers. Of the 189 candidate genes, TP53 and OBSCN were the only commonly mutated genes in both tumor types. Analysis of the mutational profile of OBSCN in other types of cancers revealed a germline mutation in glioblastoma as well as novel somatic mutations in melanoma. Moreover, whole genome array analysis of 68 gastrointestinal stromal and leiomyosarcoma tumors indicated that the differential expression of OBSCN and Prune2 could be used as a two-gene expression classifier to distinguish between the two tumor types. Collectively, these studies strongly suggested the importance of the normal expression of obscurins and the unexpected association between mutations in the OBSCN gene and the development of different types of cancer.

Accomplishments and Scientific Advancements

In this Pilot Project, the investigators studied the expression profile of obscurins in breast, colon, and skin cancer cell lines and their involvement in cell survival and growth. Immunoblot analysis demonstrated significant reduction of obscurins in cancer cells, resulting from decreased mRNA levels and/or the presence of mutant transcripts. In normal epithelium, obscurins localize in cytoplasmic puncta, the cell membrane, and the nucleus. Non-tumorigenic MCF10A breast epithelial cells stably transduced with small hairpin RNAs (shRNAs) targeting giant obscurins exhibited increased viability and reduced apoptosis following exposure to the DNA-damaging agent etoposide. Consistent with this finding, quantitative RT-PCR analysis indicated that the anti-apoptotic genes BAG4 and HAX1 were up-regulated, while initiator caspase-9 and death caspase-3 transcripts were down-regulated. From these results, critical roles for obscurins in cancer development by contributing to the regulation of cell survival were pinpointed. The investigators also showed that down-regulation of giant obscurins

Physical Sciences Perspective

Using microfabrication and microrheology tools, this team has examined how loss of giant obscurins regulates cell cytoskeletal compliance, cell motility, and invasion in physically confined spaces.

Summary of Research Highlight

Obscurins are cytoskeletal and membrane regulators, the loss of which provides epithelial cells with a growth advantage (Figure 4.2A) and enhanced migratory ability (Figure 4.2B).

What Makes It Innovative?

Our studies are the first to show that giant obscurins possess tumor and metastasis suppressing activities in breast epithelial cells, which makes them ideal diagnostic and therapeutic candidates.

in MCF10A cells mediates the epithelial-to-mesenchymal transition, as evidenced by the differential regulation of an array of relevant genes including E- and N-cadherin, β- and δ-catenin, vimentin and Slug at both the mRNA and protein levels. The researchers also observed that obscurin-depleted MCF-10A cells acquire a fibroblast-like phenotype concomitant with the disassembly of adhesion junctions, undergo extensive cytoskeletal remodeling, and become more motile and invasive as a result of metalloproteinase secretion. Based on these findings, the investigators proposed that obscurins represent a novel family of tumor and metastasis suppressors in breast epithelium.

Future Plans

Given the dual role of giant obscurins as tumor and metastasis suppressors, the investigators plan to decipher the precise mechanism(s) through which obscurins exert these activities and further evaluate their diagnostic and therapeutic potentials in breast cancer.

Publications

Perry, N.A., et al. Loss of giant obscurins promotes breast epithelial cell survival through apoptotic resistance. *Faseb J* **26**, 2764-2775 (2012).

Shriver, M., K. Stroka, K. Konstantopoulos, and A. Kontrogianni-Konstantopoulos. Loss of Giant Obscurins Induces EMT and Increases the Motility and Invasion of Breast Epithelial Cells. To be submitted to *Nature Commun.*

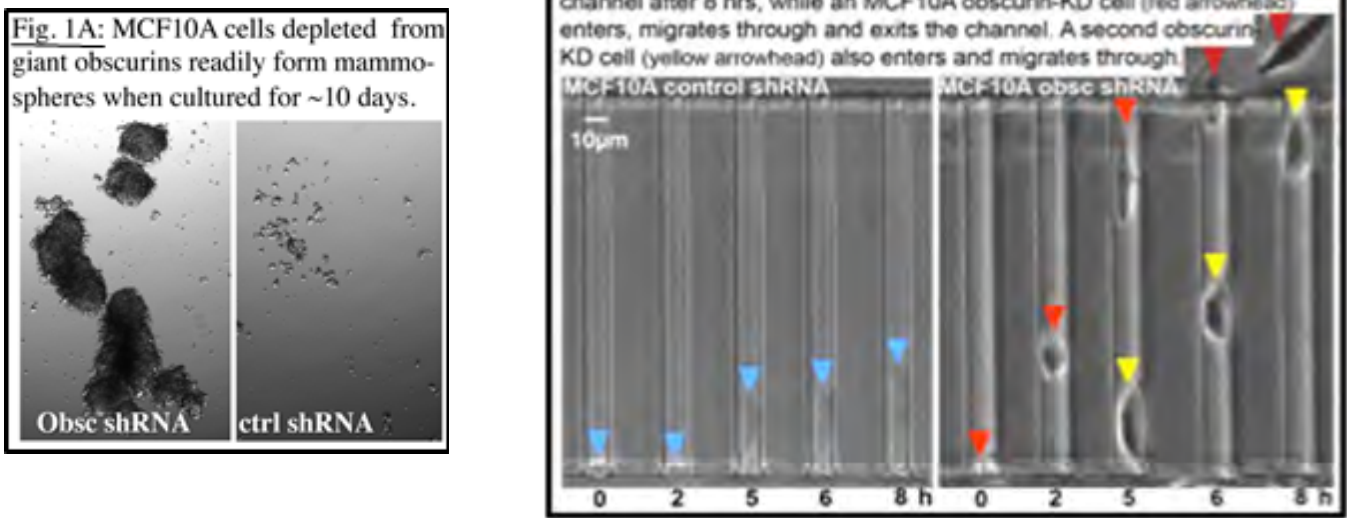


Figure 4.3. (A) MCF10A cells depleted from giant obscurins readily form mammospheres when cultured for 10 days. (B) An MCF10A control shRNA cell fails to enter the channel after eight hrs (blue), while an MCF10A obscurin-knockdown cell enters, migrates through and exits the channel (red). A second obscurin-knockdown cell also enters and migrates through (yellow).

★ Somatic Evolution Within Breast Cancer Histology

Mark Lloyd, Marilyn Bui, Kasia Rejniak, and Joseph Johnson, H. Lee Moffitt Cancer Center and Research Institute; Joel Brown, University of Illinois at Chicago

Project Objectives and Significance

In this Pilot Project, advanced microscopy, image analysis, and computational modeling were used to investigate how the somatic evolution of breast cancer can be characterized and measured. Multiparametric morphological features of single cells in histology sections were evaluated to test the hypothesis that single cell features will distinguish subpopulations of cells, both in the tumor and the physical microenvironment, which will correlate with clues of somatic evolution including phenotypic variation, heritable changes, and niche partitioning and parameterization.

Background

Heterogeneity between cancer cells has long been recognized as a property of cancer progression and resistance to therapeutic intervention and is a hallmark of somatic evolution. Somatic evolution in the progression of cancer is often investigated through genetic heterogeneity and measured in terms of single nucleotide polymorphisms, sequence mutations, and chromosomal abnormalities. While genetics and signaling networks are the basis of core traits, the adaptive evolution and even diversification of cell traits in response to the diverse conditions within the physical microenvironment may dictate trends in tumor growth dynamics. This Pilot Project focused on single-cell heterogeneity with the goal of identifying morphological features of tumor cells and relating these to tumor progression. Phenotypic heterogeneity of tumor cells provides a useful indicator of cancer progression, but such heterogeneity prompts further questions about the underlying biology such as: (1) Does phenotypic variation follow a predictable evolutionary trajectory as tumors progress? (2) Do the cells of progressive stages of higher grade cancer manifest heritable changes or phenotypic plasticity? and (3) Do changes in the physical microenvironment spur tumor cell evolution along adaptive landscapes as adaptive opportunities promote niche remodeling and diversification, which then directs further tumor progression?

Accomplishments and Scientific Advancements

This team used an optical microscopy digitization-based approach to collect individual phenotypic cell feature data and used co-matrix data analysis tools in conjunction with in silico spatial distribution modeling and graphical outlier analysis to address the three questions above. The team used these methods to evaluate potential phenotypic feature similarity between cells and clusters of cell subpopulations to determine whether certain phenotypes appear adapted to fill specific niches and to correlate that information with tumor progression to trace heritable paths of evolution. The investigators also examined tumor cell features in conjunction with their physical microenvironment in an attempt to understand niche parameterization of tumor cells within a changing environmental landscape. Lloyd and colleagues have begun to identify key features, totaling less than 50, within a more expansive multiparametric

Green star = clinical implications

Physical Sciences Perspective

Quantitative pathology and pattern recognition tools computationally elucidate physical changes in cells. By identifying the physical changes of cells, this team can determine if evolutionary principles are influencing cancer progression.

Summary of Research Highlight

Over 9 million cells in 15 breast cancer patient samples have been evaluated to date for more than 40 different physical features.

What Makes It Innovative?

The approach being used is the first means of investigating cancer as an evolutionary process using the same theory of observable physical features Darwin used in the *On the Origin of Species* (e.g., finches) at the single cell level.

morphological and molecular feature set of single cells and subpopulations of cells to identify clusters of similar cell phenotypes. Furthermore, computational characterization of tumor and physical microenvironment cell phenotypes suggests a critical relationship between the invasive tumor and the adjacent soft tissue that may be a key to cancer control. Based on these findings, the investigators are now evaluating the multiparametric feature datasets by grouping individual features and features in combinations with other traits and correlating these results with their spatial location within the tumor and the features of the neighboring host physical microenvironment. Correlating quantifiable tumor cell features with trait evolution within an adaptive landscape may yield insight into cancer cell fitness, its ability to adapt to fill niches when the opportunity arises, and, ultimately, toward new methods to understanding tumor cell invasion.

Future Plans

Future plans will evaluate the feasibility of multiparametric feature identification as a tool to be translated to the clinic to assist pathologists with their evaluation of breast cancer and are seeking patent protection for such a tool. The investigators will also link this information to breast cancer progression using cases of various pathology grade, or Nottingham score, to identify trends. If information regarding the underlying evolutionary biology of the tumor and its physical microenvironment is determined, it will be of value as a diagnostic tool.

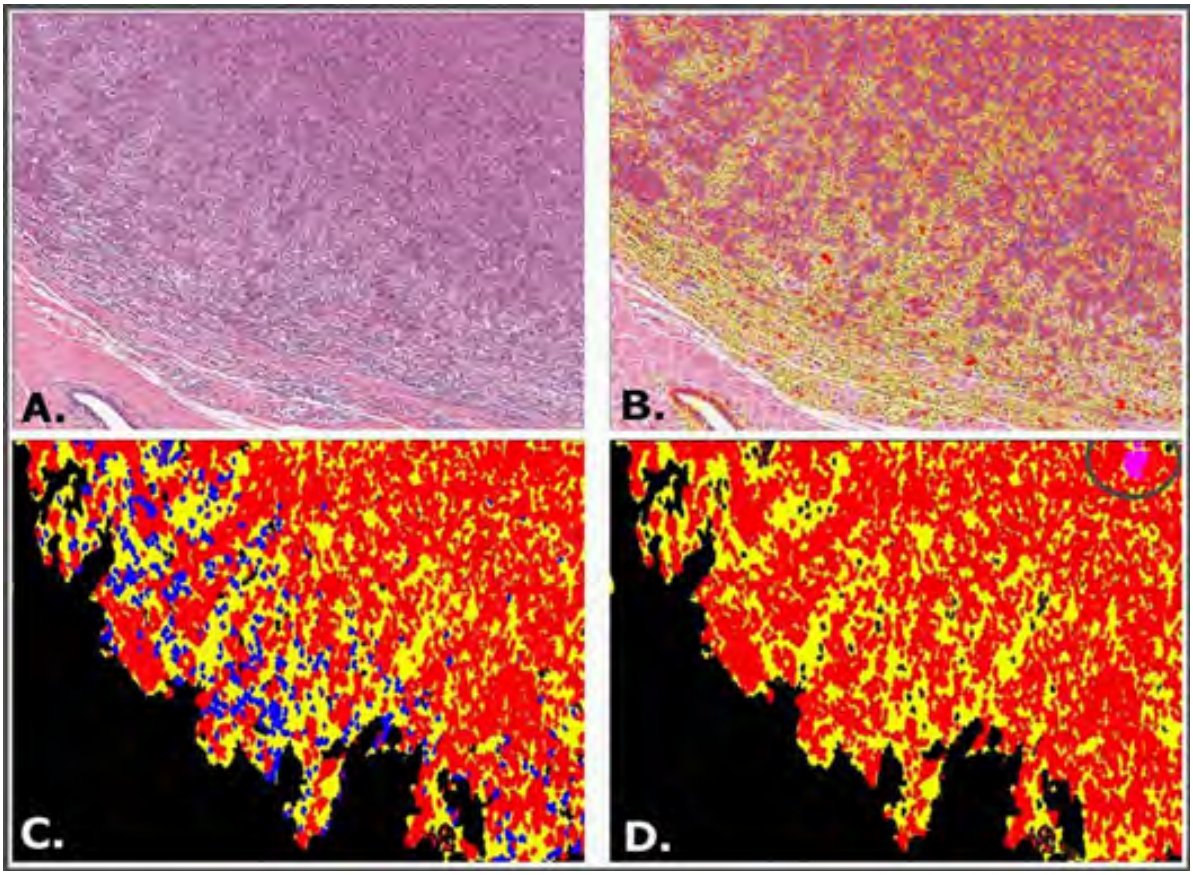


Figure 4.4. A and B show the single cell segmentation (tumor-red; inflammation, fibroblasts-yellow) of a grade III breast cancer H&E, while in C and D individual populations and co-matrix analysis of multiparametric features identified phenotypes along the invasive edge (C; blue and black) and core (D; magenta) of the tumor not recognizable by eye. This approach, still in its infancy, is already beginning to produce insights into the variability that the investigators hypothesize may be relevant in understanding tumor evolution and help explain details of tumor invasion.

Physical Sciences Perspective
The HD CTC platform will allow researchers to measure the kinetics of CTC transport across vascular compartments and follow the evolutionary dynamics of cancer prior to the onset of detectable metastatic disease.

Summary of Research Highlight
The Scripps PS-OC has developed a novel system (HD-CTC Fluid Biopsy) to capture CTCs from patients and have utilized this technology to isolate CTCs from hepatocellular carcinoma patients undergoing liver transplants. The CTCs will be characterized by physical and genetic parameters at the single cell level to determine whether the HD-CTC system can be used to predict clinical outcomes.

What Makes It Innovative?
The ultimate scientific output of this Pilot Project is a direct comparison at the single cell level of the genotype and phenotype of cells of interest. A series of key experimental challenges are being overcome by using specific experimental design processes that enable the measurement of single cell parameters in different environments on single cells that are directly extracted from the patient.

★ HD-CTCS as an Adjunct to Clinical Staging in Patients with Hepatocellular Carcinoma

Randolph Schaffer and Kelly Bethel, Scripps Clinic; and Angel Dago, The Scripps Research Institute

Project Objectives and Significance

This Pilot Project is aimed at better understanding and defining the clinical behavior of hepatocellular carcinoma in cirrhotic patients, including those who are candidates for liver transplantation. The goal of the study is to see whether the HD-CTC Fluid Biopsy can be used to detect tumor cells in patients with hepatocellular carcinoma and be used to augment the current tumor-staging modalities that are available for hepatocellular carcinoma. This Pilot Project aims to produce insights into the kinetics of the fluid phase of hepatocellular carcinoma and the heterogeneity of the disease in the solid phase. Ultimately, this study may alter the way patients with hepatocellular carcinoma are stratified as candidates for liver transplantation and may change the way hepatocellular carcinoma is diagnosed and treatment efficacy is measured.

Background

Hepatocellular carcinoma is one of the leading indications for liver transplantation world wide. In the United States, one in five liver transplants is tied to hepatocellular carcinoma. The Milan Criteria is the current gold standard for selecting eligible hepatocellular carcinoma patients for liver transplantation. Despite rigid definitions of appropriate tumor number and size, there remains a substantial hepatocellular carcinoma recurrence rate after transplantation in patients who meet these criteria. At the same time, patients found to have tumors that exceed the Milan Criteria in either tumor size or number have been shown by some transplant centers to do as well as those patients meeting Milan Criteria.

The most powerful predictor of tumor recurrence is microscopic or subsequent macroscopic vascular invasion, although imaging methodologies cannot readily identify the former. Both forms of vascular invasion, however, suggest disseminated, circulating cells as the source of recurrence. While other investigators have attempted to identify circulating tumor cells in hepatocellular carcinoma patients, their results have been inconsistent and have yet to be validated. Given the substantially improved sensitivity of the Scripps HD-CTC Fluid Biopsy and its capability as an actual biopsy for downstream characterization of cell morphology and single cell genomics, this Pilot Project aims to demonstrate a correlation between the presence of CTCs and tumor behavior in patients with hepatocellular carcinoma before, during and after liver transplantation. Hepatocellular carcinoma is a potentially highly informative tumor type for mapping studies, as it is commonly treated with resection or transplant, allowing clinical access to extensive pre-, intra-, and post-operative anatomically oriented blood sampling as well as primary tissue harvest.

Green star = clinical implications

If the Pilot Project proves successful, the investigators plan to conduct a multicenter study that would allow them to attain sufficient statistical power. If the study results prove to be significant, the subsequent phase would be prospective validation. Ultimately, a successful project would show the HD-CTC assay to be a sensitive tool used in diagnosing, assessing prognosis, and gauging response to therapy in all patients with hepatocellular carcinoma, not just those awaiting liver transplantation.

Accomplishments and Scientific Advancements

The hepatocellular carcinoma project has thus far enrolled 38 patients who are actively on the liver transplant list, with 14 patients having proceeded to definitive surgery, either transplantation or resection, with collection of extensive intraoperative CTC sampling. Primary tumor sampling includes harvesting samples of all identified tumors in various forms including touch preparation, snap frozen, and formalin fixed conditions, with fresh frozen tissue stored appropriately for subsequent analysis. The investigators completed clinical validation of the HD-CTC in this initial patient cohort, proving that cells can be identified and prepared for the downstream biopsy analysis (Figure 4.5). Ongoing assay development work is focusing on tissue specific markers such as HepPAR1 as well as single cell isolation for downstream molecular analysis. The investigators are now completing the protocols for both PCR-based mutational analysis on the Ion Torrent platform as well as single cell gene copy number variation experiments on the Illumina platform. The team has also finished primary tissue dissection and characterization, using specific geographic sections prepared from both malignant and the cirrhotic tissue. Each section is processed for downstream experiments that allow for comparisons with the fluid biopsy. For example, minced tissue is spiked into normal blood to mimic CTCs. The unique ability of the HD-CTC Fluid Biopsy to create a permanent archive of the blood samples for rare cell characterization has been a critical aspect of the team's ability to simultaneously recruit a clinically comprehensive patient cohort, develop and validate assays using a fraction of the samples, and then execute specific experiments against a subset of patient samples with known outcomes. This is the first time that CTC-based assays are being used to monitor patients over time in retrospective trials.

Future Plans

The investigators are completing assay development and will begin the first direct correlation of primary tumor and fluid phase biopsy using multiple experimental modalities, which is expected to provide insights into the system disease patterns at the single patient level.

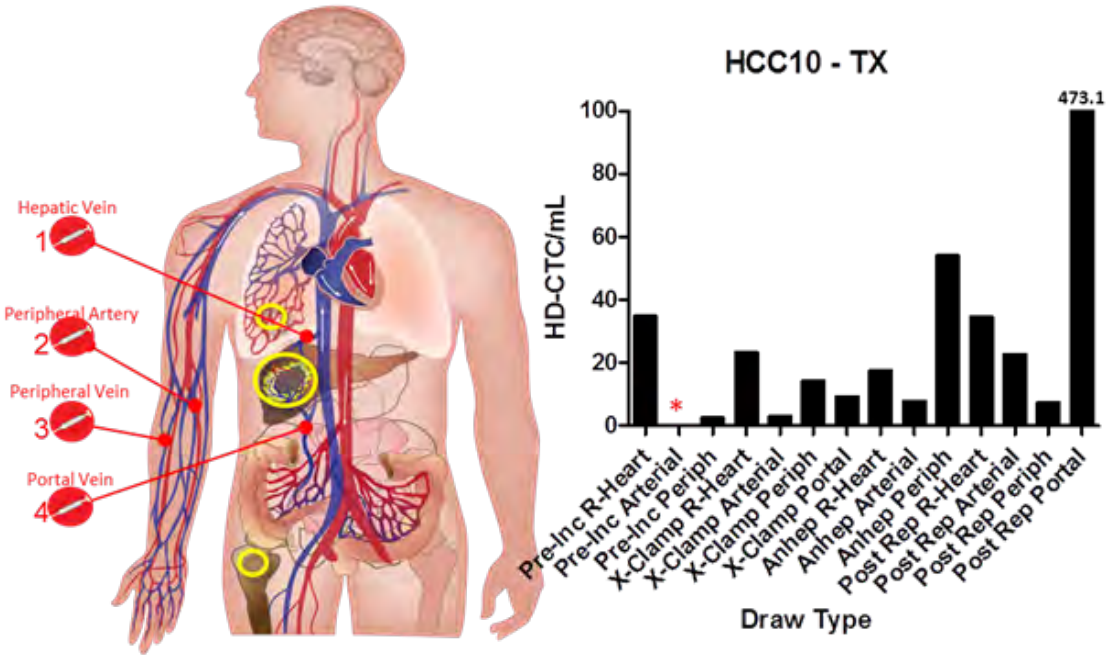


Figure 4.5 A schematic illustration of where CTCs will be collected along with the CTC counts from each collection site at various times pre- and post-surgery.

4.2. PS-OC Outreach Pilot Project

Each PS-OC sets aside about $50,000 each year to fund Outreach Pilot Projects. This funding mechanism provides Centers with the flexibility to easily bring in expertise from outside the Network to address needs within the PS-OC or explore new avenues of research. As with the Center Pilot Projects, the PS-OCs are free to administer these Outreach Pilot Projects through their Center Advisory Committees and with support from OPSO program officials (Figure 4.6). This section highlights three Outreach Pilot Projects that demonstrate the utility of the funding mechanism for recruiting top researchers from outside the PS-OC Network and for initiating cutting edge research.

- An Outreach Pilot Project funded through the UCB PS-OC is combining the Center's focus on mechanobiology with the research of Dr. Victoria Seewaldt from Duke University on the disparately high level of non-palpable, clinically "silent" breast cancers in African American women.
- An MIT PS-OC Outreach Pilot Project led by Dr. Michael Yaffe and Dr. Patrick Doyle of the Koch Institute for Integrative Cancer Research is developing a microfluidic device to measure how the physical parameters of chromatin are altered by post-translational modifications of histones that occur before and after DNA damage.
- A JHU PS-OC Outreach Pilot Project has teamed up Dr. Sharon Gerecht of Johns Hopkins and Dr. Jason Burdick of the University of Pennsylvania to investigate the role of the ECM in controlling vascular morphogenesis using an innovative hydrogel system.

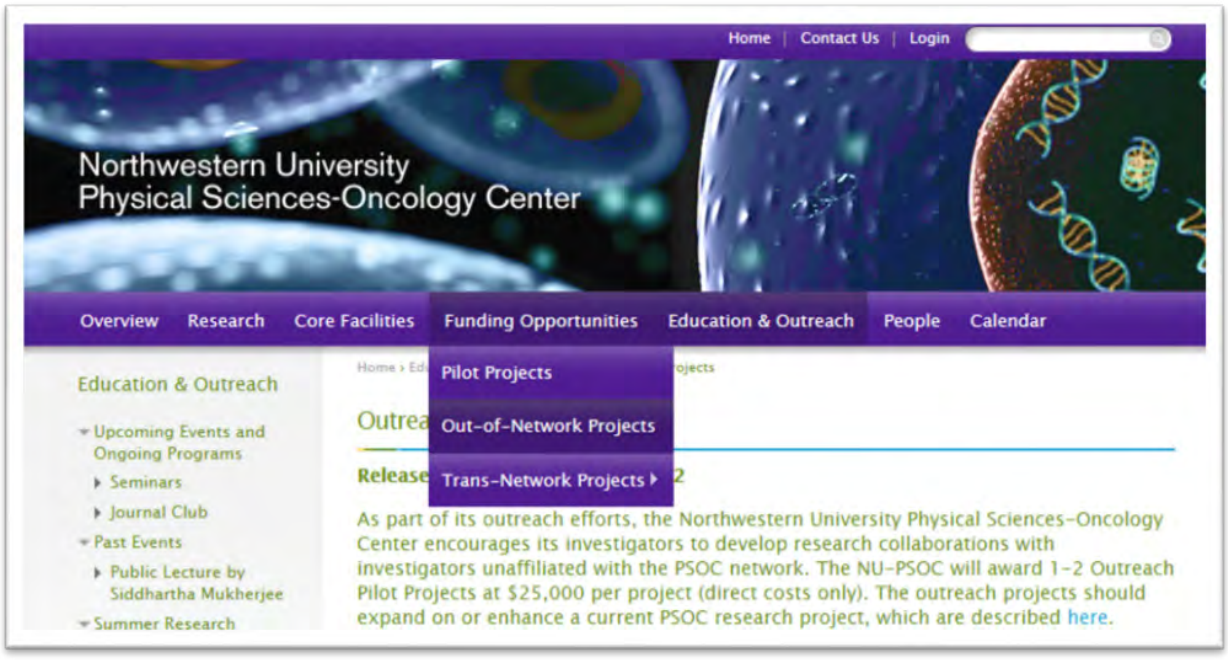


Figure 4.6. The Northwestern PS-OC uses its website to solicit proposals from investigators outside of the PS-OC Program for Outreach Pilot Projects.

★ Mechanobiology of Clinically Silent Breast Cancer in African American Women

Victoria Seewaldt, Duke University and Valerie Weaver, University of California, San Francisco

Physical Sciences Perspective

This project uses advanced manipulation and measurement technologies from the physical sciences, as well as multiscale integrated models of cell-cell and cell-matrix interactions.

Summary of Research Highlight

This team is using advanced tools and concepts from the physical sciences to understand the origin of a health disparity that leads to late detection and poor outcomes in African American women with breast cancer.

What Makes It Innovative?

Innovation consists of quantitatively inter-relating tissue mechanics, signaling, and clinical progression and outcomes in a patient population with a particular disease profile resulting in poor outcomes. This Outreach Pilot Project enables the translation of PS-OC scientific discoveries to drive clinical trials and improve community health.

Project Objectives and Significance

Many breast cancers form a palpable mass, but some do not. These non-palpable cancers are referred to as clinically "silent" and are typically detected only at an advanced clinical stage. Silent breast cancers are especially prevalent in African American women. This Pilot Project asks whether certain biological and biophysical properties of a patient's interstitial collagen are risk factors for developing silent breast cancers. In an established cohort of high-risk African American women, this research team is testing the hypothesis that the relative strengths of mammary cell versus matrix mechanics can distinguish palpable from non-palpable breast cancers and represent a target for early detection.

Background

Clinicians can detect many forms of breast cancer by palpating the tissue, which stiffens during cancer initiation and progression. This stiffening is due in part to gradual changes in the composition, organization and cross-linking of interstitial collagen. Investigators at the UCB PS-OC discovered that during breast cancer initiation and progression, interstitial collagens in the breast are remodeled and cross-linked, bundled, and linearized. Matrix stiffening increases cell contractility to enhance integrin adhesion assembly and increase a complex network of PI3K/FAK/ERK-phospho-signaling, promoting malignant progression. In this way, modifications in the mechanical and biochemical dialogue between the dynamically evolving stroma and progressively abnormal mammary epithelium may promote the formation of a palpable breast cancer. Some cancers do not appear to follow the same path. In some of those cancers, mammary epithelial cells acquire abnormalities that significantly modify their rheology and increase their contractility irrespective of matrix context. These lesions are clinically "silent" or non-palpable, and are frequently not detected by conventional breast cancer screening.

Accomplishments and Scientific Advancements

In this Outreach Pilot Project, the investigators are profiling tissue mechanics in premenopausal African American women during breast cancer initiation and in breast biopsy tissue from premenopausal African American women with palpable versus non-palpable breast cancer. The investigators have initiated tensile force mapping and corresponding analysis of phosphoprotein network signaling in prophylactic mastectomy specimens from four high-risk women. Two women had triple-negative breast cancer; two women had rapid progression based on MRI tracking. More than 100 cores have been obtained per breast. The research team has completed the phosphoprotein signaling and is now testing for modulation of force-regulated miRNA (Figure 4.7). ECM stiffness and cell rheology are being mapped with atomic force microscopy (AFM), matrix

Green star = clinical implications

organization with second harmonic imaging, and cell traction forces using traction force microscopy (TFM). In addition, the investigators are comparing the primary tumor with matched morphologically normal adjacent and distal tissue. Matrix cross linking and mechano-responsiveness are being assessed using Picosirius red (PS) and immunohistochemistry (IHC) for lysyl oxidase (LOX) and 397FAK, and the results are correlated with physical measurements.

Future Plans

It is possible that the dynamic and reciprocal interaction between tissue mechanics and premalignant epithelial cells creates a "high-risk" segment of the breast that allows breast cancer to progress without also stiffening the sourrounding tissue. This may be especially true in a subset of clinically aggressive breast cancers such as triple-negative or inflammatory breast cancer. Quantitative technologies developed by the PS-OC may enable phylogenetic linage assessment of epigenetic events that promote progression of aggressive, clinically silent breast cancers.

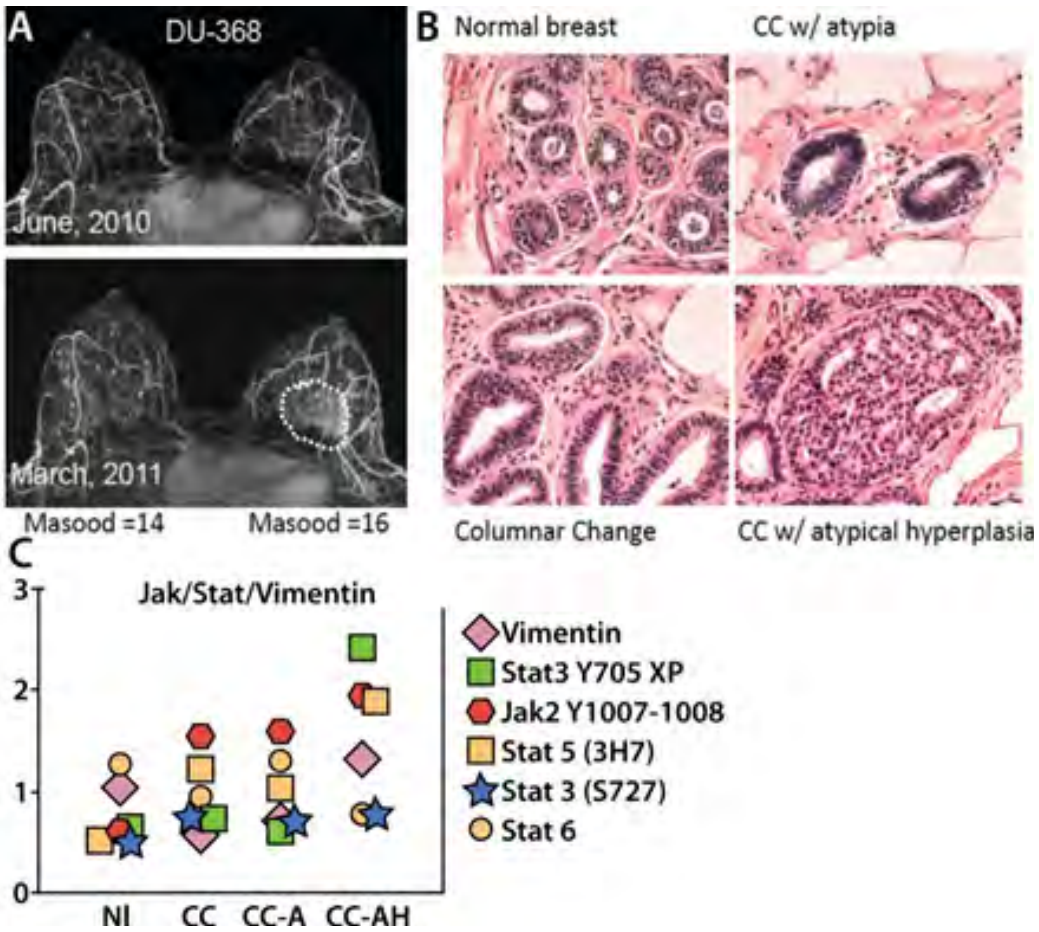


Figure 4.7. Temporal and spatial mapping of phospho-protein activation during breast cancer initiation in DU-368. More than 100 cores were obtained per breast. DU-368 is a 38-year-old African American woman with four family members with premenopausal breast cancer. (A) Breast MRI progression. (B) Early precancerous changes, normal (NL), columnar change (CC), CC with atypia (CC-A), CC-with atypical hyperplasia (CC-AH). (C) Activation of phospho-protein signaling is first observed in CC-AH.

Examining DNA Damage, Signaling and Repair at the Single Molecule Level

Michael Yaffe and Patrick Doyle, Koch Institute for Integrative Cancer Research

Project Objectives and Significance

Cancer is associated with recurrent mutations in chromatin regulators that affect histone modifications and chromatin remodeling. A major thrust within the PS-OC Network is aimed at understanding how higher-order chromatin structure influences chromosome stability, chromosome damage, and structural rearrangements as well as coordination of gene expression. A central goal of this Outreach Pilot Project is to examine how the physical properties of chromatin are altered by post-translational modifications of chromatin proteins, particularly histone acetylation, methylation, and phosphorylation, both before and following DNA damage. The investigators are concentrating primarily on HDAC inhibitors since the most profound changes in chromatin properties associated with the DNA damage response are observed with these drugs. In addition, several of these agents are now in clinical trials for cancer therapy. The investigators first performed a comprehensive screen of HDAC inhibitors to determine which compounds caused the greatest alterations in chromatin structure and which caused the least change in chromatin structure following DNA damage produced by 10 Gy of ionizing radiation. Dose-response relationships by assaying for chromatin opening/γH2AX foci formation were determined using a quantitative high-content microscopy assay. In addition, single molecule techniques were used to examine the spatial and temporal recruitment of specific DNA damage signaling molecules to collapsed replication forks. This project brings together expertise in signaling pathways and networks that control cell cycle progression and DNA damage responses in cancer and cancer therapy with precision measurement of single molecules advanced microfluidic devices.

Background

In response to DNA damage, eukaryotic cells activate a complex network of signaling pathways that localize to the DNA lesion, sense the specific type of damage, arrest the cell cycle, and recruit DNA repair machinery. The DNA damage response functions as a major barrier to tumorigenesis. Mutational or epigenetic inactivation of the DNA damage response is a critical event that must occur during the early stages of tumor development in order for emerging cancers to bypass oncogene-induced senescence. Fortuitously, defects in DNA damage response pathways renders many cancers susceptible to various types of DNA damaging chemotherapeutic agents, many of which constitute the first line of anti-cancer drugs currently used in most clinical treatment regimens. There is a growing, but incomplete understanding of the DNA damage response. The net result of the process appears to be the orderly assembly of signaling proteins and DNA repair factors at or near the sites of damage through the combined actions of protein kinases, ubiquitin ligases, and chromatin remodeling enzymes together with modular binding domains that specifically recognize phosphorylated, ubiquitinated, and/or methylated sequences on chromatin or chromatin-associated molecules. Exactly how this occurs at the single DNA molecule level remains mysterious, since most studied to date have involved biochemical or

Physical Sciences Perspective

A central goal of this Pilot Project is to examine how the physical properties of chromatin are changed by current anti-cancer drugs, and how this results in enhanced chromatin susceptibility to endogenous and exogenous events that result in DNA damage.

Summary of Research Highlight

This Outreach Pilot Project team has used a novel slit-like nanochannel device in which single DNA molecules are flow directed to a stagnation point and then extended by application of an electric field. This device allows for the study of physical properties of chromatin following post-translational modifications of chromatin-associated proteins.

What Makes It Innovative?

Most studies to date have involved biochemical or cell biological assays on large ensembles of cells or imaging studies involving whole nuclei. How single chromatin molecules behave physically has not been well studied.

cell biological assays on large ensembles of cells or imaging studies involving whole nuclei.

Accomplishments and Scientific Advancements

The investigators have designed and built a new slit-like nanochannel device in which single DNA molecules are flow directed to a stagnation point and then extended by application of an electric field (Figure 4.8). By turning an electric field off and then back on, the rate of DNA compaction and re-extension can be measured and analyzed to provide accurate values of the force constant for single DNA molecules. This approach allows the investigators to exchange the buffer solution surrounding the immobilized DNA with cell lysates, including those deficient in chromatin-binding molecules that are important in DNA damage and repair. These devices have been successfully used to measure force constants for single control chromatin molecules and are currently in the middle of making measurements for chromatin isolated from cells treated with HDAC inhibitors and DNA methylation inhibitors before and after DNA damage. Results indicate that HDAC inhibition results in a dramatic reduction in the elastic modulus of chromatin, resulting in direct DNA damage by relatively small extension forces. These observations suggest that one mechanism by which HDAC inhibitors can increase the amount of DNA damage in tumor cells is by physical disruption of the chromatin architecture that buffers weak mechanical forces. Such physical alterations in chromatin might enhance DNA damage when tumor cells undergo replication during S-phase and/or during mitosis, and this team is planning to perform those experiments. In addition, this physically weakened chromatin may be more susceptible to DNA damage by secondary agents such as ionizing radiation and chemotherapy. The investigators have also developed improved methods for distinguishing replication forks that have collapsed upon mild DNA damage stress and are using the nanochannel device to examine the recruitment of different DNA damage signaling molecules to collapsed forks on single DNA molecules as a function of time.

Future Plans

Future plans will continue to measure the alterations in elastic modulus in chromatin upon HDAC inhibition, using both single molecule techniques and also rheological methods to sample a broad ensemble of molecules in order to understand the range of variability. To address sample heterogeneity, the team is now purifying a single genomic locus from mouse embryonic fibroblasts. These experiments should give the investigators a big-picture view on the range of chromatin physical properties at a single locus and in the entire nuclear chromatin fraction. Investigators will then examine whether HDAC inhibitor treated chromatin preferentially forms breaks in S-phase and/or results in enhanced chromatin instability during chromosome separation in mitosis. Finally, the investigators will continue to optimize these methods and explore the kinetics of DNA damage signaling at the single molecule level on collapsed replication forks.

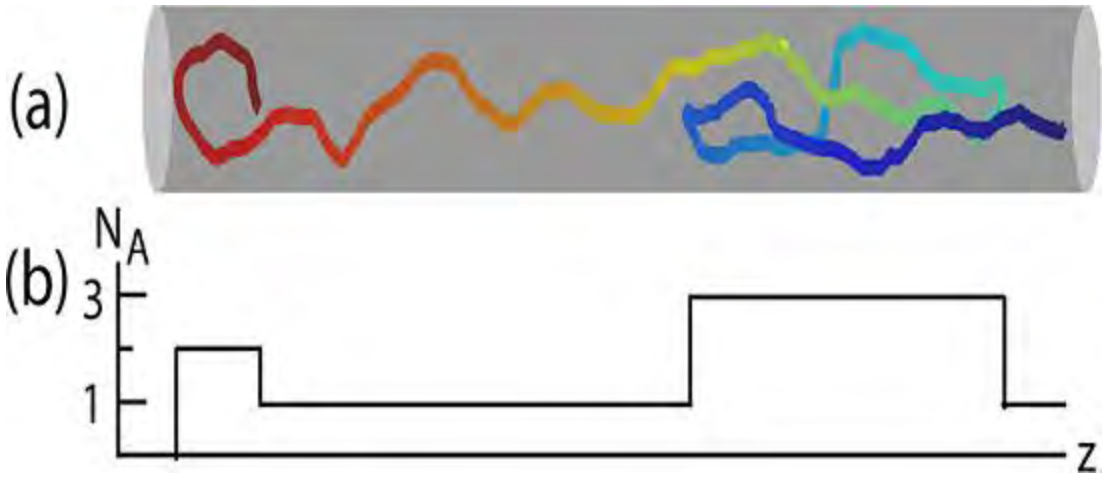


Figure 4.8. (a) Snapshot of equilibrated Monte Carlo conformation of DNA (w = 5 nm, P = 50 nm, L = 8 μm) inside a D = 50 nm channel. Only a section of the chain is shown. (b) Number of segments profile along the channel.

Physical Sciences Perspective

Using materials science and tissue engineering techniques a device was fabricated to mimic the complexity of the environment in order to study the dynamic interactions of cancer and endothelial cells.

Summary of Research Highlight

We recently developed a technique where the hydrogel chemistry can be controlled to alter cell-mediated degradation with spatial control. Since degradation is needed to permit the formation of vascular networks, this approach has allowed us to spatially control vasculogenesis and angiogenesis within hydrogel structures.

What Makes it Innovative?

Controlling cell mediated degradation with spatial control is an innovative technique that opens up further complexity for future in vitro studies of cellular interactions.

Modulated Three-Dimensional Environments to Study Cancer-Endothelial Cell Interactions

Jason Burdick, University of Pennsylvania, and Sharon Gerecht, Johns Hopkins University

Project Objectives and Significance

Understanding the role of the extracellular matrix (ECM) in vascular morphogenesis has been possible using natural ECMs as in vitro models. However, little is known about vascular morphogenesis in synthetic matrices where properties can be tuned toward the basic understanding of tubulogenesis in modular environments that are able to mimic the developing tumor surroundings. The objective of the project was to investigate how cues in the microenvironment affect the interplay between matrix and hypoxia as related to cancer-endothelial cell interactions.

Background

In the preliminary studies, the research team investigated synthetic, tunable hyaluronic acid (HA) hydrogels and determined both the adhesion and degradation parameters that enable human endothelial progenitor cells (EPCs) to form efficient vascular networks. Entrapped EPCs underwent tubulogenesis dependent on the cellular interactions with the HA hydrogel during each stage of vascular morphogenesis. Vascular networks formed within HA hydrogels containing EPCs formed connections with the host's circulation and supported blood flow in the hydrogel following transplantation. This provided the basis for the Pilot Project related to the interaction of EPCs and cancer cells.

Accomplishments and Scientific Advancements

A range of accomplishments were completed with this Outreach Pilot Project award. The first stage involved the investigation of EPC tubulogenesis in HA hydrogels under normoxic and hypoxic conditions. A mathematical model was first developed to investigate levels of dissolved oxygen throughout hydrogels, including oxygen transport via diffusion at interfaces with air, growth media, and through the gel. The investigators found that the dissolved oxygen level within the hyrdogel was dependent on the initial level of human umbilical vein endothelial cell (HUVEC) seeding density. In order to assess cancer cell cycle and proangiogenic responses in HA hydrogels in normoxic and hypoxic conditions, HT1080 cells were encapsulated in HA hydrogels of various stiffness, and the team observed that viscoeleasticity has minimal effects on the cell-induced apoptosis. However, the investigators observed down-regulation of pro-apoptosis/autophagy gene BNIP3/BNIP3L and up-regulation of VEGF and GLUT1 gene as time progressed under hypoxia condition.

Drs. Burdick and Gerecht proceeded to build on their previous efforts in HA hydrogel design, moving to spatial manipulation of cell-mediated gel degradation to control both vasculogenesis and angiogenesis. Vascular tube branching and sprouting that are enabled by matrix metalloproteinase dependent mechanisms were observed only in the "permissive" (primary addition-crosslinking-only) hydrogels, while ECFCs in the "inhibitory" (secondary radical-crosslinking) hydrogels could not degrade the

matrix and were arrested at the vacuole and lumen stage (Figure 4.9). Furthermore, the sequential crosslinking system was used to create three-dimensional regions within a single HA hydrogel that permit or inhibit cell-mediated degradation during in vitro vasculogenesis or angiogenesis, supporting patterned structures.

Future Plans

The investigators will continue to collaborate on the use of hydrogel scaffolds as templates to control EPC vasculogenesis. Specifically, working on a new hydrogel system in which the gel is processed into fibrous structures where fibers can be manipulated with respect to mechanical properties, adhesion, and the release of growth factors locally will be the main focus.

Publications

Hanjaya-Putra, D., et al. Spatial control of cell-mediated degradation to regulate vasculogenesis and angiogenesis in hyaluronan hydrogels. *Biomaterials* **33**, 6123-6131 (2012).

Hanjaya-Putra, D., et al. Controlled activation of morphogenesis to generate a functional human microvasculature in a synthetic matrix. *Blood* (2011).

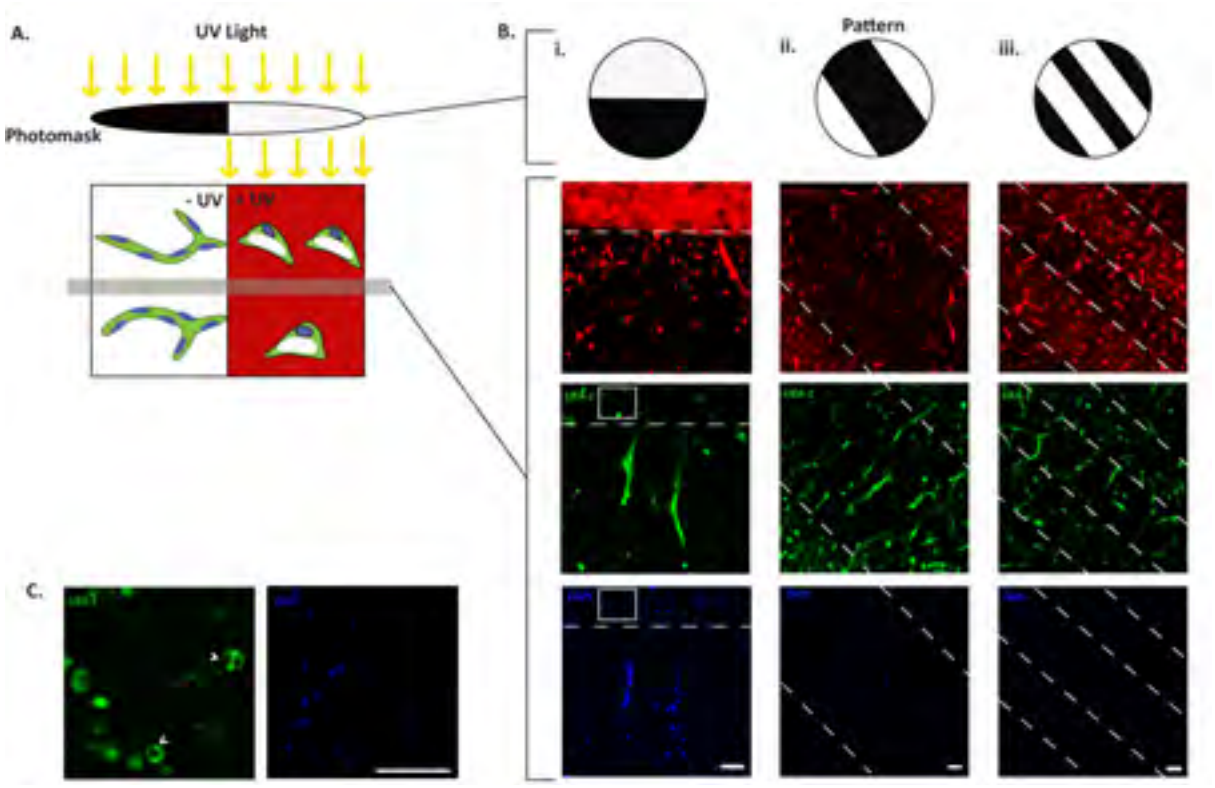
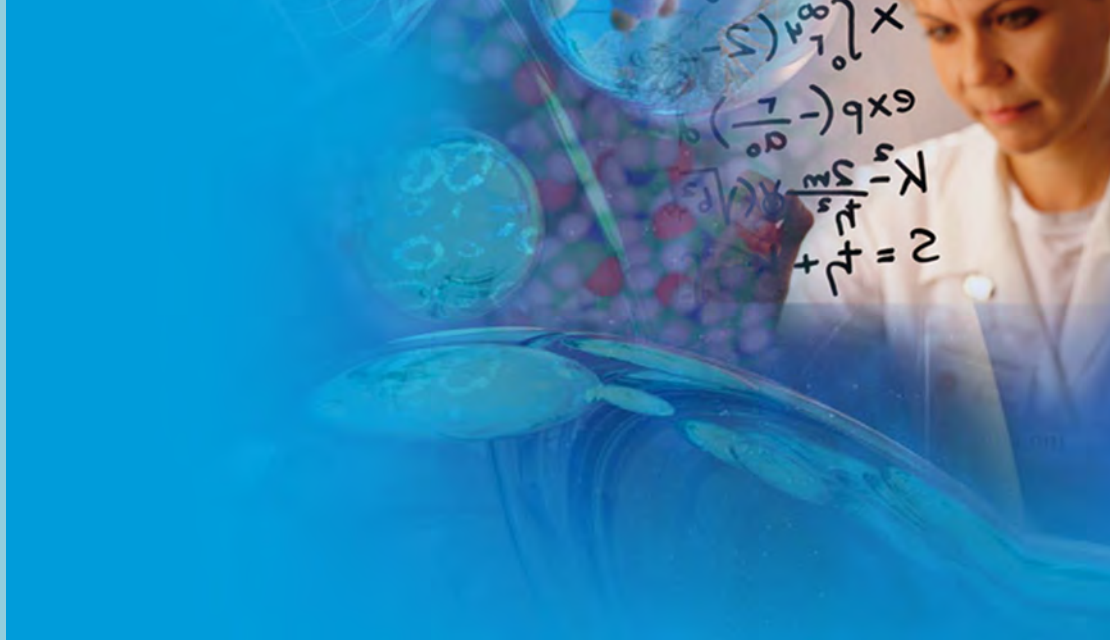


Figure 4.9. Spatial control over in vitro vasculogenesis. (A) Schematic diagram of photopatterning HA hydrogels using UV light and photomasks to create –UV and +UV regions. (B) Representative confocal images of photopatterned AHA hydrogels with various patterns. Vascular tube formation is "permitted" within the –UV regions and "inhibited" within the +UV regions. (C) Vascular morphogenesis by ECFCs is arrested at the stage of vacuole and lumen formation within the "inhibitory" +UV regions (arrowhead); high magnification inset of the boxed area in (B). ECFCs are stained with Fluorescein-conjugated UE-I lectin (green) and DAPI (blue). Scale bars are 100 μm.



5. PS-OC Network Projects and Activities

The PS-OC was built as a virtual Network of 12 centers to promote scientific collaboration and Network-wide dissemination and education of a new transdisciplinary field of physical sciences in oncology. Each PS-OC has its own unique strengths and infrastructure that are directed toward achieving its Center goals. However, the PS-OC Network places particular emphasis on combining these strengths and infrastructure to tackle "Big Questions" in cancer, develop new theories, integrate orthogonal datasets to gain new knowledge, and disseminate to the scientific community through joint workshops and seminars. Since the initiation of the PS-OC Program, there have been a number of different projects and activities designed to enable these collaborations, including Trans-Network Projects, a joint cell line characterization exercise, PS-OC working groups, and a PS-OC investigator retreat. Each of these activities is described in more detail in the following section.

5.1. Trans-Network Projects: Answering the "Big Questions"

A critical and unique component for realizing the full potential of the PS-OC Program is the availability of funds for Trans-Network Projects. Awarded to each of the Centers are approximately $100,000 of restricted funds that are allocated specifically to support Trans-Network Projects. The Trans-Network Projects are designed to answer major questions or overcome critical barriers in cancer research through unconventional physical sciences perspectives and robust collaborations between PS-OC Network investigators.

The PS-OC Steering Committee has overseen the Trans-Network programs and ensured that the programs have met the needs of the PS-OC Network and supported investigators at all levels, from trainees to senior scientists. A summary of the Trans-Network awards is provided below:

- Thirteen large Trans-Network Projects have been funded. The awards have ranged in size from $100,000 to $600,000 in total costs and collaborations between two to six participating PS-OCs. These projects have been initiated either via open calls for proposals on any topic, a call for proposals addressing questions in heterogeneity, or a call focused on a selection of "Big Questions."

- Three Trans-Network Impact Experiment (TIME) awards have been awarded at $30,000. These are designed to support innovative experiments that address the existing aims of at least one of the participating Centers.

- Twelve Young Investigator Trans-Network awards have been funded to support small collaborations among trainees at up to three PS-OCs. Five Young Investigator awardees have since moved to independent assistant professor positions, and four of these awardees are still actively involved in the PS-OC Network.

Overall, the Trans-Network Projects Program has been critical to the interactions of the broader PS-OC Network. By providing funding to support collaborative research between Centers, the Trans-Network Projects lower the barriers to collaboration and promote the formation of a truly integrated Network. Further details on selected projects are provided in this section.

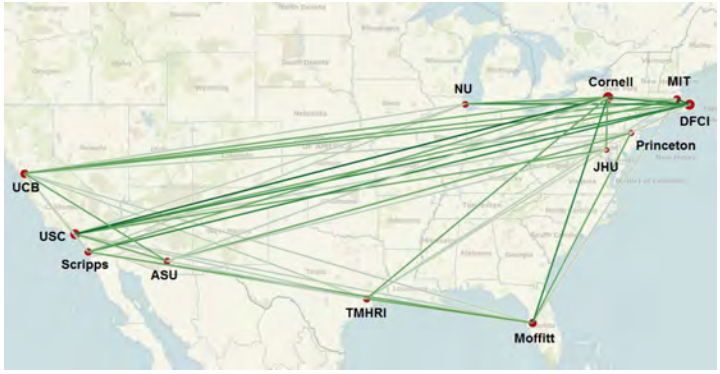


Figure 5.1. A map of the Trans-Network Projects within the PS-OC Program. Each line represents a Trans-Network Project between two PS-OCs. The darkness of the line depicts the number of Trans-Network Projects. Dark lines indicate more projects.

Physical Sciences Perspective
Physical Sciences search for clean model systems that can represent the complexity of nature yet are amendable to quantitative analysis and predictive response. This team has shown that using fundamental principles of evolution dynamics coupled with modern microfabrication technologies an environment can be created that vastly accelerates the evolution of resistance in cancer cells.

Summary of Research Highlight
Understanding the dynamics of the tumor-bone marrow system in individual MM patients can be obtained only through computational models that integrate ex vivo interrogation of the evolutionary dynamics of MM cells using advance microfluidic technology with patient-specific cellular and micro-environmental parameters obtained through bone marrow aspirates and biopsies.

Impact on Cancer Research
This project moves us toward developing "personalized" treatment for cancer patients by matching the physical characteristics of their tumor with specific therapeutic strategies.

★ Emergence of Therapy Resistance in Multiple Myeloma: The Roles of Genomic and Microenvironmental Heterogeneity

Robert Gatenby, Ariosto Silva, and Zayar Khin, H. Lee Moffitt Cancer Center and Research Institute PS-OC; Robert Austin, Amy Wu, James Sturm, and Liyu Liu, Princeton University PS-OC; Guillaume Duclos, Institute Curie

Project Objectives and Significance

This Trans-Network Project is investigating the evolution of drug resistance in patients treated for multiple myeloma (MM). Typically, patients with MM respond well to initial therapy, but relapse is inevitable as therapeutic resistance emerges. Thus, MM remains a fatal disease, and length of survival is determined by the evolutionary dynamics of drug resistance. To study the dynamics, the investigators in the Princeton PS-OC used a novel micro-ecology platform, names the "Death Galaxy", to generate clinically relevant drug gradients in vitro. These data, together with patient-specific cellular and micro-environmental data obtained from bone marrow aspirates and biopsies, allowed the investigators to parameterize computational models of the underlying evolutionary dynamics in individual patients.

Background

MM is an incurable hematologic cancer that develops in the bone marrow, where it remains confined until late stages of the disease. While most patients respond well to initial therapy, all eventually relapse. This team has hypothesized that the heterogeneous microenvironment of the marrow, with regions of low drug exposure, associated with the natural fluidity of the marrow, would accelerate the emergence of drug resistant clones. As with many cancers, the stroma is an important factor for progression and drug resistance in MM. Cell-adhesion mediated drug resistance (CAMDR) is generally accepted as a major factor responsible for permitting residual disease following initial therapy. This provides an evolutionary bridge from de novo to acquired drug resistance. Currently, MM patient therapy is determined by a "snapshot" provided by bone marrow aspirate and biopsy. As a result, the dynamics of tumor response and adaptation to therapy cannot be predicted. To examine these dynamics, the team interrogates MM cells ex vivo using a Death Galaxy microfluidic system that mimics the key elements of the bone marrow, including extracellular matrix, stroma, and regions of stable drug gradients, as well as micro fabricated walls that act as surrogates for the trabecular bone sieve.

Accomplishments and Scientific Advancements

This team has recreated a three-dimensional representation of the bone marrow micro-environment, composed of red fluorescent human MM cell lines, a green fluorescent stromal cell line, and extracellular matrix composed of Matrigel™. A stable gradient of doxorubicin was established through this microhabitat for 10 days, during which time the investigators used fluorescent imaging to quantify cell death in high-drug concentration regions, replication in low-drug concentration regions, and migration. At the end of these experiments, the investigators observed that some cells were still

Green star = clinical implications

alive and capable of replication in regions with chronic high concentrations of chemotherapy. These cells were collected, and after expansion showed a significant increase in the expression of p-glycoprotein pumps, a common mechanism of drug resistance observed in patients treated with doxorubicin. This team has now used the three-dimensional bone marrow reconstruction to study the dose response of primary cells from eight MM patients.

Based on these results, the researchers propose that its methodology provides a more clinically relevant model system to study the emergence of drug resistance than standard drug resistant cell lines. The latter are selected for drug resistance in single culture and thus express only intrinsic drug resistance mechanisms, a selection that is both slower and less clinically relevant. In contrast, this team's approach exposes MM cells aspirated from individual patients to gradients of drug that recapitulates the in vivo selection pressures. In addition, this approach includes the interactions between cancer cells and the tumor micro-environment, such as adhesion and soluble factors, which are clearly important to drug resistance. As an example of this advantage, consider that the doxorubicin resistant MM cell line used in this study, 8226/DOX, was selected by continuously exposing the parental cell line to an initial drug concentration of 10nM, and gradually increasing this concentration until 100nM after 10 months. In the drug-gradient experiment described here, clones capable of replicating at 200nM were obtained in less than two weeks.

Future Plans

These results suggest that the microfluidics system used to select for drug resistance in MM cell lines may be used for patient primary cells. Although the selected clones in patient primary cells may not be able to be expanded they should be able to be sequenced.

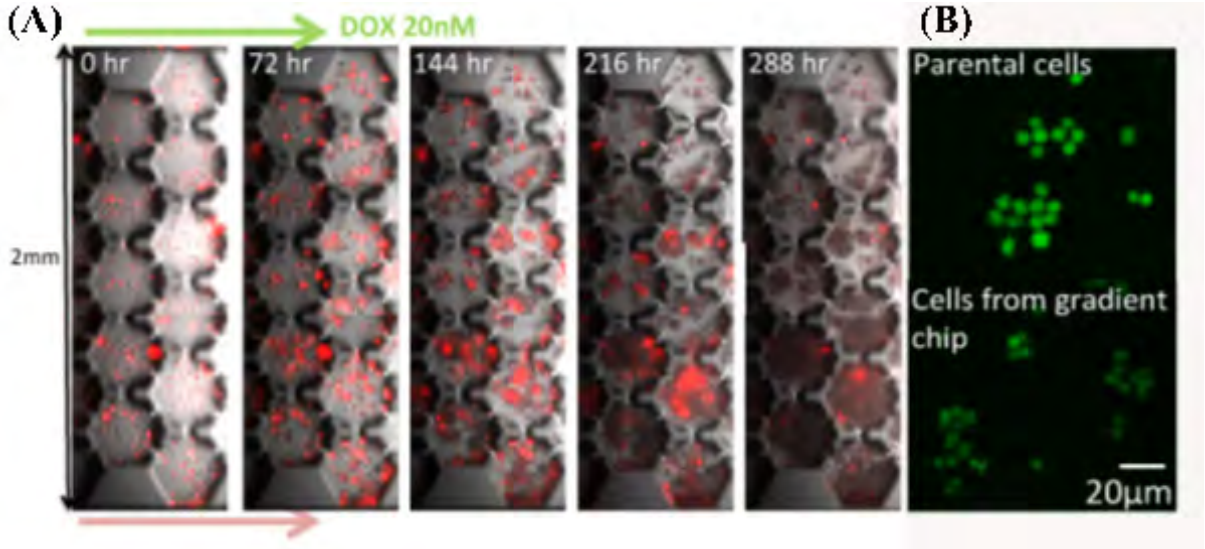


Figure 5.2. (A) Growth of myeloma cells as a function of time in a hex-array across a doxorubicin gradient (DOX), in this example 0-20 nm DOX. (B) Fluorescence images of the internal concentration of the dye Calcein AM. Evolved cells from the chips showed a significant increase in the expression of p-glycoprotein pumps and are one-half as bright as the control parental cells.

Physical Sciences Perspective
In this project, the investigators chose to describe tumors as a complex adaptive system. Their use of evolutionary mathematical models in concert with an efficient, high-throughput experimental platform allows them to simulate events that are typically difficult to directly measure and additionally to test in silico a large space of scheduling, dosing, and drug combination choices; a complete search of this space would be experimentally infeasible.

Summary of Research Highlight
The team utilized mathematical modeling in conjunction with quantitative experiments to identify optimal erlotinib and paclitaxel NSCLC treatment strategies that prevent or delay tumor progression.

What Makes It Innovative?
The development of this computational tool helps us to ask fundamental questions about tumor behavior and heterogeneity and to investigate and optimize treatments over a multidimensional space of scheduling strategies. The dynamic ability to inform and refine the model with experimental calibrations will facilitate the design of clinically relevant therapeutic strategies that influence the evolution of resistance and improve patient outcomes.

Development of Quantitative Models of Penetration of Resistance

Parag Mallick, Shannon Mumenthaler, David Agus, Josh Labaer, and Laura Gonzalez, University of Southern California PS-OC; Franziska Michor, Jasmine Foo, and Kevin Leder, Dana-Farber Cancer Institute PS-OC; Thea Tlsty, Philippe Gascard, and Luis Estevez-Salmeron, Princeton University PS-OC

Project Objectives and Significance

The goal of this Trans-Network Project is to investigate quantitatively the role of differential selection pressure on tumor composition dynamics during growth and in response to therapeutic intervention. This team's integrated experimental and computational approach allows investigators to ask questions about the role of evolutionary forces in the development of a tumor and the process by which drug-resistant cells dominate the population of a tumor leading to poor clinical outcomes. The application of their model to develop novel treatment schedules may ultimately lead to novel strategies for limiting penetrance of drug resistant cells, possibly through the use of treatment cycling.

Background

The development of diagnostic tools to accurately describe, evaluate, and predict an individual's response to cancer therapy is a field-wide priority. The vast majority of cancer therapeutics rapidly lose effectiveness over time, in part through the tumor's evolution of resistance (Figure 5.3A). Despite extensive study, overcoming resistance remains a major obstacle to improving remission rates and achieving prolonged disease-free survival. Recent findings highlight the prevalence of intratumoral heterogeneity and emphasize the likelihood of tumor adaptation, posing a challenge to personalized medicine. Previous work has addressed how increasing selective pressure may lead to the emergence of drug resistant colonies. Therefore, understanding the spatial-temporal dynamics of tumor heterogeneity and genetic diversity as a result of therapeutic intervention is imperative for designing optimal treatment strategies. The goal of this Trans-Network Project is to develop a computational model-based tool, informed by experimental measurements of tumor behavior, to diagnose the level of tumor heterogeneity and guide the design of personalized treatment strategies that improve patient outcome.

Accomplishments and Scientific Advancements

This team has taken an interdisciplinary approach combining computational and experimental approaches to develop, calibrate, and test a computational tool that aims to predict the impact of evolutionary pressures, such as therapy and microenvironmental factors, on the penetrance of drug resistant cells throughout a tumor. Early in this collaboration, it became clear that the team needed to develop a novel, accurate experimental platform that would generate detailed cell growth and death measurements. The investigators accomplished this using the Cellomics Arrayscan, a high-throughput, automated fluorescence microscopy approach that allows the team to monitor cell heterogeneity in an extremely detailed, accurate, and reproducible manner and generate truly quantitative cell growth data to calibrate the model. The team applied these tools to the clinical problem of acquired resistance to

the EGFR-tyrosine kinase inhibitor (TKI) erlotinib in non-small cell lung cancer (NSCLC) patients who develop a T790M point mutation in EGFR. The team's mathematical model predicted the population dynamics of mixtures of sensitive and resistant cells, describing how the tumor composition, initial fraction of resistant cells, and the degree of selective pressure influence the time until progression of disease. Using this approach, the investigators systematically explored the space of combination treatment strategies (Figure 5.3B) and demonstrated that optimally timed combination strategies (Figure 5.3C) yielded large improvements in time-to-progression relative to monotherapies at the same concentrations. In some cases, complete regression was achievable. The overall outcome was sensitive to timing, dose, and initial ratio of sensitive to resistant cells, and the team was able to identify regimens that overcame TKI resistance. Although cell growth rates may differ in vivo compared to in vitro, two main conclusions can be drawn from this model: The timing of drug scheduling in combination therapies can have a striking impact on the overall outcome of therapy, and mathematical modeling provides an effective and efficient method to investigate and optimize scheduling strategies in multidimensional space. These realizations serve as a starting point for future investigations that will address more complex scenarios arising in vivo as well as additional resistance mechanisms and drugs.

Future Plans

The tumor microenvironment plays an active role in tumor progression and metastasis, and factors such as hypoxia, pH, glucose concentration, drug treatments, and secreted factors influence the overall fitness of cancer cells and drive the evolution of a tumor. The team is in the process of refining its computational model to determine the impact of these microenvironmental selection pressures on tumor behavior. These new parameters will be calibrated using quantitative two-dimensional and three-dimensional experiments measuring changes in growth rates as a function of varying microenvironmental conditions.

Publication

Mumenthaler, S.M., Foo, J., Leder, K., Choi, N.C., Agus, D.B., Pao, W., Mallick, P., and Michor, F. Evolutionary Modeling of Combination Strategies to Overcome Resistance to Tyrosine Kinase Inhibitors in Non-Small Cell Lung Cancer. *Mol Pharm* **8**(6):2069-2079. (2011).

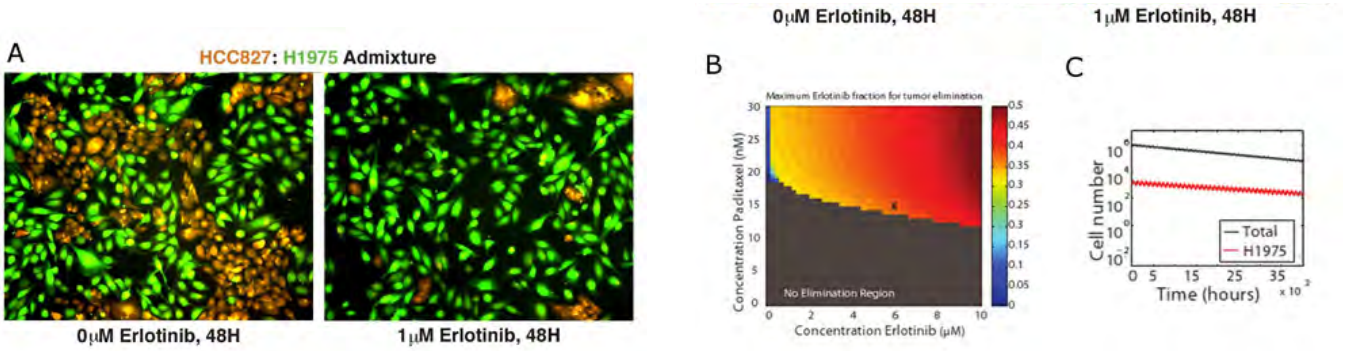


Figure 5.3. (A) Fluorescent images illustrating the evolution of drug resistance under treatment with a molecular targeted therapy (EGFR TKI-erlotinib). HCC827 sensitive cells (orange) and H1975 resistant cells (green), labeled with CellTracker, were admixed 1:1 and treated with 1uM erlotinib. (B) Experimentally calibrated model predictions reveal a range of optimal sequential combination schedules (erlotinib:paclitaxel) that can achieve elimination of a NSCLC population. This heat map illustrates the maximal erlotinib fraction for sequential treatment strategies that achieve tumor elimination for each dose-pair in the elimination (upper) region. (C) Dynamics of total tumor (black line) and H1975 resistant (red line) population sizes under optimal sequential combination strategy, using a maximal fraction of time on erlotinib (37 percent time on erlotinib at 6 μM, 63 percent of time on paclitaxel at 15 nM), which is denoted by the "x" in panel b. This dosing strategy resulted in the elimination of both the sensitive and resistant cell populations. The initial population size in these maps was 10^6 cells with 0.1 percent initial resistance frequency.

★ Heterogeneity of Cytoskeletal Architecture as an Indicator of Treatment Response

Peter Kuhn, Scripps Research Institute PS-OC; Brian Kirby, Cornell University PS-OC; Kasia Rejniak Moffitt Cancer Center PS-OC; Mitchell Gross, University of Southern California PS-OC

Project Objectives and Significance

The goal of this Trans-Network Project is to explore the hypothesis for the existence of a direct relationship between tumor heterogeneity, assessed by cellular biophysical measurements, and therapeutic response. The project integrates approaches developed at each of the four participating PS-OCs to develop a clinical model system to evaluate a taxane-based treatment in patients with advanced prostate cancer. The overall impact of this work spans length scales ranging from nanoscale processes such as microtubule assembly and androgen receptor expression to interactions between circulating tumor cells with endothelial cells to the whole organism in assessing an individual patient's overall clinical response to taxane-based chemotherapy.

Background

A fundamental question in caring for patients with advanced forms of prostate cancer and other cancers involves understanding differences in therapeutic response during the course of treatment. In relation to taxane-based treatments, laboratory studies have associated specific molecular alterations, such as tubulin isotype switching or expression of multidrug resistance efflux pumps, with therapeutic resistance. This project aims to study this question at the single-cell level by combining the Scripps PS-OC fluid phase biopsy assay and the Cornell PS-OC Geometrically Enhanced Differential Immunocapture (GEDI) microfluidic device to capture and analyze circulating tumor cells (CTCs). The computational fluid-dynamics methods based at the Moffitt PS-OC will then perform a series of fully controlled theoretical experiments testing various physical properties of individual tumor cells and their role in cytoskeletal changes and pattern formation. The clinical expertise of the USC PS-OC provides clinical samples and maintains the overall therapeutic relevance of this project to patient care decisions.

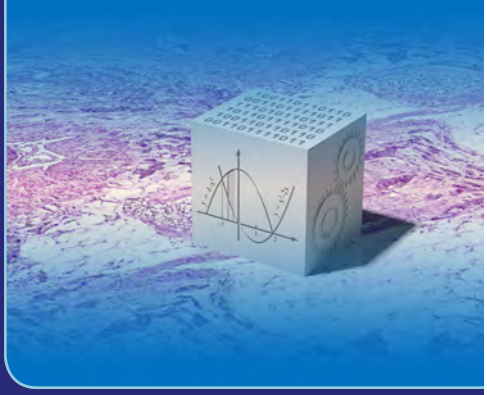
Accomplishments and Scientific Advancements

The identification of the appropriate patients is an important aspect of this exploratory project. A clinical trial was activated to provide prospective collection of blood samples for analysis at Scripps using the HD-CTC and GEDI CTC assays. Patients receiving routine clinical care for metastatic prostate cancer who were about to begin a new line of treatment with either a taxane or an androgen-directed therapy were identified and invited to participate in an observational, correlative science protocol. Blood for CTC enumeration and analysis is being collected at baseline and immediately after taxane-treatment, if applicable; after 2 to 5 weeks of therapy; and after 9 to 12 weeks of therapy or at the time of disease progression. All samples are immediately processed and sent via overnight courier to the Scripps PS-OC for analysis. Figure 5.4C shows the tracking of patient response with the HD-CTC assay.

Green star = clinical implications

Physical Sciences Perspective

The PSP is the direct correlation of quantitative physical measurements at the cellular level using biological insights to first correlate and then predict clinical outcome. At the program level, PSP is the integrated effort between clinic, engineering, physics, and mathematics that comes to bear in this project. As with any large physical sciences program, the integration across disciplines is required to achieve that next level. The data collection, data reduction, and analysis paradigms of the HD-CTC Technology are derived from the experimental and statistical boundary conditions of rare event identification problems. The derived parameters are quantitatively measured to be consistent with the required



The fluid phase biopsy technology has been technically and clinically validated as well as demonstrated in a clinical utility study during the first two years of the Network. This clinical validation provided the rationale for the hypothesis underlying this proposal. In a multicolor assay, the investigators are quantitatively analyzing parameters derived from the HD-CTC imagery. As a first step, the modified assay was developed to include alpha-tubulin as a marker. While cell line development was straightforward, the validation of the assay in patients is still ongoing. The investigators are collaborating across the PS-OC Network to advance this further. Patient samples from the clinical trial are being preprocessed and stored in a biorepository for use upon completion of the assay development phase. Figure 5.4A-B show the combined four-color assay of untreated versus treated cells where DAPI blue is showing the nucleus, red represents cytokeratin, and green is tubulin. At the same time, the GEDI device assay has been modified for cross-Network use in two ways:

- Cell characterization, quantification, and interpretation has been automated. Intact cells are identified by DAPI stain levels and morphology, and circulating tumor cells are identified by immunostain marker levels/immunostaining and morphology. Cross-platform comparisons between different microscope systems are in progress to confirm that results are site-independent.
- An on-site training protocol and SOP has been developed for outside users to learn implementation of GEDI devices from Cornell PS-OC staff; this has been implemented for non-Cornell PS-OC users and is being tested with out-of-Network investigators.

This team has also developed a three-dimensional computational mechanical model of the circulating tumor cells with growing microtubules and algorithms to determine microtubule bundlednessbundling using quantitative rather than visual measures. Under the assumption of tubulin stabilization due to the taxane-based drug action, the investigators have simulated microtubule growth, taking into

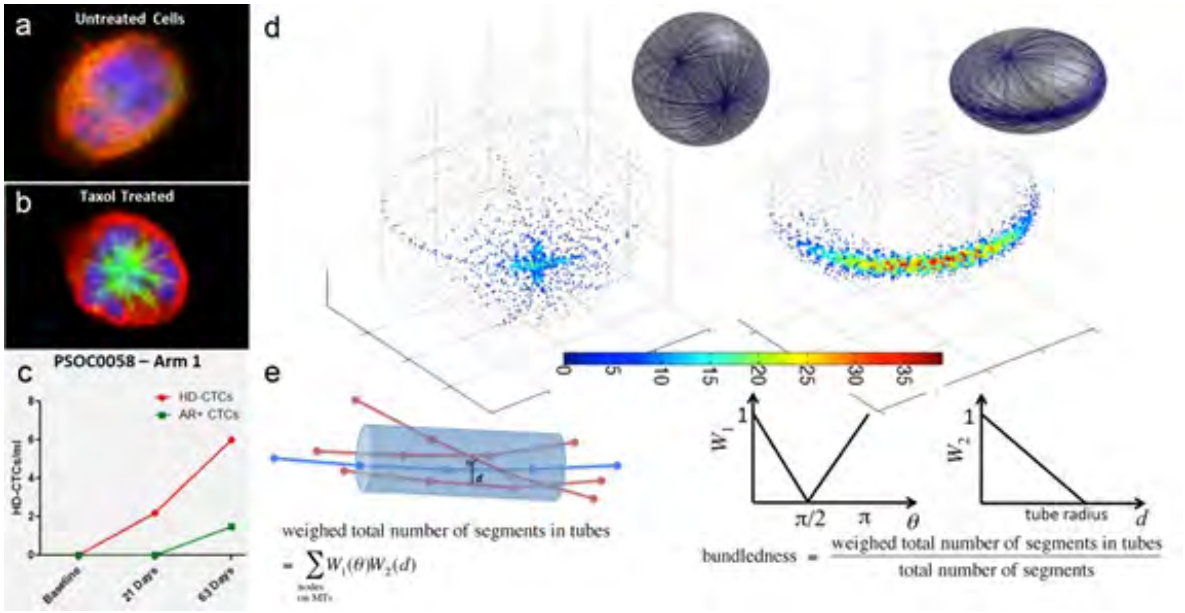


Figure 5.4. Microtubule patterns and its quantification. (A-B) composite pictures of untreated (A) and taxane-treated (B) Staining: cytokeratin (red), DAPI (blue), FITC/tubulin (green); (C) prostate cancer patient treated with Abiraterone (Zytiga) who progressed over the course of treatment. The number of CTCs (red) and the number of CTCs expressing androgen receptor (AR, green) are shown. (D) computational method for microtubule bundle quantification with high intensity (yellow to red) indicating the bundle (right), and low intensity implying no bundles even in the case of microtubule crossing (left). (E) Schematic illustration of the bundle quantification algorithm.

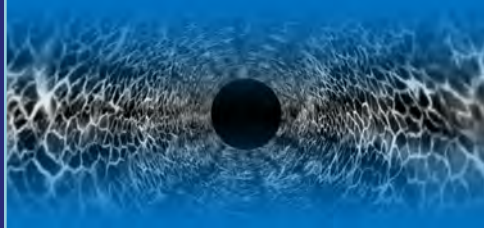
consideration a range of physical parameters, such as microtubule stiffness, speed of microtubule assembly, and viscosity of the cell cytoplasm that allowed the investigators to observe various microtubule patterns. In order to quantify these patterns, the team members developed a method to assess both the angles between nearby microtubule rods and the persistence of the direction of microtubule growth that together allows them to distinguish between microtubules that are aligned from those that only cross each other (Figures 5.4D and 5.4E).

Future Plans

Clinical validation of the assay is expected to conclude shortly. The initial cell line data are being used to develop the first draft of the mathematical model. With each of the individual components reaching its technical milestones, the team will then test the system in the patient population. It is the team's expectation that the refined model will substantially aid in the monitoring of the response of these patients. This would then require a larger and statistically powered clinical utility trial.

What Makes It Innovative?

The concept of characterizing cellular morphology changes to identify treatment response is innovative. This is the first time that highly complementary fluid biopsy approaches are being applied in concert toward addressing a particular cancer biology question based on direct analysis of patient-derived specimens. Further, this project incorporates mathematical models on subcellular/nanoscale levels with clinical information at the whole organism level. This team expects the results of this project to provide both direct information concerning changes in response to taxane-based therapies as well as provide mechanistic insight into physical changes in circulating tumor cells assessed at the single-cell level.



5.2. PS-OC Cell Line Pilot Study

The Cell Line Pilot Study was initiated in November 2009 by the PS-OC Steering Committee and overseen by the NCI OPSO project manager Nastaran Kuhn to test the feasibility of providing a standardized "benchmark" protocol for showcasing the diverse physical science technologies across the PS-OC Network. Center PIs and other Network physical scientists provided input into the specific types of technologies that could be applied to cell lines, while Center SIs and other cancer biologists provided insight into specific cell lines that could be used for the Pilot Project. The PS-OC Steering Committee reached a majority consensus that two human mammary epithelial cell lines would be used for the studies: MCF-10A and MDA-MB-231. These two cell lines could be used for comparative studies of "premalignant" versus "malignant" signature. While it was realized that no cell line would fill all criteria for being representative of any given cancer, the purpose of the chosen lines was not to represent the disease per se but to have common cell culture standards for pilot experiments to showcase the various new technologies being developed or utilized by the multiple Centers.

While it may seem paradoxical that members of the PS-OC Network were asked to blaze novel avenues of research using two cancer cell lines that have been in common use for several decades, it made sense for the selected pilot experiments. The notion was that every group needed a common language in order to communicate, and applying the multitude of different technologies to one or two common cell lines in a pilot study helped to initiate that process within the PS-OC Network. In collaboration with a lead PS-OC investigator, Dr. Thea Tlsty (UCSF; Princeton PS-OC), a common in vitro culture protocol was developed for the cell lines. Dr. Tlsty provided a single lot number of each cell line at the same passage to a representative lab in each Center along with a starter package of cell culture medium and serum to limit the number of technical variables among participants. The cell lines were distributed to the PS-OCs in February 2010, only four months after the Program launch. All 12 PS-OCs have participated in the Cell Line Pilot Study, generating data from an array of technologies across a range of length scales. Investigators from nine PS-OCs presented preliminary results at the first annual PS-OC Network Investigator's Meeting in April 2010, only two months after initial receipt of the cell lines. A follow-up 2nd Cell Line Pilot Study Meeting was held in June 2010 at the USC PS-OC where progress was presented by several of the participating PS-OCs.

A decision was made at the 2nd Cell Line Pilot Study Meeting to publish the experimental results as a PS-OC Network publication. Consequently, a Publication Team was formed consisting of two PIs, two SIs, and two project leaders, respectively: Robert Austin (Princeton PS-OC), Denis Wirtz (JHU PS-OC), Thea Tlsty (UCSF; Princeton PS-OC),

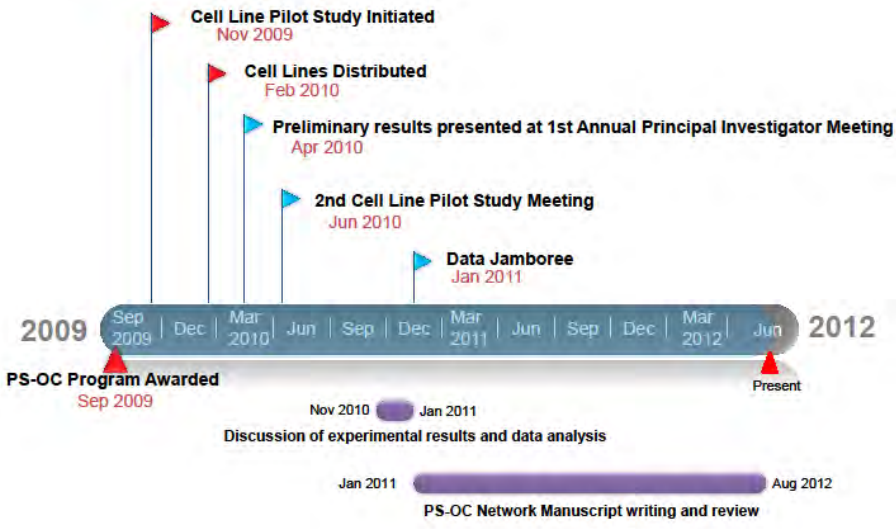


Figure 5.5. Timeline of key events in the PS-OC Cell Line Pilot Study.

To investigate the transition from a premetastatic to a metastatic state from a physical sciences perspective, the PS-OC Network performed molecular and biophysical assays on two breast cancer cell lines.

Experiments were performed in 20 laboratories from all 12 PS-OCs and revealed dramatic differences in mechanics, migration, adhesion, oxygen response, and proteomic profiles.

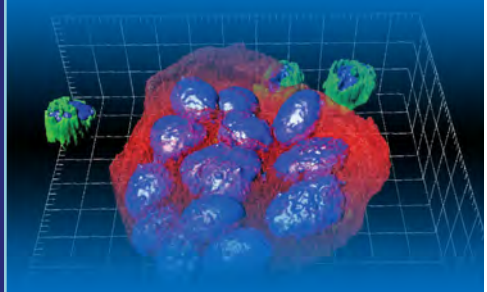


Figure 5.6. Images from PS-OC trainees and investigators interacting at the PS-OC Data Jamboree in January 2011.

Valerie Weaver (UCSF; UCB PS-OC), Parag Mallick (Stanford; USC PS-OC), and Owen McCarty (Oregon Health Sciences University; Scripps PS-OC). Additionally, a pilot data portal was created by the USC PS-OC Core Leader Carl Kesselman to facilitate data sharing among the study participants. The publication team held bimonthly teleconferences to discuss the status of the paper and to organize teams for data analysis (Table 5.1). A data freeze was set in mid-November 2010, by which time all

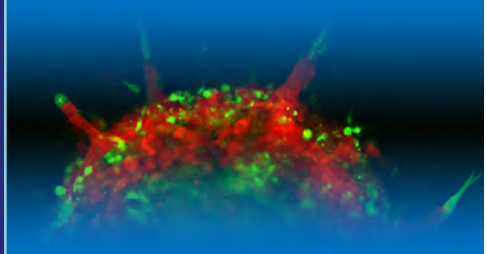
participants completed experiments and uploaded data to the portal. The PS-OC Program staff organized a Data Jamboree at the end of January 2011 in order to convene all participants to discuss the data and the structure of the paper. Prior to the Data Jamboree, team members for each figure of the paper participated in a series of teleconferences that occurred monthly from November 2010 through January 2011 during which experimental results and data analysis were discussed.

At the end of January 2011, all participants of the Cell Line Pilot Study convened at the Data Jamboree in Scottsdale, Arizona. At the meeting, each team leader presented the results for his or her assigned figure. Presentations were followed by detailed discussion, and several suggestions were made by publication team members. Valerie Weaver stated that "the purpose of the cell line pilot study was to show the cancer research field that scientists from different fields can work together," and she hoped that the Network would be able to use the data collected to generate interesting, novel hypotheses. The organization and structure of the paper was entirely reworked at the meeting. Several hours were dedicated to breakout sessions during which teams reassembled figures and discussed at length the topic areas for each figure: (1) morphology, (2) motility, (3) survival and stress response, and (4) network model. The redesigned figures were presented by the team leaders. A final discussion prior to meeting adjournment included the topics of journal preference, writing and figure assignments, and deadlines. Despite the challenges faced at the meeting, the Data Jamboree proved to be successful for establishing trans-Network collaborations and devising a strategy for producing the first PS-OC Network publication. The PS-OC Network manuscript was written by Publication Team member, Denis Wirtz, and is currently under review at a leading open-access journal.

Table 5.1. Members of the PS-OC Cell Line Committee from each PS-OC

PS-OC	PS-OC Cell Line Committee Member(s)
ASU	William Grady, Stuart Lindsay,
Cornell	Barbara Hempstead, Michael King
JHU	Peter Searson, Sharon Kostas
MIT	Alexander van Oudenaarden
Moffitt	Robert Gatenby, Robert Gillies
NU	Jonahtan Licht, John Marko
Princeton	Robert Austin, Thea Tlsty
Scripps	Owen McCarty
UCB	Jan Liphardt, Valerie Weaver
USC	Parak Mallick

"The goal of the UN of Cell Modulus project is to standardize, define, and integrate cell stiffness/modulus measurements to gain an understanding of the mechanical heterogeneity of a cell and disseminate information about the techniques to the broader scientific community."



5.3. "United Nations" (UN) of Cell Modulus

Introduction

Mechanical and shear forces are increasingly recognized as major regulators of cell phenotype and tissue structure (see Section 3.2.2 for more details). The modulus of the cell, its viscoelastic properties, is a key factor in how cells sense these forces and interact with other cells and the extracellular matrix. The modulus of a cell is commonly measured by several different methods, including atomic force microscopy, intracellular nanorheology, optical stretching, microrheology, magnetic bead twisting, and parallel plate rheology. These methods differ widely in how the measurements are taken and what area of the cell is measured. A quick comparison of these techniques reveals the heterogeneity and complexity of the cell mechanical properties. The large variation in measurements derived from these techniques impedes the use of cell modulus measurements in more clinically relevant applications.

Increasing interest among PS-OC investigators to understand the modulus of the cell and its role in cancer progression led to the formation of the UN of Cell Modulus. The goal of the UN of Cell Modulus project is to standardize, define, and integrate cell stiffness/modulus measurements to gain an understanding of the mechanical heterogeneity of a cell and disseminate information about the techniques to the broader scientific community. The UN of Cell Modulus recruited experts in cell modulus measurements from the PS-OC Network and the broader research community to participate in the study. A total of eight research labs are participating in the project. Each lab has performed its measurement using the same cell line and reagents from the PS-OC Bioresource Core Facility (PBCF). The output is expected to be a manuscript for the field to reference when discussing cell modulus measurements and its role in disease.

Methods

Each participant in the UN of Cell Modulus project was asked to perform cell modulus measurement on MCF-7 cells obtained from the PBCF. Cells were supplied to each participant along with a starter kit that contained media, serum, growth factors, and specific protocols for culturing conditions. To standardize measurements, all measurements were performed on sterilized glass (except those that required suspended cells). Dr. Denis Wirtz, JHU PS-OC, supplied a protocol for sterilization of the glass surface. Also, in an attempt to minimize differences in experimental protocols, simple guidelines were provided for measurements detailing the passage number, cell density, and incubation time prior to measurement.

Table 5.2. Summary of UN of Cell Modulus Participants and Techniques

Name	Institution	Technique
Dr. Atef Asnacios	Paris Diderot	Parallel plates rheology
Dr. Dennis Discher	University of Pennsylvania	Mircoaspiration
Dr. Jochen Guck	Cambridge University	Optical stretching
Dr. Paul Janmey	University of Pennsylvania	AFM (tip, cytoplasm)
Dr. Albrecht Ott	Univeristy of Saarland	Monolayer shear rheology
Dr. Robert Ros	Arizona State University	AFM (tip, over nucleus)
Dr. Igor Sokolov	Clarkson University	AFM (bead)
Dr. Richard Superfine	University of North Carolina	Magnetic bead pulling
Dr. Ning Wang	University of Illinois	Magnetic bead twisting
Drs. Denis Wirtz/Yiider Tseng	Johns Hopkins University/ University of Florida	Ballistic intracellular nanorheology

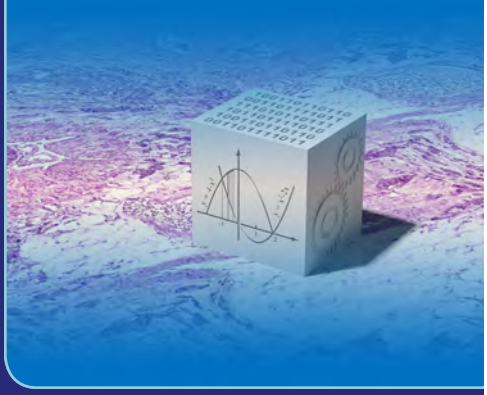
Results to Date

Preliminary results from the UN of Cell Modulus are being complied by the leader, Denis Wirtz. Each participant has provided a detailed description of his methodology and the modulus measurement of MCF-7 cells using his technique (Table 5.2). As expected, each approach obtains a different value for the cell modulus because the methods interrogate different aspects of cell viscoelasticity. For example, some techniques probe the cell's cytoplasm, while others probe the cell surface. Some techniques measure viscoelasticity over short time scales, while others take measurements over longer time scales. A summary of the data collected is available in Table 5.3. The values collected to date vary two orders of magnitude (0.11 to 8.7 kPa). This range demonstrates the complex mechanical behavior of the cell in response to forces and emphasizes the importance of choosing the correct technique depending on the experimental question being asked. The next steps will be to discuss the implications of these measurements and their relevance to various biological events.

Table 5.3. Preliminary Data from Cell Modulus Measurements

Technique	Cell Modulus (kPa)
AFM (tip, cytoplasm)	8.7
Magnetic bead twisting	1.871
AFM (bead)	1.8
AFM (tip, nucleus)	0.65
Parallel plate	0.86
Nanorheology	0.011

Several working groups have been established for the PS-OC Network to foster collaboration and facilitate achievement of program goals. Each working group is facilitated by at least one OPSO program official, with Network investigators serving as chairs or co-chairs of the groups.



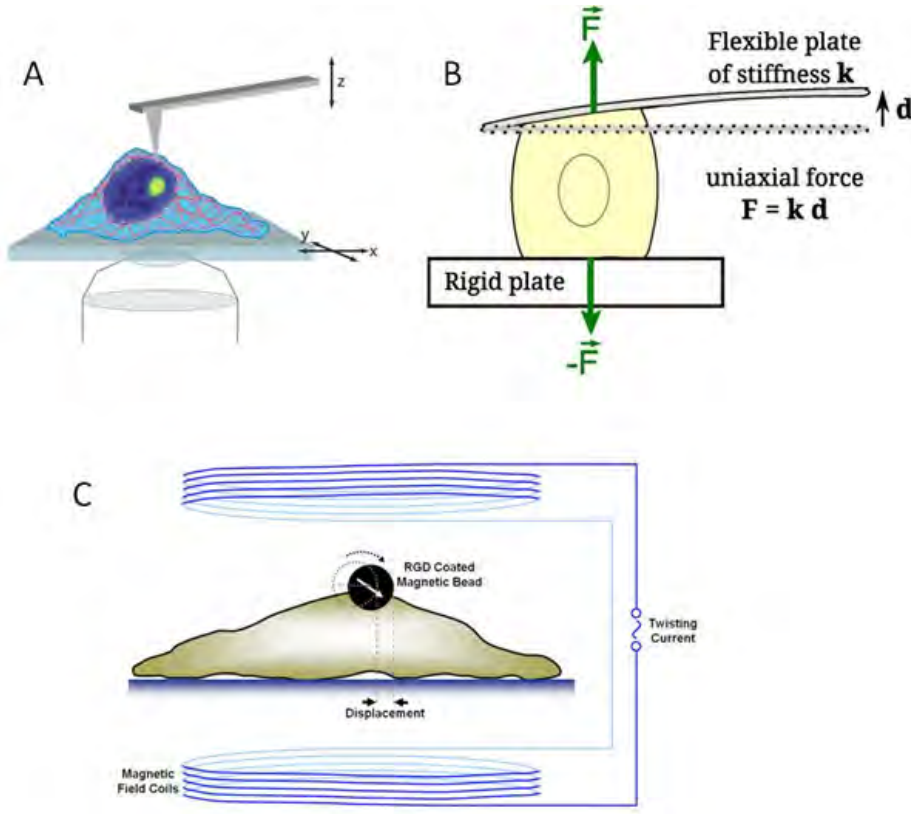


Figure 5.7. Examples of Cell Modulus Measurements. A) Atomic force microscopy with a fine point tip measuring the local elasticity of the cell membrane in response to a point deformation B) Parallel plate microrheology measuring the global elasticity of the cell in response to cell compression C) Magnetic bead twisting measuring the local elasticity of the cell membrane in response to a tangential force.

Future Plans

The UN of Cell Modulus is currently writing a manuscript to report the findings of the study. The group plans to continue working together and expand to different types of cells and measuring cellular perturbations with these different methods.

5.4. PS-OC Scientific Working Groups

Several working groups have been established for the PS-OC Network to foster collaboration and facilitate the achievement of Program goals. Each working group is facilitated by at least one OPSO program official with Network investigators servings as chairs or co-chairs of the groups. Additional working groups may be established on the basis of need and interest. Working groups have been created for the areas that follow.

5.4.1 Evolution of Drug Resistance Working Group

The PS-OC Evolution of Drug Resistance Working Group was created in March 2010, six months after the Program launch, with the goal of fostering communication and cultivating collaborations among the PS-OC members working in the field of drug resistance in cancer. Members of the working group consist of investigators representing six PS-OCs, and the group has held seminars during the past three annual PS-OC Network Investigators' Meetings, as well as semi-annual conference calls with invited guest speakers including Anirban Maitra from Johns Hopkins University. Additionally, the working group contributed to a special theme issue on the evolution of drug resistance in cancer published in Molecular Pharmaceutics on December 5, 2011 (Volume 8, Issue 6) (Figure 5.8). The special issue comprised several articles submitted by the Moffitt, Princeton, DFCI, and USC PS-OCs, including the first publication that resulted from a Trans-Network Project award. The Dana-Farber and USC PS-OC team published their results using evolutionary mathematical modeling to overcome targeted therapy resistance in non-small cell lung cancer. In addition, there was a preface authored by OPSO program officials Nastaran Kuhn and Larry Nagahara.

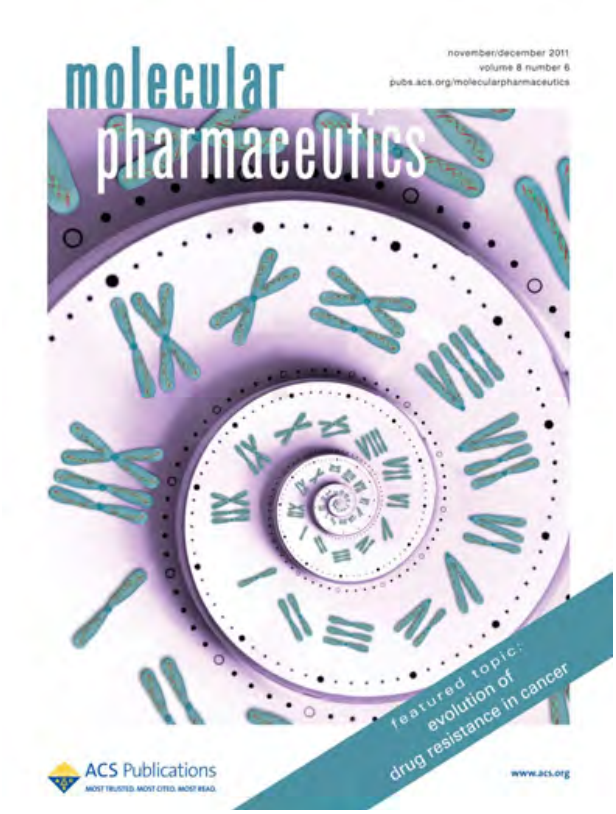


Figure 5.8. The journal cover for the special issue in Molecular Pharmaceutics on evolution of drug resistance in cancer.

"…physicists working at different length scales collaborated to develop a working model that describes the overall process of energy generation, and transfer and explains much of what researchers observe and measure. The question remains, will this approach transfer to cancer research?"

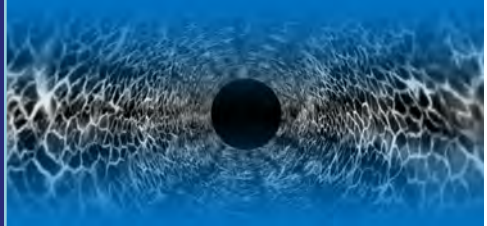


Table 5.4. Members of the PS-OC Evolution of Drug Resistance Working Group.

PS-OC	Evolution of Drug Resistance Working Group Members
ASU	John Pepper
Cornell	Paraskevi Giannakakou
DFCI	Franziska Michor
DFCI	Jasmine Foo
DFCI	Ross Levine
MIT	Christopher McFarland
MIT	David Basanta
MIT	Leonid Mirny
Moffitt	Alexander Anderson
Moffitt	Jessica Cunningham
Moffitt	Joel Brown
Moffitt	Robert A. Gatenby
Moffitt	Robert Gillies
Princeton	Beverly Emerson
Princeton	Donald S. Coffey
Princeton	Robert Austin
Princeton	Robert Getzenberg
Princeton	Thea Tlsty
USC	Parag Mallick
USC	Shannon Mumenthaler
NIH/NCI	Nastaran Kuhn

5.4.2 Physics Working Group

Overview and Background

The history of physics emphasizes the importance of team work to solve challenging problems. For example, to gain a complete perspective of how the sun works, physicists working at different length scales collaborated to develop a working model that describes the overall process of energy generation and transfer and explains much of what researchers observe and measure. The question remains, will this approach transfer to cancer research? The Physics Working Group was initiated at the start of the PS-OC Program as a way to integrate several of the physics tools and principles implemented by the PS-OC Network.

The Physics Working Group is made up of more than 40 investigators representing all 12 PS-OCs. The group leaders, Claudia Fischbach-Teschl (Cornell PS-OC) and Denis Wirtz (JHU PS-OC), hold meetings twice each year to initiate discussions and identify ways to integrate the different scientific approaches taken by the various PS-OCs.

Objectives

The Physics Working Group established four objectives at its first meeting. These objectives were designed to initiate communication and integration across the PS-OC Network and disseminate information as a group about the PS-OC Program. The objectives are listed below:

- Identify common areas of physical measurements across the PS-OC Network and discuss and implement standardized reporting criteria.
- Share and develop physical science techniques across the PS-OC Network.
- Organize workshops and minisymposia at relevant conferences to inform the broader scientific community on the integration of physics and oncology.
- Partner with professional societies of interest to promote interdisciplinary research in physics and oncology.

Activities

The Physics Working Group initiated a subgroup to focus on integrating techniques that measure the modulus of the cell. The UN of Cell Modulus project was established to standardize cell modulus measurements for the cancer research community. Please see Section 5.3 for more information.

As a result of discussion during the Physics Working Group meetings, several interactions have resulted that integrate physical sciences measurements across the PS-OC Network. There are two formal collaborations that have started because of these interactions.

- "Comparison of elasticity and disorder strength of cell nuclei " – ASU PS-OC and Northwestern PS-OC
- "Identification and Characterization of Circulating Tumor Cells by Partial Wave Spectroscopy" – Scripps PS-OC and Northwestern PS-OC

To date, the Physics Working Group has organized symposiums related to the physics of cancer with the goal of disseminating information to investigators within the PS-OC Network and the broader scientific community.

- 2010 Annual Biomedical Engineering Society Meeting

  "The Physics and Engineering of Cancer Cells and Their Microenvironment " — Organized by Cynthia Reinhart-King, Cornell PS-OC, and Jerry Lee and Nastaran Kuhn, NCI

- 2011 Annual PS-OC Network Investigators' Meeting Session

  "Partial Wave Spectroscopy" — Vadim Backman, Northwestern PS-OC

  "The Intracellular Physical Microenvironment: Isoelectric Localization of Proteins" — Robert Gatenby, Moffitt PS-OC

Future Plans

The Physics Working Group will continue to meet and discuss areas where the PS-OC Network can integrate measurement to gain a more complete picture of cancer. The group plans to start additional subgroups focused on a physical property and has preliminary plans to submit a proposal for a Keynote or Gordon conference in this area.

Objectives of the CTC Transport Working Group

- Discuss barriers to understanding the mechanisms of CTC transport
- Challenge current assumptions with physical science principles
- Identify collaborative experiments to test new hypothesis in this area

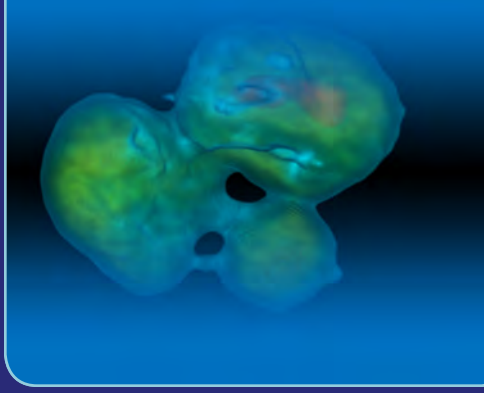


Table 5.5. Members of the PS-OC Physics Working Group

PS-OC	Physics Working Group Members
ASU	Roger Johnson
ASU	Stuart Lindsay
ASU	Robert Ros
Cornell	David Erickson
Cornell	Claudia Fischbach-Teschl
Cornell	Michael King
Cornell	Cindy Reinhart-King
Cornell	Abraham Stroock
Cornell	Mingming Wu
Cornell	Ying Zheng
DFCI	Gregoire Altan-Bonnet
JHU	Robert Getzenberg
JHU	Konstantinos Konstantopolous
JHU	Yiider Tseng
JHU	Denis Wirtz
MIT	Scott Manalis
MIT	Alexander van Oudenaarden
Moffitt	Robert Gillies
Moffitt	Keiran Smalley
Moffitt	Gargi Chakraborty
NU	Vadim Backman
NU	Vinayak Dravid
NU	Mark Hersam
NU	John Marko
NU	Teri Odom
NU	Jinsong Wu
Princeton	Robert Austin
Princeton	Guillaume Lambert
Princeton	James Sturm
Princeton	Robert Veltri
Scripps	Owen McCarty
TMHRI	Paolo Decuzzi
TMHRI	Steven Curley
UCB	Dan Fletcher
UCB	Jan Liphardt
UCSF	Valerie Weaver
USC	Vittorio Cristini
USC	Paul Macklin
USC	Parag Mallick
NIH/NCI	Nastaran Kuhn
NIH/NCI	Larry Nagahara
NIH/NCI	Jerry Lee

5.4.3 Circulating Tumor Cell (CTC) Transport Working Group

Overview and Background

The mechanisms of circulating tumor cell (CTC) transport from a primary tumor to a metastatic lesion are not well understood, and current assumptions of the process tend to neglect the physics and physical forces. Several of the PS-OCs are investigating the clinical implications of CTCs using a physical sciences perspective. To combine efforts in this area, the PS-OC Network initiated the PS-OC CTC Transport Working Group in the fall of 2011. Organized by Owen McCarty, Scripps PS-OC, the group is made up of a team of seven investigators currently studying CTC transport mechanisms within the PS-OC Network. These members represent five PS-OCs and four different disciplines and includes biomedical engineers, chemical engineers, oncologists, and mathematicians. The initial objectives of the group were to discuss barriers to understanding the mechanisms of CTC transport, challenge current assumptions with physical science principles, and identify collaborative experiments to test new hypothesis in this area.

Objectives and Milestones

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 physical biology of the intravasation process. The process of metastasis is highly inefficient. This paradigm was first established by experiments showing that more than a million cells per gram of tumor can be shed daily, with less than 0.01 percent of these cells establishing a secondary tumor. Recent studies have reported the presence of tens to hundreds of CTCs per milliliter of blood in the venous circulation of carcinoma patients. Nonetheless, the presence of CTCs has not been shown to be predictive of overt metastases. The majority of studies have focused on characterizing the later

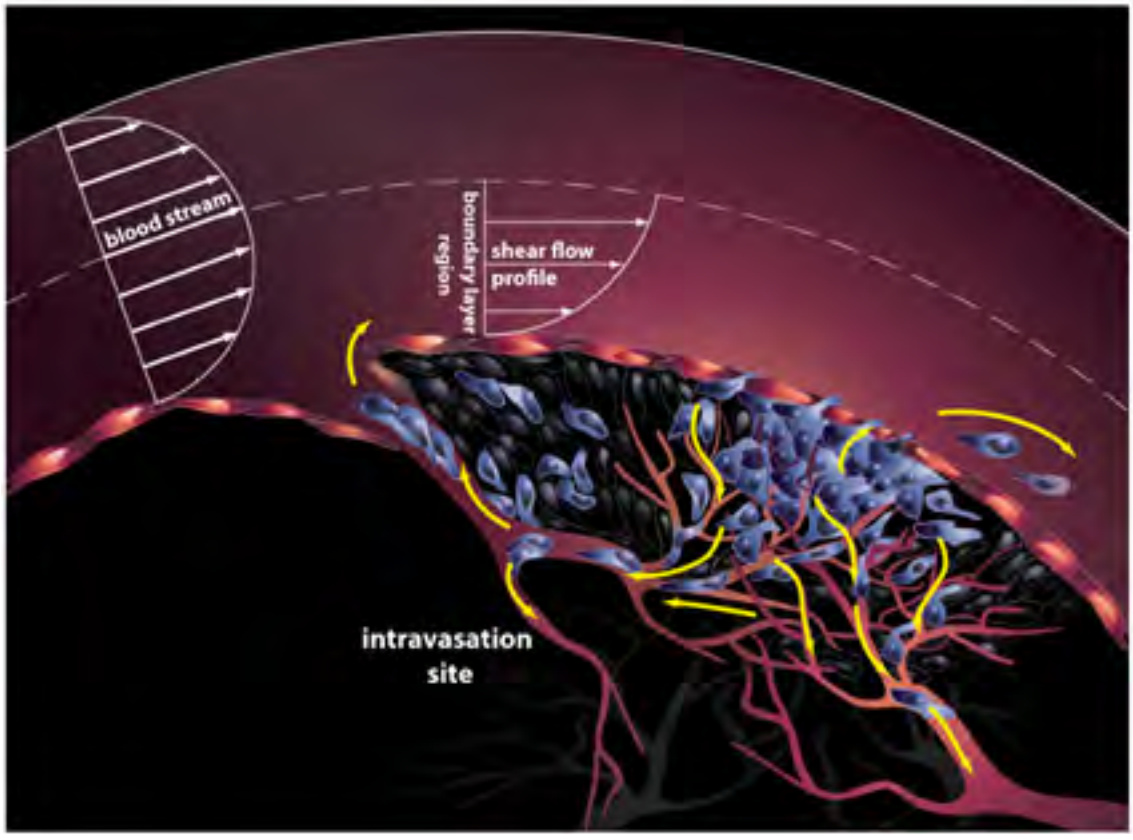
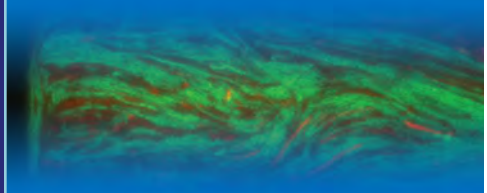


Figure 5.9. A graphical schematic depicting the forces involved in the intravasation of cancer cells. (Courtesy of the Scripps PS-OC).

"The CTC Transport Working Group plans to expand the number of members and include scientists and clinicians with different expertise in the discussion. Future efforts will focus on identifying assumptions and questions in the transport of CTCs and designing groups to answer each question."



steps of the invasion-metastasis cascade, namely, survival in the circulation, arrest at distant sites, extravasation, micrometastasis formation, and tumor colonization at secondary sites. The biochemical and physical parameters that regulate the entry of CTCs into the circulation have received far less attention.

The loss of the ability to adhere to neighboring tumor cells and the gain of a migratory and invasive phenotype is a requisite for cancer cells to leave the primary tumor and to disseminate to distant organs. Two fundamentally different patterns of invasion have been observed in histological sections: single cell invasion and collective cell invasion. Mesenchymal invasion is initiated by an epithelial-mesenchymal transition (EMT) of individual cancer cells at the invading front of the primary tumor. Characteristics for mesenchymal invasion are the spindle-shaped morphology of the cancer cells and the expression of proteases to degrade the basement extracellular matrix proteins. In contrast, during coordinated collective invasion, cancer cells invade the surrounding tissue without losing contact with their neighboring cells and/or the primary tumor. It is unclear whether a passive or active mechanism of intravasation would favor either single or collective cells.

Accomplishments

1. Two Trans-Network collaborations that turned into funded PS-OC Trans-Network Projects.
    - Ross Levine (DFCI PS-OC) and Owen McCarty (Scripps PS-OC) — Characterization of procoagulant leukemic cells
    - Mike King (Cornell PS-OC), Paolo DeCuzzi (TMHRI PS-OC), Owen McCarty (Scripps PS-OC) — What makes a microenvironment permissible for tumor growth?
2. Collaborative Research Projects: Kostas Konstantopoulos (JHU PS-OC), Owen McCarty (Scripps PS-OC) — Role of CD44 in metastasis under coagulation and shear
3. Exchanging students between investigators in the CTC Transport Working Group
    - Flor Cianchetti — graduate of Chris Schaffer's Group (Cornell PS-OC) took a post-doctoral fellowship position in Owen McCarty's group (Scripps PS-OC)
    - Ishan Patel — McCarty group (Scripps PS-OC) member awarded an American Heart Association Fellowship to work in Ross Levine's group (DFCI PS-OC) this summer

4. In-progress publications as a result of the working group discussions:
    - Ross Levine (DFCI PS-OC) and Owen McCarty (Scripps PS-OC) — writing a mini-review for Frontiers in Oncology entitled "Do circulating tumor cells play a role in coagulation and thrombosis?"
    - Special Issue: Editors are Mike King (Cornell PS-OC) and Owen McCarty (Scripps PS-OC)
5. Held PS-OC Network Meeting on this topic at the Third Annual Investigators' Meeting. Michael King (Cornell PS-OC) presented his recent finding on the flow of cancer cells in an ex vivo system to model cell rolling and sticking.

Future Plans

The CTC Transport Working Group plans to expand the number of members and include scientists and clinicians with different expertise in the discussion. Future efforts will focus on identifying assumptions and questions in the transport of CTCs and designing groups to answer each question. The group will continue to collaborate on writing a review with a list of some of the key questions being investigated.

Table 5.6. Members of the PS-OC CTC Transport Working Group

PS-OC	CTC Transport Working Group Members
Cornell	Chris B. Schaffer
Cornell	Michael R. King
DFCI	Ross Levine
JHU	Katarzyna (Kasia) Rejniak
JHU	Konstantinos Konstantopoulos
Scripps	Owen McCarty
TMHRI	Paolo Decuzzi

5.5. PS-OC Network Retreat

As a Network, PS-OC investigators learn about the research and development that take place at other Centers once a year during the Annual PS-OC Network Investigators' Meeting. In order to bring the investigators closer, encourage more Trans-Network collaborations, and learn about the PS-OCs' research progress, OPSO organized the first PS-OC Retreat on December 15-16, 2011. During this meeting, each PS-OC was asked to present three vignettes that showcased its most promising research. The vignettes presented included how theoretical physics could help explain metastasis, using methods from statistical physics to analyze patterns in DNA mutations and mathematical modeling of drug resistance, transport in cancer, and tumor homing phenomena. Other PS-OCs presented different technologies to isolate and analyze CTCs, in addition to several other breakthrough technologies and concepts in cancer research. During the second part of this meeting, several centers presented their Trans-Network Project proposals. These proposals came out of the "physical sciences perspective challenge. A few weeks before this meeting, the OPSO challenged the PS-OCs to come up with a particular scientific question that is still not answered in cancer research and to try to resolve it using a physical sciences perspective. These projects brought the mathematicians, engineers, cancer biologists, oncologists, and physicists together to work on these challenging questions. Four of these proposals were subsequently funded.

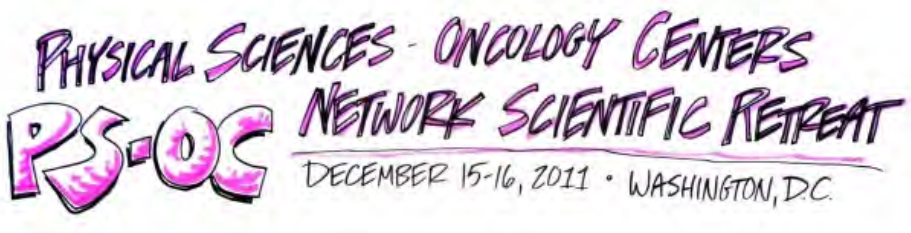
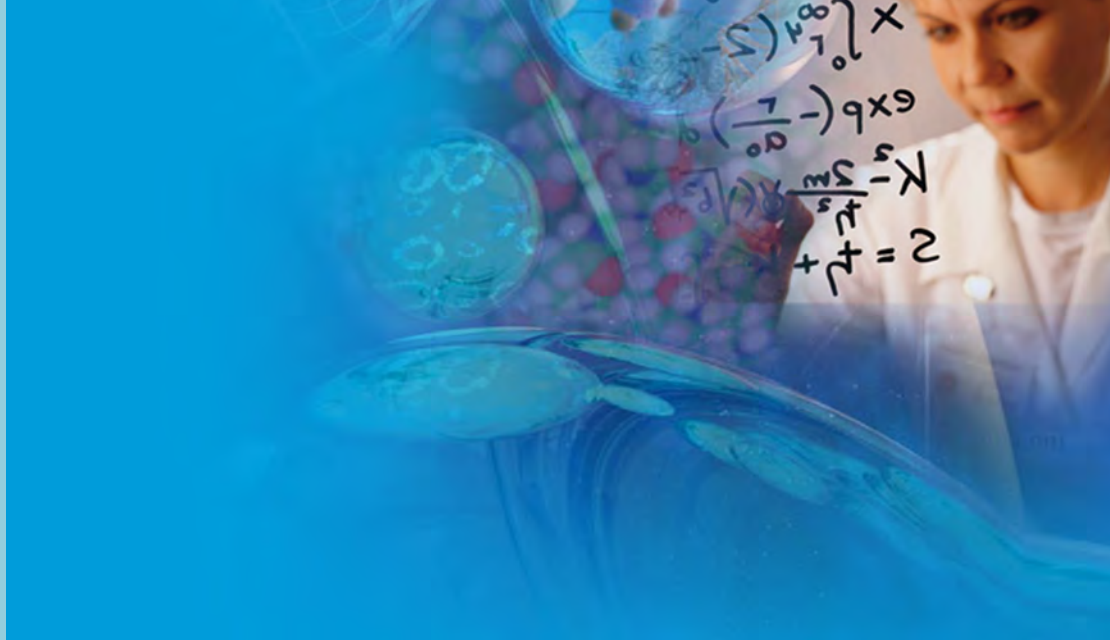


Figure 5.10. A graphical introduction to the PS-OC Network Scientific Retreat.



6. PS-OC Program Center Highlights

The PS-OC Network uses a Center mechanism to enable team science and provide supporting infrastructure, with the goal of solving tough problems in cancer using a physical sciences perspective and training a new generation of cancer researchers. The Center structure has provided several advantages to the investigators as they are challenged to meet these expectations. These advantages include the following:

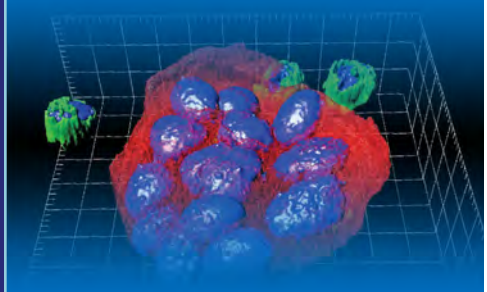
- Establishing a Center-wide overarching Framework that addresses questions in cancer using a physical sciences perspective. Each Center is made up of three to five projects and cores that intersect under the overarching Framework. This allows teams to address problems with multiple length and time scales within one grant mechanism.
- Fostering new transdisciplinary collaborations among scientists of diverse scientific backgrounds with a common research goal. The formation of new transdisciplinary collaborations is fueled by the support of the Center and has led to new Pilot Projects, publications, and grants (Figure 6.1).
- Educating a new generation of transdisciplinary scientists who will bring a physical sciences perspective to cancer research. Within a Center, trainees are exposed to multiple disciplines and have the opportunity to gain knowledge or skills in another field.

Each PS-OC is unique in how it has established its Framework and infrastructure. This section highlights the overarching accomplishments of four Centers: Dana-Farber Cancer Institute PS-OC, Northwestern University PS-OC, The Scripps Research Institute PS-OC, and the University of Southern California PS-OC. To see the accomplishments of all the PS-OCs, please refer to Appendix.

Figure 6.1. Example of Center collaborations reported by the Cornell PS-OC. Each node represents an investigator affiliated with the Cornell PS-OC. The edges indicate a collaboration between two investigators. The colors indicate the project that the investigator is associated with in the PS-OC. White nodes are affiliated with more than one PS-OC.

Physical Sciences Perspective

The principal mission of the PS-OC is to promote the understanding of cancer evolution utilizing approaches from the physical sciences. The goal of this PS-OC is to advance the understanding of the physical principles that govern cancer initiation, progression, response to treatment, and the emergence of resistance.



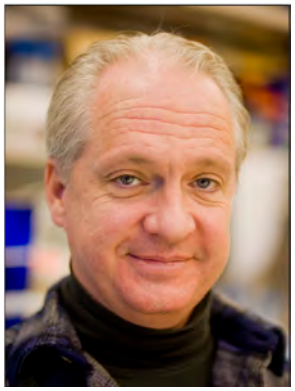
Dana-Faber Cancer Institute PS-OC: Exploring and Understanding Evolution and Evolutionary Theory in Cancer from a Physics Perspective

Principal Investigator: Franziska Michor, Ph.D.
Senior Scientific Investigator: Eric Holland, M.D.

Summary

The overarching Framework of the Dana-Farber Cancer Institute (DFCI) PS-OC is "Exploring and Understanding Evolution and Evolutionary Theory in Cancer from a Physics Perspective." The Center combines evolutionary theories with experimental approaches from the physical sciences to better define, understand, and control cancer at all levels. From a physical sciences view, cancer should be thought of as a complex adaptive system that is most appropriately studied in the context of evolution and evolutionary theory. From an evolutionary standpoint, tumors are collections of cells that accumulate genetic and epigenetic changes, which are then subjected to selective pressures within a tissue. These heritable variations can lead to adaptation of the cells and the induction of processes such as angiogenesis, drug resistance, immune evasion, and faster growth – factors that lead to the clonal selection of cells with greater "fitness." Mutations advantageous to the cancer cell, however, are detrimental to the organism and ultimately result in the death of both the patient and the tumor. Therefore, neoplastic processes serve as an example of selection acting on different hierarchical levels: clonal evolution generally selects for increased proliferation, survival, and evolvability on the cellular level and leads to progression, invasion, and resistance; the latter effects are selected against on the level of multicellular organisms.

Franziska Michor, Ph.D.



Eric Holland, M.D.

Key Center Accomplishments

Optimization of drug dosing for lung cancer with evolutionary cancer modeling. Non-small cell lung cancers (NSCLCs) that harbor mutations within the epidermal growth factor receptor (EGFR) gene are sensitive to the tyrosine kinase inhibitors (TKIs) gefitinib and erlotinib. Unfortunately, all patients treated with these drugs eventually acquire resistance, most commonly as a result of a secondary mutation within EGFR (T790M). Because both drugs were developed to target wild-type EGFR, this team hypothesized that current dosing schedules were not optimized for mutant EGFR or to prevent resistance. To investigate this further, team members developed isogenic TKI-sensitive and TKI-resistant pairs of cell lines that mimic the behavior of human tumors and used these to determine that the drug-sensitive and drug-resistant EGFR-

mutant cells exhibited differential growth kinetics, with the drug-resistant cells showing slower growth. The investigators then incorporated these data into evolutionary mathematical cancer models with constraints derived from clinical datasets. These mathematical models consist of a stochastic birth and death process with rates identified from in vitro experiments. This modeling work predicted alternative therapeutic strategies that could prolong the clinical benefit of TKIs against EGFR-mutant NSCLCs by delaying the development of resistance and suggested that erlotinib should be continued beyond progression of disease. The team is currently working to implement these strategies in clinical trials.

Toward a mechanistic framework of the generation of somatic genomic alterations in cancer. An unstable genome is a hallmark of many cancers. It is unclear, however, whether some features of the genome are mutagenic and can drive somatic alterations in cancer. The DFCI PS-OC team performed a genome-wide analysis of 663,446 DNA breakpoints associated with somatic copy-number alterations (SCNAs) from 2,792 cancer samples classified into 26 cancer types. Many SCNA breakpoints are spatially clustered in cancer genomes. The investigators observed a significant enrichment for G-quadruplex sequences (G4s) in the vicinity of SCNA breakpoints and established that SCNAs show a strand bias consistent with G4-mediated structural alterations. Notably, abnormal hypomethylation near G4s-rich regions is a common signature for many SCNA breakpoint hotspots. They proposed a mechanistic hypothesis that abnormal hypomethylation in genomic regions enriched for G4s acts as a mutagenic factor driving tissue-specific mutational landscapes in cancer. Furthermore, they integrated data on DNA replication timing, long-range interactions between genomic material, and 331,724 SCNAs from 2,792 cancer samples classified into 26 cancer types. Team members reported that genomic regions of similar replication timing are clustered spatially in the nucleus, that the two boundaries of SCNAs tend to be found in such regions, and that regions replicated early and late display distinct patterns of frequencies of SCNA boundaries, SCNA size, and a preference for deletions over insertions. They then showed that long-range interaction and replication timing data alone can identify a significant proportion of SCNAs in an independent test dataset. From these findings, the investigators proposed a model for the generation of SCNAs in cancer, suggesting that data on spatial proximity of

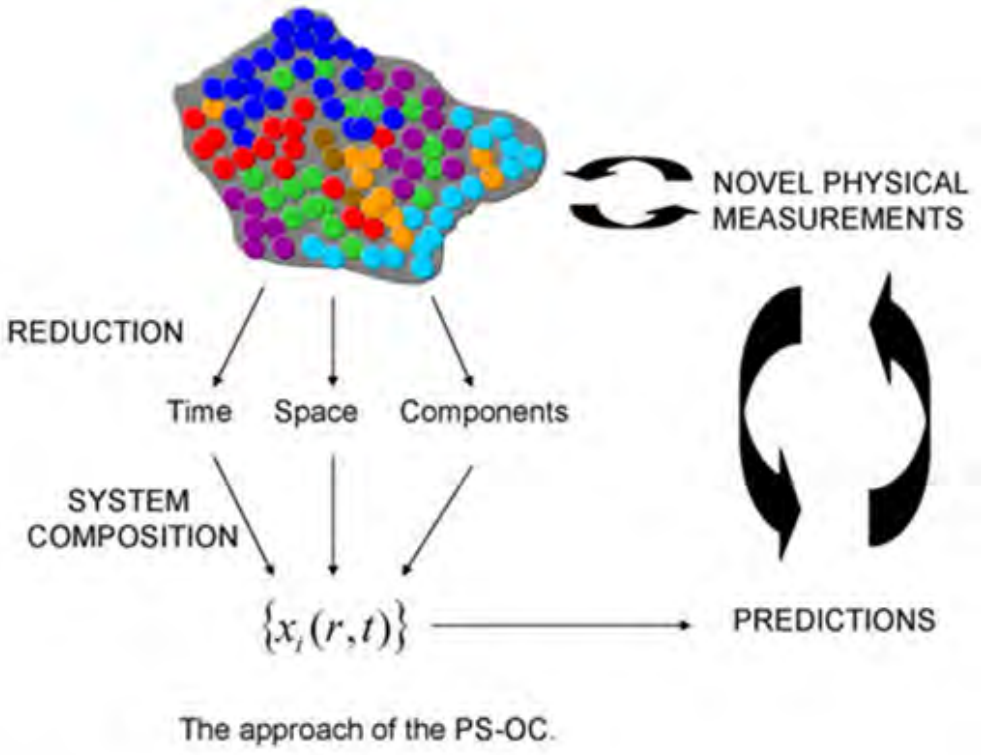
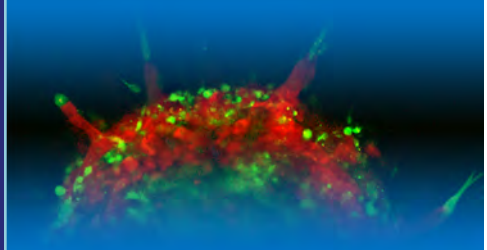


Figure 6.2. The overall approach of the DFCI PS-OC.

DFCI PS-OC Accomplishments

The Center has used evolutionary modeling and experimental approaches to optimize the dosing schedules for NSCLC cells treated with tyrosine kinase inhibitors (TKIs) and the radiation protocol used to treat a mouse model of glioma.

In both cases, the predictions of the mathematical models increased the effectiveness of these existing treatments, and for TKIs, delayed the onset of resistance. These models are currently being used to inform the design of new clinical trials.



regions replicating at the same time can be used to predict the mutational landscapes of cancer genomes.

Optimization of radiation dosing for brain cancer with evolutionary cancer modeling. Patients suffering from glioblastoma (GBM), the most common and malignant primary tumor of the brain, have very poor survival. The standard of care is surgery when possible followed by radiation and chemotherapy; it has essentially been unchanged over the past 50 years, as has the overall survival for this disease. Over the years, several attempts to update radiation therapy for these tumors have been undertaken. Dose escalation studies demonstrated that survival improvements are seen up to an overall dose of 60 Gray (Gy). Beyond this point, there are little if any improvements in survival at the cost of increased toxicity. Typically the dosing schedule is 2 Gy per day five days per week for six weeks. Alternative schedules have been attempted such as hypo-fractionated dosing of 3-6 Gy per session or hyper-fractionated dosing of 1 Gy fractions twice per day. Neither of these strategies has shown improvements over the standard 2 Gy in one dose per day. This team designed a mathematical model of proneural GBM stem and progenitor cells to investigate the effects of radiotherapy on cell numbers. Using this model, team members then optimized over all possible strategies that deliver 10 Gy in one week, identifying an administration schedule that was predicted to outperform standard treatment strategies. This schedule, when administered to mice engineered to develop proneural glioma, led to a significant improvement in survival compared to the standard schedule. In fact, the Kaplan-Meier curves were indistinguishable animals treated with twice the amount of radiation used in the optimized strategy. The team is currently submitting this validated optimized protocol as a clinical trial at Memorial Sloan-Kettering Cancer Center.

New Infrastructure Built to Support the PS-OC

The Single Cell Analysis Core Facility develops innovative technologies for quantitative single-cell assays and facilitates the research proposed in the DFCI PS-OC. This core contributes to the PS-OC by providing quantitative measurements used to parameterize "cellular fitness" as a convolution of cell cycle and apoptosis dynamics and signaling events in colorectal, glioma neurospheres, and leukemia cell lines. These measurements are then inserted into the modeling Framework of the PS-OC.

Interactions with the PS-OC Network

The DFCI PS-OC has recently started a Trans-Network Project to study the impact of higher-order chromatin structure and cellular context on chromosome stability and gene expression. There are six PS-OCs involved, and the project is being led by Franziska Michor (DFCI PS-OC) and Jonathan Licht (Northwestern PS-OC). Cancers are characterized by aberrant levels of and mutations in chromatin regulators that affect histone modifications and chromatin remodeling. The specific question this project aims to investigate is: Why is cancer associated with specific mutations in chromatin regulators? The team has hypothesized that certain mutations in chromatin regulators corrupt the global chromatin configuration within the nucleus in specific ways and that these genome-wide "spatial" defects are the root cause of subsequent amplifications and deletions in cancer genomes. Team members will utilize recently developed

theoretical approaches and precision measurement tools to investigate the three-dimensional structure of the human genome and its relationship to the generation of copy number alterations in cancer genomes and changes in gene expression. The investigators anticipate that alterations in histone methyl-transferases, chromatin remodelers, and epigenetic regulators will impact the higher order structure of the genome. They also expect to find that modifying the cellular context, such as the mechanical properties of the extracellular matrix, will potentiate this effect. Furthermore, they hope to predict the mutational landscapes of these cancer types, in terms of copy number variations, from data on replication timing as well as the three-dimensional structure of the nucleus. These outcomes will allow the team to predict and identify low-frequency events in cancer and to relate cancer incidence and genomic changes to the microenvironmental landscape. The data will ultimately facilitate the development of novel diagnostics and therapeutics designed to prevent the changes causing structural alterations in cancer.

Future Plans

- Continue progress toward mathematical modeling to identify optimum clinical strategies.
- Continue progress toward a mechanistic understanding of the generation of somatic genetic events during tumorigenesis.
- Continue progress toward a mechanistic understanding of the generation of somatic epigenetic events during tumorigenesis.

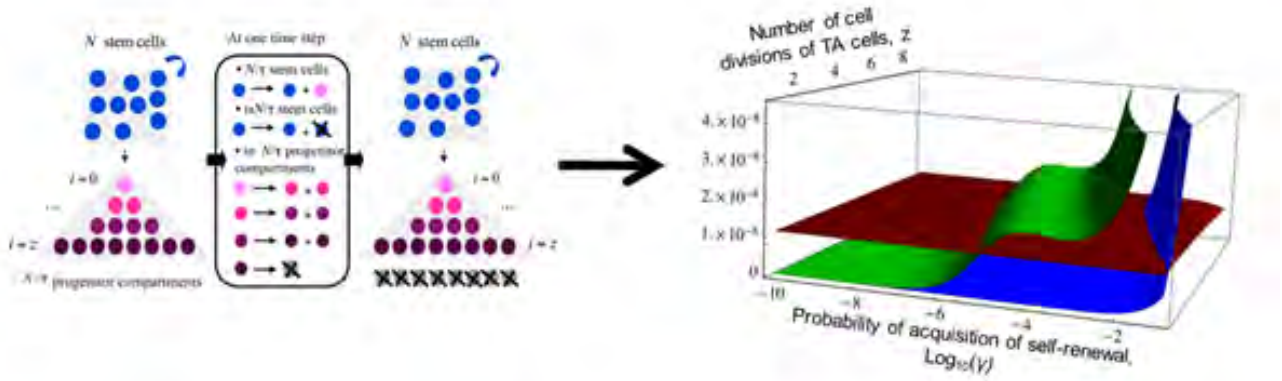


Figure 6.3. Applying evolutionary theory to the response of cancer cells to radiation in a mouse model of glioma enabled researchers to develop an optimized radiation dosing regimen. This model assumed that tumor growth was driven by a small population of tumor stem cells and that radiation had differential effects on the probability of survival on stem cells compared to the bulk population.

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

Principal Investigator: Tom O'Halloran, Ph.D.
Senior Scientific Investigator: Jonathon Licht, M.D.

Summary

The overarching theme of the Northwestern University PS-OC is the "Coding, Decoding, Transfer, and Translation of Information in Cancer." The goal of the Center is to investigate the molecular basis of the flow of information in cancer cells. The underlying hypothesis of this PS-OC is that physical sciences approaches including nano and atomic scale investigation, advanced optics, high-level computational power, and mathematical modeling can yield new insights into fundamental processes of the cell.

Tom O'Halloran, Ph.D.

Jonathon Licht, M.D.

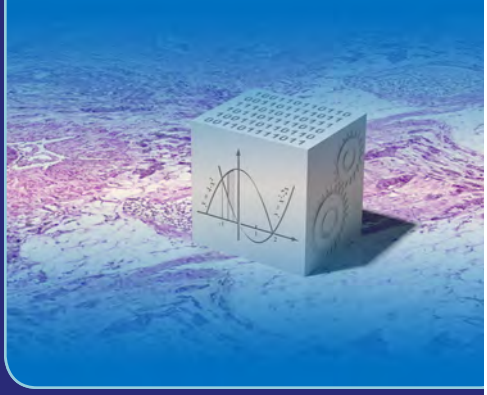
Key Center Accomplishments

The research of the Northwestern PS-OC has focused on applying physico-chemical concepts and methodologies to understand the information content of chromatin at various hierarchical levels, ranging from investigating the sequence dependence of the mechanical properties of naked DNA to the development of approaches to measure the elasticity of metaphase chromosomes. An important goal of this research is to identify links between the chemical and physical properties of chromatin and its biological functions. A further goal is to characterize the differences in these properties between normal and tumorigenic cells and to examine whether these differences contribute to the tumorigenic phenotype. The discoveries and accomplishments that have arisen from these approaches are outlined below:

Discovery that DNA Bending Depends on Base Pair Composition. To assess the sequence dependence of DNA flexibility, a physical parameter that influences nucleosome formation, Rob Phillips of the California Institute of Technology has developed both in vitro and in vivo methods to measure the propensity of short DNA segments to form loops. DNA flexibility showed both a length and sequence dependence in vitro but in vivo was only dependent on length. The presence of the bacterial HU architectural protein in vivo may explain this difference. In parallel, George Schatz of Northwestern developed a computational physics model that uses all-atom force fields and molecular dynamics/mechanics to describe the dependence of DNA bending on base pair composition. This model suggests that the observed propensity for nucleosomes to favor DNA sequences that have the dinucleotides AA, TT, or TA in 10 bp steps and to avoid extended A-tracks is derived from the physics of DNA.

Physical Sciences Perspective

This is the first time that theoretical and physical methods have been applied to the study of each level of information processing in the cell within the context of a unified, systematic construct. Through the use of a unifying model system, MMSET variant acute myeloid leukemia (AML) cell lines, this team has undertaken a range of studies that bring both depth and breadth to an understanding of the forces that govern gene expression and their ramifications in malignancy.



The Development of High Accuracy Nucleosome Mapping Techniques. To increase the accuracy with which nucleosomes can be mapped, the Widom lab has developed a novel technique for use in yeast cells that replaces MNase digestion of DNA flanking nucleosomes with a chemical cleavage approach, allowing nucleosome centers to be directly mapped. In collaboration with Ji-Ping Wang, leader of the Northwestern PS-OC's Bioinformatics Core, who developed a novel method that allowed analysis of the complex data generated by this approach, this technique has revealed details of competing preferential nucleosome positions genome-wide in yeast.

Verification of partial wave spectroscopy for measurement of nuclear disorder in tumor cells. Vadim Backman and his collaborators have pioneered the development of a new diagnostic technique that can detect the presence of malignancies by measuring optical changes in histologically normal-appearing cells located within the field of a tumor. This approach has been heralded as a major advance in early cancer detection. In research supported by the Northwestern PS-OC, partial wave spectroscopy (PWS), one of the methods being used by the Backman group to detect altered light scattering properties, has now been shown to measure the degree of nuclear disorder in neoplastic cells. This disorder is correlated with the degree of heterochromatization, the sizes of chromatin clumps in the nucleus, and the detachment of chromatin from the nuclear membrane.

New Transdisciplinary Collaborations Within the PS-OC

Neil Kelleher has teamed up with Jonathan Licht to combine Chip-Seq and mass spectrometry to measure the effects of histone methylation on nucleosome positioning and aberrant methyltransferases in lymphoma. They have deployed mass spectrometry-based measurement and modeling of histone methylation kinetics ("M4K") integrating stable isotopes, quantitative mass spectrometry, and computational

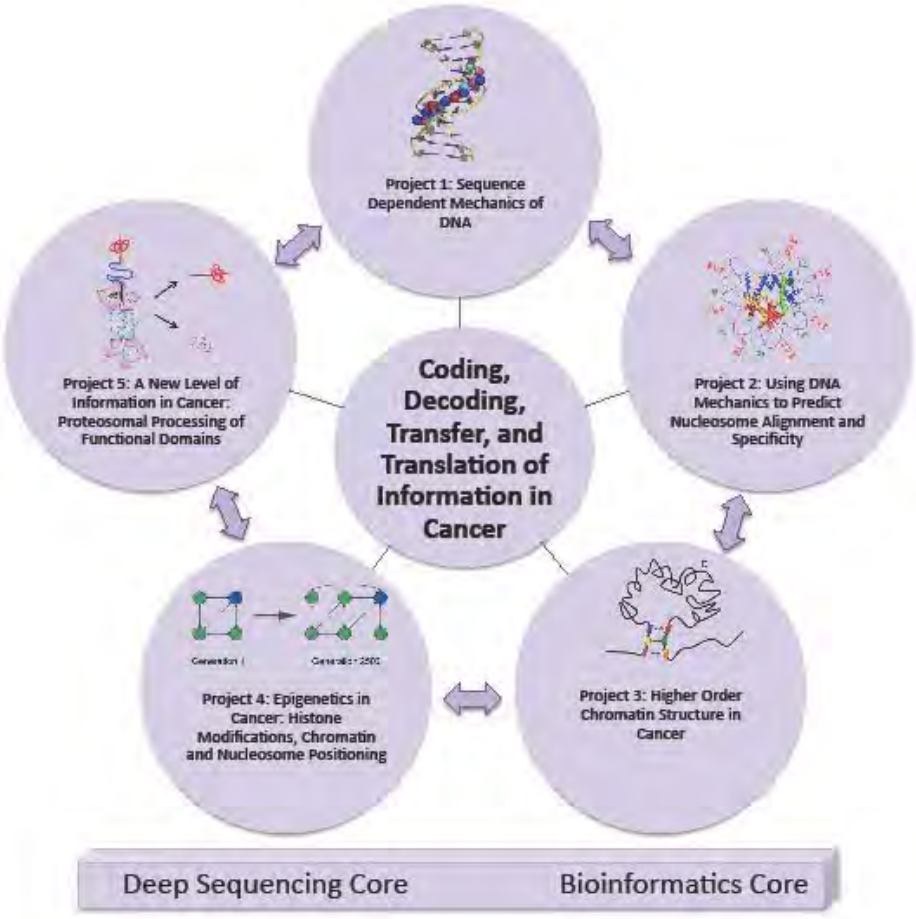


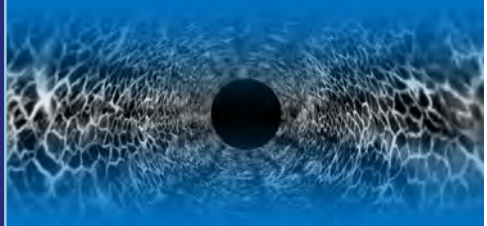
Figure 6.4. Overall approach and Framework of the Northwestern PS-OC.

Northwestern PS-OC Accomplishments

The Center has made strong progress toward many of its aims and has developed several new techniques that could have a transformative impact.

Among the accomplishments is a new technique to resolve the localization of nucleosomes at single base pair accuracy. This level of detail will help researchers better understand how DNA sequence controls nucleosome distribution.

The Center has also pioneered the use of Partial Wave Spectroscopy (PWS) as a cancer diagnostic tool. This technique measures light scattering techniques that correlate with changes in chromatin remodeling.



modeling to investigate how histone methylation patterns are established in living cells. They have used the M4K approach to determine the full matrix of effective rate constants for two methylation sites on histone H3: H3K36 and H3K27. Two of the histone methyltransferases that target these sites, MMSET and EZH2, are frequently deregulated in multiple myeloma (MM) and lymphomas respectively. M4K revealed that dimethylation on either H3K36 or H3K27, sharply reduces the rate of further methylation on the other site, suggesting a bi-directional antagonism in the methylation of these two sites.

Adilson Motter is collaborating with Steven Rosen to apply a novel mathematical framework to the analysis of cancer metabolism and, in particular, to search for potential therapeutic interventions by targeting genes that support the Warburg effect. Systematic application of this approach allowed them to identify gene knockouts that maintain the survival of normal differentiated cells and the growth of normal proliferating cells while suppressing cancer growth. Four out of the top nine predictions obtained by this method are genes that are targets of either approved or experimental drugs, while the other hits genes are novel targets. Highlighting the power of this approach, knock-out of one of the genes predicted to be critical to the Warburg effect, G6PD, in MM cells resulted in tumor cell death.

New Infrastructure Built as a Result of the PS-OC

The Northwestern PS-OC has catalyzed an entire series of collaborations that would not have existed otherwise by introducing mathematicians, clinicians, physicists, materials scientists, and engineers to each other and potential topics of mutual interest. These interactions have been accelerated through the Center's workshops and training sessions for physical scientists on various cancer topics, which have lowered the barrier to productive interactions. The roster of physical scientists who have joined the Lurie Cancer Center has grown markedly as more physical scientists have engaged in collaborative cancer research, spurred on by the PS-OC.

The Center has also built a vigorous education and training program across the physical sciences-oncology spectrum for a large group of graduate students and postdocs (more than 80) who will become the foundation for the next wave of transdisciplinary cancer researchers. The Education Program has also created a web-based repository of education and training tools that are freely available online (http://PS-OC.northwestern.edu/education-outreach/video-archive).

The Northwestern PS-OC Bioinformatics Core has developed a valuable tool for prediction of nucleosome positioning in vivo. The method has been implemented in a software tool called NuPoP in three formats: an online nucleosome positioning prediction engine available at http://nucleosome.stats.northwestern.edu, an R package available at http://www.bioconductor.org, and a stand-alone Fortran program. The Bioinformatics Core has also made progress in developing a nucleosome browser for visualizing the nucleosome positions and other genomic annotations including genes, regulatory factors, transcription starting sites, linker length index, etc. This website is made freely available to the community at http://ncode.northwestern.edu.

Interactions with the PS-OC Network

The PS-OC Network has contributed toward progress on the overall goals of the Northwestern PS-OC by providing a framework for collaboration between Centers that has contributed to the development of a project that will examine the impact of higher-order chromatin structure and cellular context on chromosome stability and gene expression. This collaboration with the DFCI PS-OC, Cornell PS-OC, MIT PS-OC, UCB PS-OC, and USC PS-OC will enable Northwestern PS-OC researchers to extend the Center's fundamental studies of chromatin organization to determine whether the genomic signatures of underlying failure mechanisms such as micro-environmental changes, somatic mutations, and mistakes in micro-RNA control of translation can be predicted from sequence data.

Future Plans

- To build upon collaborations between the O'Halloran and Schatz labs to further define the role of base pair sequences in the regulation of transcription factor binding
- To incorporate and expand the Kelleher Pilot Project on histone methylation dynamics in tumor cells by incorporating this project into Project 2

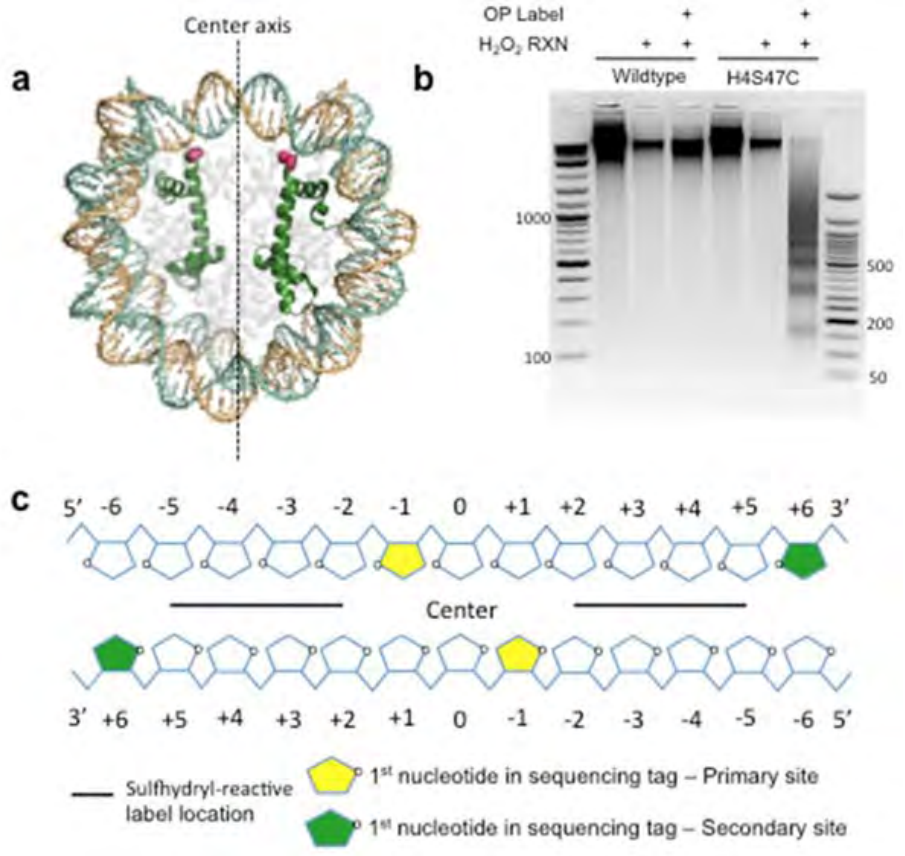
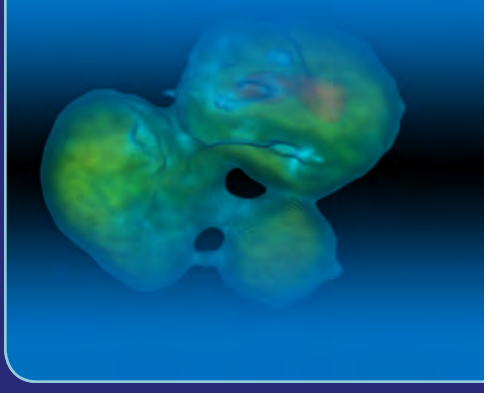


Figure 6.5. Mapping nucleosome centers by site-specific chemical cleavage. a, Nucleosome structure, highlighting histone H4 (green) and residue serine 47(pink), which is mutated to a cysteine where the sulfhydryl-reactive label covalently binds. b, Ethidium bromide stained agarose gel showing the chemical mapping results in a DNA banding pattern, which occurs only when the reaction includes (indicated by "+") the sulfhydryl-reactive label, copper, H2O2, and the H4S47C mutant yeast. Brogard K, Xi L, Wang JP, Widom J. Nature-2012.

Physical Sciences Perspective

The Scripps PS-OC is developing mathematical models that apply techniques previously used in the areas of digital communication and fluid dynamics. Employing experimental design strategies adapted from high energy physics and biophysics, the Center uses physical measurements and computational approaches to quantify the many parameters that define the fluid phase of solid tumors.



The Scripps Research Institute PS-OC: Four-Dimensional Fluid Biopsy Center (4DB)

Principal Investigator: Peter Kuhn, Ph.D.
Senior Scientific Investigator: Kelly Bethel, M.D.

Summary

The Scripps PS-OC uses physical and mathematical sciences to understand the fluid phase (blood circulation phase) of solid tumors (carcinomas), including relationships between the primary tumor, circulating tumor cells (CTCs), and distant metastatic deposits of tumor. This understanding will enable the use of blood samples to monitor the efficacy of treatments for solid tumors and predict patient responses. Prescribing the right treatment at the right time at the right dose to the right patient can prolong productive life, reduce side effects, reduce hospital time, and reduce the waste caused by ineffective treatments. Personalized cancer care management, enabled by clinically non-invasive blood sampling, will lead to better treatments for individual patients and reduce the healthcare burden on the economy.

Peter Kuhn, Ph.D.

Kelly Bethel, M.D.

Multiple types of carcinoma are being studied, including lung, prostate, pancreatic, liver, ovarian, breast, and colon cancer. All research is conducted with clinical implications in mind, using autopsy and time resolved sampling strategies from either primary or metastatic tumor tissue and patient blood samples. The evaluation of very rare cells from solid tumors present in the bloodstream is performed using a newly developed assay, termed the HD-CTC (High Definition-Circulating Tumor Cell) assay. The high definition component of the assay refers to the ability of this assay to capture the cytomorphologic details of single cells and the ability to stain cells for specific molecular targets.

Key Center Accomplishments

Using Fluid Phase Biopsies to Guide the Treatment of Non-Small Cell Lung Cancer. The Fluid Biopsy and HD-CTC assay delivers results that can potentially alter the management of individual lung cancer patients, with the highest impact being in early-stage disease and at the time of diagnosis. The HD-CTC assay utilizes physics-inspired approaches to detect rare CTCs hidden in the large number of normal cells in a patient's blood. The method spins down all cells from a patient sample onto microscope slides and then uses an automated image analysis algorithm to identify cells with the properties of CTCs. The "no cell left behind" strategy results in a data tsunami that is analyzed by Microsoft's High-Performance Computing platform. Clinicians are

presented with a summary of the results and can visualize individual cells using modern internet browser tools. The HD-CTC assay has demonstrated its immediate prognostic potential in a research setting for non-small cell lung cancer, a disease that is considered difficult to treat, diagnose, and monitor. Single case studies now indicate that the information provided by the HD-CTC assay could be used to guide how clinicians manage this disease at the time of diagnosis.

Predictions based on mathematics that can alter the approach of cancer management. Using a Markov Chain model, the same mathematical approach that underlies both the Google page ranking and the Viterbi Algorithm for cell phone communications, a mathematical model of lung cancer spread was developed. The results of this model can suggest both the likelihood of a particular route of metastatic spread and the time sequence of detectible tumor at metastatic sites. Using this approach, the model indicated that the adrenal gland has a behavior similar to lymph nodes and develops metastases early on in the disease evolution. This result is consistent with existing but not well known or widely accepted data that show anatomic connections in the lymphatic system between the lung and the adrenal gland. The model is proving to be a tipping point for thoracic oncologists to understand and specifically treat adrenal metastases. The model identifies organs that function as both spreaders and sponges in the development of metastases and could suggest new ways of managing metastatic disease in patients.

New Transdisciplinary Research

Liver transplantation is one of the primary treatment strategies in patients with hepatocellular carcinoma. However, in many cases, despite the absence of detectable secondary tumors, this treatment fails because of metastatic lesions occurring in the new liver. Using the fluid phase biopsy on hepatocellular carcinoma patients before, during, and after liver transplantation surgery could help clinicians better understand the factors that predict success and develop better strategies to manage this disease. Preliminary data indicate that the early identification of systemic disease using the HD-

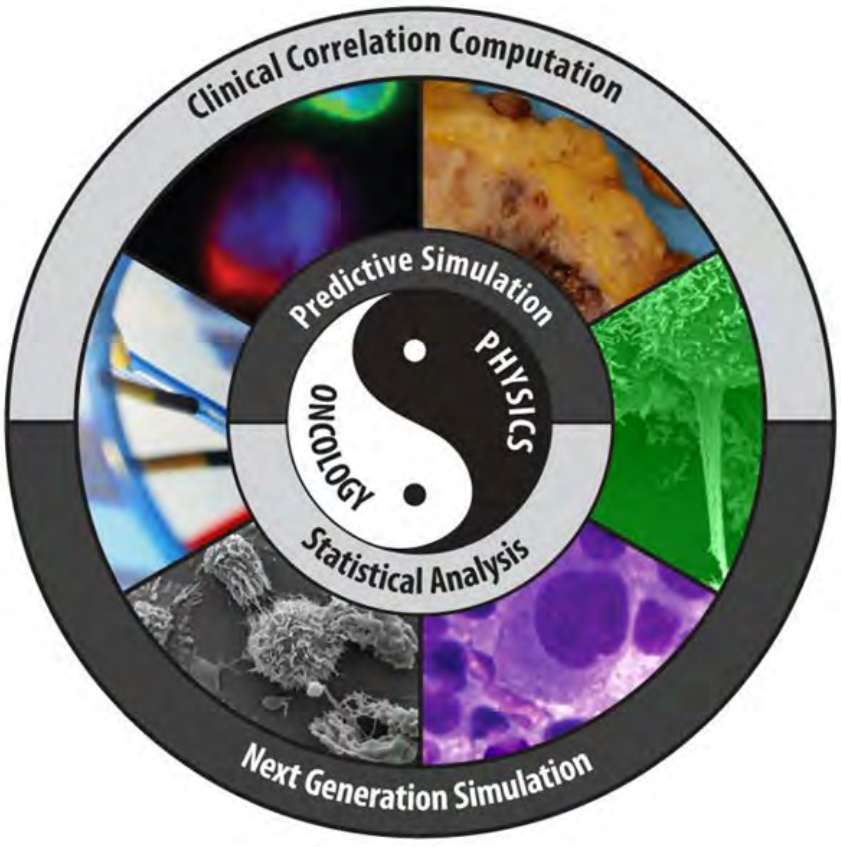
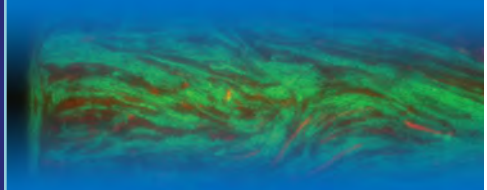


Figure 6.6. The overall Framework of the Scripps PS-OC.

Scripps PS-OC Accomplishments

The Center has used physical science perspectives to study the problem of metastasis. Using new technologies to detect and assay circulating tumor cells (CTCs) from patient samples, the Center has obtained results that could directly impact the clinical management of non-small cell lung cancer and hepatocellular carcinoma.



CTC assay, even in the absence of detectable metastasis, could be a robust predictor of short-term transplantation failure. This could provide a complementary diagnostic approach to help oncologists forecast which patients are most likely to benefit from liver transplants and enable better management of transplantation waiting lists.

New Infrastructure

Pathology Boot Camp: Integral to the process of recognizing, studying, and characterizing circulating tumor cells is recognizing cancer cells in their original birthplace in the primary tumors. Consequently, the Scripps PS-OC has run a series of microscope tutorials to familiarize both biological scientists and physical scientists with the features of human cancers as they actually exist in the clinic and hospital. From demonstrations of actual biopsy needles, to whole tumor slice microanatomy, to microscopic features of tumors, their blood supply, and their cellular morphologies, the Center's Pathology Boot Camps have educated numerous cancer researchers about the realities and opportunities in human cancer tissue research.

Correlative science clinical trials: Early access to the initial clinical data was provided to the entire PS-OC Network, enabling a number of oncologists and surgeons to ask specific clinical questions that could be answered by fluid biopsies. There are now 18 such studies under way at the Scripps PS-OC in collaboration with 15 clinical sites around the country. The questions have been on topics such as the timing of systemic disease onset, as identified by the occurrence of CTCs, and how the imaging of the primary and fluid phase cells for specific biomarkers might correlate with drug response.

Interactions with the PS-OC Network

While the Scripps PS-OC developed the original HD-CTC technology in a clinically relevant context and is putting together the mathematical models to advance the understanding of metastatic patterns, it is now through the PS-OC Network that these methods are being clinically validated. Early hypotheses are being formed by members of the other Centers. It is only through joint efforts that these early concepts can be turned into high-impact discoveries. For example, the Markov Chain model produced an observation of a probability edge returning back to the primary tumor, but it was the PS-OC Network meeting that Larry Norton from the DFCI PS-OC attended that allowed for the connection to be made to the seeding and self-seeding discoveries of his group.

Future Plans

- Utilize clinical results to drive hematology model experiments with the goal of understanding cancer cell survival in the circulatory system (Research Project 1).
- Establish a complete mathematical framework that is consistent with existing clinical observations but that will lead to testable hypotheses of modifications of clinical practice (Research Project 3).
- Continue to deploy HD-CTC assay in clinical trials to demonstrate clinical utility. Currently running 18 clinical studies with results expected to accrue over the next two years (Clinical Sample Core).
- Pursue genomic analysis of CTCs to further investigate tumor heterogeneity and its implications for treatment response heterogeneity.

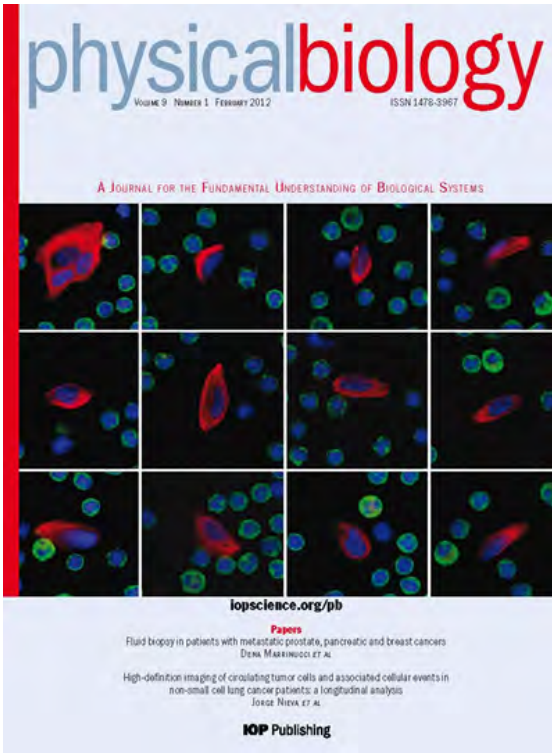


Figure 6.7. Cover of *Physical Biology* journal highlighting the research from the Scripps PS-OC.

University of Southern California PS-OC: Building A Virtual Patient

Principal Investigator: W. Daniel Hillis, Ph.D.
Senior Scientific Investigator: David Agus, M.D.

Summary

The focus of the University of Southern California PS-OC (USC PS-OC) has been on the causes and consequences of drug resistance from a multiscale perspective. Typically, drug resistance is investigated at a cellular level asking questions about the role of particular genes or pathways in determining a cell's fate in response to exposure to drug. However, some of the factors that contribute to drug resistance at a cellular level may also have complex regulatory mechanisms or may have arisen through complex evolutionary processes.

W. Daniel Hillis, Ph.D.

To address these questions, the USC PS-OC is working to develop accurate methods for simulating cancer behavior. These simulations could be used to predict and characterize responses and outcomes, which in turn could increase the odds of designing therapies that succeed. The goal of this work is to create a virtual cancer model that can accurately predict the steady state growth and response to therapy of a cancer in a human host. In addition to helping ask and answer fundamental questions about cancer mechanisms, complexity, and evolution, a virtual cancer model will enable a new paradigm in treatment. Center investigators anticipate it will become possible to take a small number of measurements from a patient, at a variety of scales, use these measurements to calibrate the virtual cancer, and then simulate disease progression with and without therapy. This approach could allow researchers to test a variety of treatment and dosing options in sillico and find the treatment that is most likely to lead to a favorable outcome. In addition, the team members are working to identify signals, such as changes in a tumor or serum protein, that indicate that a patient is truly responding to therapy, leading to a radical improvement in the standard of care.

David Agus, M.D.

Key Center Accomplishments

As a starting biomodel system, The USC PS-OC has focused on a set of mouse models of lymphoma generated by the Lowe lab. In these models Eμ-myc mice over express the c-myc oncogene in the B-cell lineage, and the resulting B-cell malignancies resemble human non-Hodgkin's lymphomas. The Eμ-myc model was one of the first transgenic strains produced and has been widely used to identify cancer genes and other aspects of cancer biology. For example, loss of either the ARF or p53 tumor suppressor genes can cooperate with Myc to promote aggressive B cell lymphomas in

Physical Sciences Perspective

The USC PS-OC approaches cancer as a dynamical system and views the state of a system as point in a multidimensional state space and the time evolution as a path through that space. Cancer, in this model, can be viewed as an unstable region of the space. This approach, originally developed for the design of atomic weapons, has been applied successfully to many complex physical systems in physical chemistry, astronomy, and quantum physics, and well as engineering problems in telecommunications, aerodynamics, and finance. The Center aims to use this innovative approach to create stochastic simulations following specification of initial states that could predict how patients will respond to therapy.

mice. The response of the genotypes can be quite variable to conventional chemotherapy. For example, lymphoma lacking ARF respond particularly well to cyclophosphamide and many animals are cured. In contrast, those lacking p53 respond poorly.

Through a set of coordinated experiments that involved the entire Center, the USC PS-OC identified some unexpected contributors and consequences of drug resistance. A surprising role for pro-angiogenic pathways in tumor progression and drug resistance emerged from the tumor-scale project. Using the Eμ-myc lymphoma model, it was found that lymphoma cells do not initially proliferate in the inguinal lymph node. Instead, they proliferate in the spleen and bone marrow for several days, then very rapidly—within 12 hours—"burst" or migrate into the lymph node around day 9-10 post-injection of lymphoma cells. Additional study revealed a role for angiotensin II and reactive oxygen species in this burst phenomenon. This study integrated both computational modeling and pushed development of a novel intravital microscopy platform. This observation challenged assumptions about the role of angiogenesis-related genes. Prior to this observation, these genes were predominantly thought of as drivers of vascular infiltration, and a role in tumor homing and drug response had not been considered.

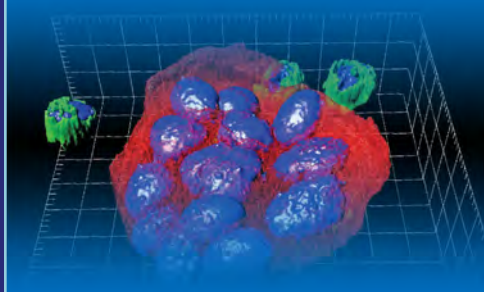
Parallel inquiries within the Center's host-scale project observed that angiogenesis-related genes such as Thy-1 showed significant time-dependent variation in auto-antibody levels during tumor progression. These studies utilized a novel high-dimensional auto-antibody profiling platform in combination with rigorous mathematical modeling. A possible explanation for these results is that the burst phenomenon might concurrently trigger a host immune response.

The Center's evolution project looked at how selective pressures might alter both genetic and epigenetic heterogeneity in tumors and at the potential consequences of those changes. These studies found that drug treatment altered the methylation profile of many genes in a drug-sensitive cell line. For a subset of genes, the methylation pattern had much less cell-to-cell variation after drug treatment, and this correlated with changes in gene expression. Among the

Figure 6.8. Overarching scientific Framework of the USC PS-OC.

USC PS-OC Accomplishments

The Center has made progress toward understanding a mouse model of lymphoma at multiple time and length scales using experimental, computational, and theoretical approaches. A major finding has been the observation that lymphoma cells initially proliferate in the spleen and bone marrow and then migrate in a transient burst to lymph nodes. Subsequent studies demonstrated that this process is regulated by angiotensin II and reactive oxygen species. These data suggest that angiogenesis-related genes may play a role in tumor homing. Parallel studies also indicate that the burst phenomenon may also trigger a host immune response.



genes identified in by this approach were FGFR1 and other regulators of angiogenesis. These data demonstrate the importance of the interplay between selective pressures, epigenetics, and the emergence of resistance.

Collectively, work from the USC PS-OC suggests a number of potentially, dogma-challenging hypotheses about the role of inflammatory cytokines and pro-angiogenic pathways in tumor progression and response to therapy. These factors may play a key role in propagating signals from the cellular scale to the host scale. In addition, the handshake between the physical sciences and life sciences was critical in the experimental design, data generation, and data analysis.

New Transdisciplinary Research

The USC PS-OC is predicated upon interactions between life scientists and physical scientists, and each project includes clinicians, cancer biologists, experimental technologists, physical scientists, and computational modelers. Specific examples include the collaboration between the Cristini and Gambhir labs around rigorous models of tumor growth and a new measurement modality. In addition, the relationship between the diverse modeling groups within this Center and the cell and cancer biology experts in the Lowe and Nolan labs would also have been unlikely to emerge without the impetus of the PS-OC. Lastly, it should be noted that even within the computational community, there are numerous sorts of models and approaches. This Center employs a combination of complex systems models, physical principles models, stochastic process models, and statistical models. These communities are typically quite distinct, but they have been unified because of the PS-OC Program.

New Infrastructure Built for PS-OC

This Center has focused the efforts of nearly two dozen investigators on building a unified, multiscale model of cancer. The calibration and testing of this model is based upon data generated by many labs working on a single common biological model system. In addition to the interdisciplinarity of individual projects, cross-project and cross-Center efforts have been critical to this Center's efforts.

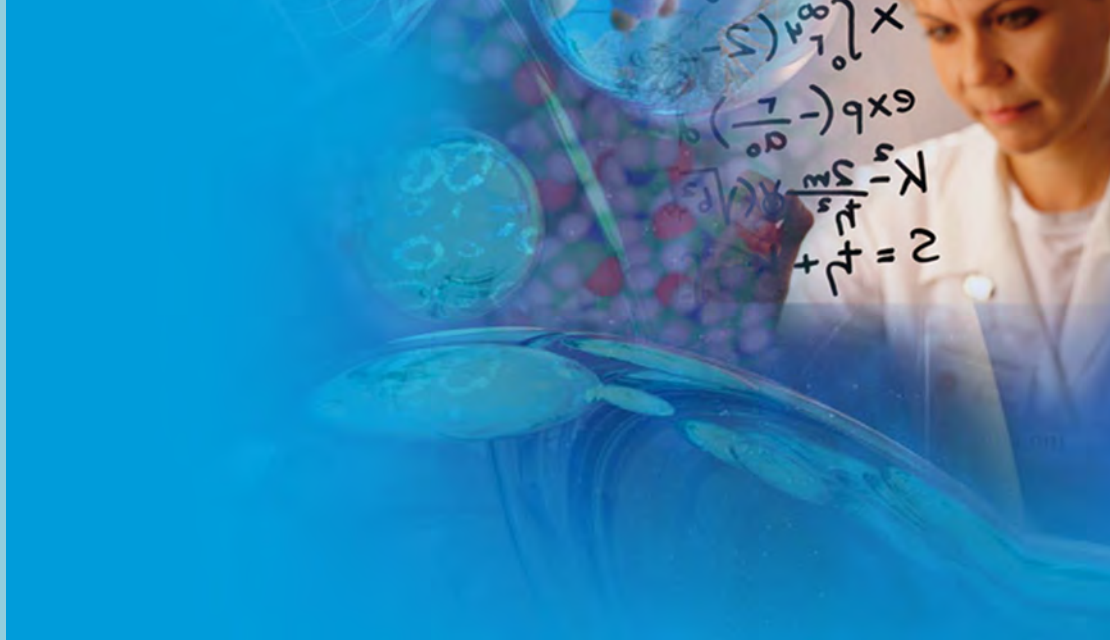
The USC PS-OC has been active in education and outreach, too. In addition to hosting several symposia, seminar series, and short courses, Center team members have held a series of brainstorming meetings during which they introduced diverse biologists, clinicians, and physical scientists to this area of research. In addition, the Center's senior co-investigator has been active in promoting the role of complex systems approaches to the study and management of cancer. In addition to these initiatives, the Center has been active in developing the first-ever multiscale data warehouse. This resource will enable diverse researchers to begin testing their own multiscale hypotheses in a way that was previously not possible.

Interactions with the PS-OC Network

The PS-OC Network has allowed the USC PS-OC to greatly leverage the diverse expertise prevalent within other labs in other Centers. For example, this Center did not possess any significant internal expertise in cell mechanics, but it has engaged in collaborations with the Wirtz and Manalis labs to leverage their knowledge and integrate this information into the Center's complex systems modeling. In addition, through collaborations with the Moffitt and ASU PS-OCs, the USC Center has been able to explore diverse aspects of the tumor microenvironment.

Future Plans

- Perform more detailed molecular analysis of lymphoma cells from different niches before and after the migratory burst to better characterize the molecular changes accompanying this tumor-scale phenomenon.
- Investigate how altering the timing and magnitude of the tumor burst alters the dynamics of auto-antibody response and drug response. The USC PS-OC is also investigating the impact of modulating FGFR1 and related genes on cellular responses to treatment as well as tumor progression.
- Continue to create specific educational tracks in both the life sciences and physical sciences curricula that promote developments in the area of physical sciences in oncology.



7. Outreach Activities

The PS-OC Program has consistently worked to inform the broader scientific community of the importance of bringing physical sciences into cancer research and to highlight exciting research at the interface of physical sciences and oncology. The first step in this mission began prior to the launch of the PS-OC Program with the requirement that each Center include an Outreach and Dissemination Unit. These Units organize workshops at institutions, scientific sessions at professional conferences, and share research highlights and funding opportunities through Center websites. In addition, the Outreach and Dissemination Unit leaders meet quarterly to discuss joint activities and prepare the PS-OC Program Newsletter, another tool to disseminate information about this emerging field. The PS-OCs also convey their work via the media. Numerous PS-OC investigators have been interviewed by journalists from around the world about their work at the intersection of physical sciences and cancer.

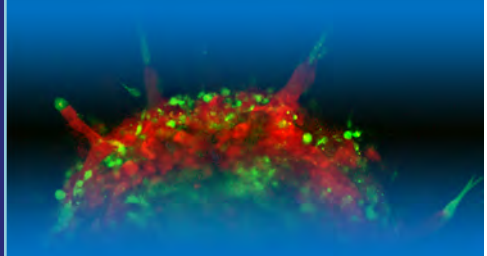
The OPSO also works to share the opportunities and accomplishments of the PS-OC Program as a whole with the broader research community. The OPSO website highlights information on research taking place across the PS-OC Network, upcoming events hosted by the OPSO and individual Centers, and relevant funding opportunities available through NCI and other funding agencies. OPSO scientific staff also write commentaries and editorials, and original research papers and develop collections of prepublished research and review papers to keep the broader research community abreast of advances being made by the PS-OC Program. Additionally, the OPSO organizes workshops and symposia at a breadth of scientific conferences and collaborates with other agencies to expand support for a growing physical sciences in oncology community. The OPSO and several divisions within the National Science Foundation have collaborated to develop funding opportunities for new investigators working at the intersection of physical sciences and cancer biology. The OPSO is also collaborating with the NCI Biorepositories and Biospecimen Research Branch (BBRB, previously the Office of Biorepositories and Biospecimens Research [OBBR]) to investigate factors affecting biospecimens using high-content screening.

7.1. PS-OC Outreach Programs

Each PS-OC has an Outreach and Dissemination Unit funded to both develop Outreach Programs and to support Outreach Pilot Projects. Each Center was given the latitude to develop the Outreach Programs that would best serve the Center's goals. The Centers have used these funds to organize workshops and symposia within their institutions and at relevant national and international conferences to inform the broader scientific community of the accomplishments and opportunities of the PS-OC. Additionally, Outreach and Dissemination Units have developed Center websites to highlight the value of applying physical science perspectives to cancer research. The PS-OC Outreach and Dissemination Programs developed by a number of Centers are highlighted below.

Figure 7.1. Dr. Jan Liphardt, PI of the UCB PS-OC, moderates a session during an outreach symposium at NIH titled "New Frontiers in Physical Sciences and Oncology."

The Center has sponsored symposia and workshops that have attracted a large audience of students, trainees, and faculty members not affiliated with the PS-OC. The intent of the workshops was to provide physical scientists and engineers, who reside primarily on Northwestern's Evanston campus, with fundamental concepts and knowledge about cancer biology and cancer therapy. Both of these workshops drew large audiences, with more than half of the attendees at the first workshop and two-thirds of the attendees at the second workshop coming from outside of the PS-OC.



Northwestern PS-OC Outreach and Dissemination Unit

A cornerstone of the Northwestern PS-OC Outreach and Dissemination Unit is its comprehensive website, which is used to inform the greater scientific community and the public about the Center's research projects, educational offerings, and funding opportunities. The website houses an extensive video archive of presentations from PS-OC-sponsored seminars, symposia, and workshops. Traffic at the website has been increasing over time. Specifically, the website's video archive, which currently contains 25 hours of seminar and workshop presentations, has averaged 251 views per video web page.

The Center has sponsored symposia and workshops that have attracted a large audience of trainees and faculty members not affiliated with the PS-OC. Since its inception, the Northwestern PS-OC has sponsored two major symposia in November 2010 and June 2011 (Figure 7.1), as well as summer workshops in July 2010 and July 2011. The intent of the workshops was to provide physical scientists and engineers, who reside primarily on Northwestern's Evanston campus, with fundamental concepts and knowledge about cancer biology and cancer therapy. Both of these workshops drew large audiences, over 100 people, with more than half of the attendees at the first workshop and two-thirds of the attendees at the second workshop coming from outside of the PS-OC. The large audience and the enthusiastic response to the two summer workshops held so far suggest that the PS-OC is providing a type of outreach and educational programming that is in high demand and otherwise unavailable on the Evanston campus.

Table 7.1. The reaction to the Center's two-day "Fundamentals of Tumor Biology" workshop.

Category	Response
Workshop Enhanced Knowledge of Cancer Biology?	97% "Absolutely" or "Very Much"
Workshop Increased Likelihood of Participating in Cancer Research?	95% "Absolutely" or "Very Much"
Enjoyed Workshop?	90% "Absolutely" or "Very Much"
Recommend to Colleagues?	90% "Absolutely" or "Very Much"

The signature outreach event of the Center to date was a public lecture on May 1, 2012, by Pulitzer Prize winning-author Siddhartha Mukherjee, who discussed his book The Emperor of All Maladies: A History of Cancer (Figure 7.2). This event was widely advertised across the Chicago metropolitan area. Brochures describing the work of the Center were distributed during the event, and a public reception featured video monitors displaying a slide show regarding the Center and the PS-OC Network.

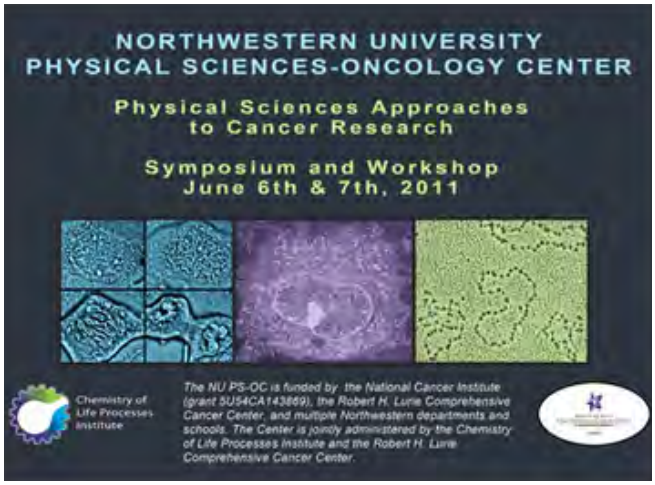
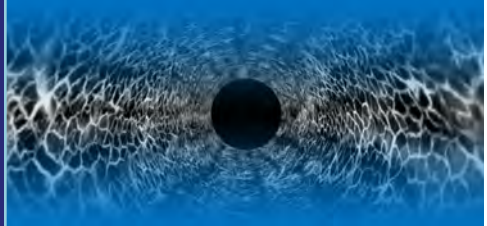


Figure 7.2. The Northwestern PS-OC has sponsored several major symposia and workshops. Images above are from the June 2011 workshop on Physical Sciences Approaches to Cancer Research. In May 2012, the Northwestern PS-OC sponsored a guest lecture on the "History of Cancer" with invited speaker, Siddartha Mukherjee, the Pulitzer prize-winning author. Approximately 600 people attended the lecture.

The Center conducts three workshops a year that have focused on topics including "Cancer as a Dynamical System," "Quantum Mechanics and Cancer Biology," and "Electrical Properties of Cells." Each workshop includes approximately 20 participants, including many from other PS-OCs, who discuss and develop challenging problems in cancer research. To bring the new ideas of workshop participants to the wider scientific community, the Outreach and Dissemination Unit interviews selected participants and makes the interviews available on the Center website.



Arizona State University PS-OC Outreach and Dissemination Unit

The Arizona State University (ASU) PS-OC Outreach and Dissemination Programs aim to share the promises of uniting the physical sciences and cancer biology with both the general public and the broader scientific community. It hosts a comprehensive website (cancer-insights.asu.edu) that highlights the Center's research and describes the Center's various activities. Another unique feature of the Center's Outreach Program is the interviews conducted by Pauline Davies with the many scientists who participate in the Center's research workshops. These interviews allow dissemination of cutting-edge ideas to a general and scientific audience.

To facilitate outreach to the local scientific and lay community, the ASU PS-OC hosts a regular seminar series during which cancer biologists, oncologists, and physical scientists highlight their latest thinking and research. These lectures are open to the general public but are mainly attended by scientists from ASU, the Mayo Clinic, Scottsdale, and the Translational Genomics Research Institute (TGEN) in Phoenix. Center team members are particularly proud of their special public lectures by eminent cancer researchers. The Center has hosted 5 such events so far that have been attended by more than 1,500 people in total.

Figure 7.3. The ASU PS-OC booth and the ASU Homecoming event distributing information about the PS-OC Program.

The Center also has a display every year at the Homecoming site at ASU (Figure 7.3), which is seen by thousands of people who attend the event.

The Center videocasts its seminars and makes them accessible in real time. Links can be found on the Center's website. Another feature of the ASU PS-OC is the research workshop program it runs. The Center conducts three workshops each year that have focused on topics that have included "Cancer as a Dynamical System," "Quantum Mechanics and Cancer Biology," and "Electrical Properties of Cells." Each workshop has approximately 20 participants, including many from other PS-OCs, who discuss and

develop challenging problems in cancer research. To bring the new ideas of workshop participants to the wider scientific community, Center staff interview selected participants and make the interviews available on the Center website. To date, the ASU PS-OC has compiled about 40 of these interviews, and in 2012 the Center also began providing a transcription of the workshop summaries.

The PS-OC Network Newsletter has proved very helpful in disseminating the work of the PS-OC. It is on display at public lectures and during Homecoming and is distributed to research workshop participants to provide an overview of the PS-OC Network. The ASU Press Office has copies to show interested journalists, and individual departments display the newsletter to showcase the research of their members. In addition, it enables the ASU PS-OCs to keep abreast of the work of the other PS-OCs.

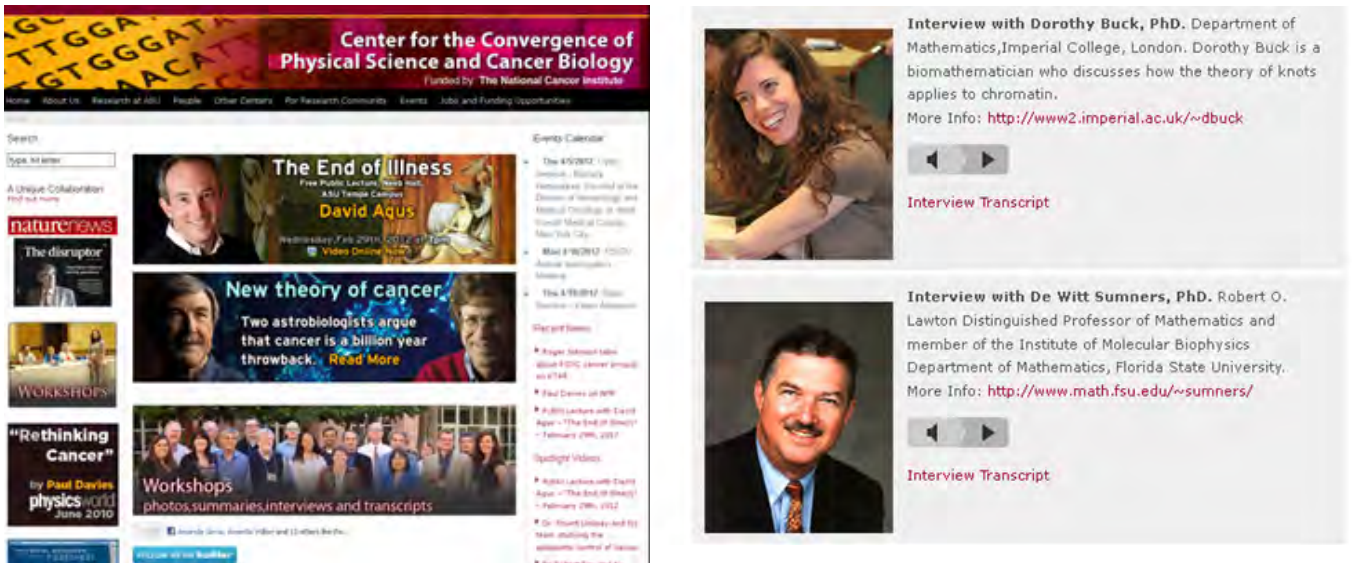
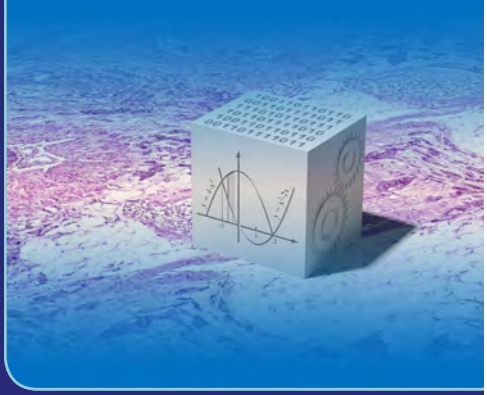


Figure 7.4. The ASU PS-OC website homepage http://cancer-insights.asu.edu. To bring the new ideas of workshop participants to the wider scientific community, the Outreach and Dissemination Unit interviews selected participants and makes the interviews available on the Center website.

"the strengthened links between the Centers has led to Moffitt and Cornell's co-hosting of the Cancer Brainstorming Club and a number of Young Investigator Trans-Network Project proposals that were submitted at the April 2012 meeting"



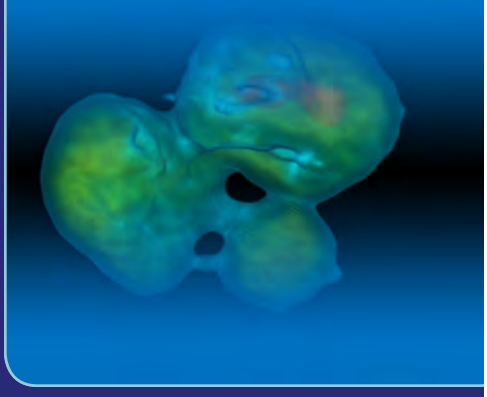
Cornell PS-OC Outreach and Dissemination Unit

The Cornell PS-OC Outreach and Dissemination Unit has focused on reaching out to the broader scientific community, not simply by creating information and making it available, but by creating opportunities for learning that are of value to outside researchers. This Unit has disseminated the research of Cornell PS-OC and other PS-OCs via its seminar series and symposia. The first two research symposia were attended by investigators from other PS-OCs and have been the starting point in fostering more in-depth collaborations. Some examples include seminars given by Robert Gillies, SI of the Moffitt PS-OC, and Mauro Ferrari, PI of the TMHRI PS-OC. After these seminars, the strengthened links between the Centers has led to Moffitt and Cornell's co-hosting of the Cancer Brainstorming Club (Figure 7.5) and a number of Young Investigator Trans-Network Project proposals that were submitted at the April 2012 meeting, including one collaboration that was selected for funding. TMHRI and Cornell have collaborated on both the Translational Cancer Research Symposium (October 2011) and the Translational Oncophysics Workshop (January 2012), with further plans for collaborations on grant proposals.

The Unit's series of workshops and minicourses serve to create a truly interdisciplinary group of researchers. These outreach activities provide training to researchers who are new to interdisciplinary research and who do not have experience with specific topics. The training rapidly brings researchers up to speed on practical lab techniques, protocols, and vocabulary, enabling them to accomplish their desired research and communicate with researchers in these interdisciplinary topics with greater effectiveness. To date, 81 researchers have attended Cornell PS-OC minicourses on the topics of surface modification, cell culture, and microfluidics.

Figure 7.5. The Cornell and Moffitt PS-OCs Cancer Brainstorming Club meeting.

"The goal of the PS-OC Outreach and Dissemination Working Group is to share the promise and accomplishments that arise from applying a physical science perspective to cancer research with the broader scientific community."



7.2. Outreach and Dissemination Working Group

Overview

The goal of the PS-OC Outreach and Dissemination Working Group is to share the promise and accomplishments that arise from applying a physical science perspective to cancer research with the broader scientific community. The working group serves as a resource to help coordinate and integrate outreach and dissemination projects across the Network and is made up of outreach and dissemination coordinators from each PS-OC and OPSO program officials. The working group meets quarterly, including a face-to-face meeting during the Annual PS-OC Network Investigators' Meetings. The Outreach and Dissemination Working Group acts as a forum where the members inform the group of the outreach activities in their Centers, exchange ideas, and learn from each other's experience how to reach out to the broader scientific community. During these meetings, the working group also discusses the topics to feature in the newsletter and collaborations between Centers in organizing symposia at different scientific conferences.

Some of the challenges for the PS-OC Network Outreach and Dissemination Working Group the difficulties associated with sharing progress and challenges associated with the complex interdisciplinary questions being addressed by the PS-OC Network. During the origins of the Network, the Outreach and Dissemination Working Group facilitated transdisciplinary, intra-Center, intra-Network, and external communication and collaboration. Issues such as dealing with discipline-specific jargon, visualization conventions, and priorities all benefitted from providing outreach and dissemination coordinators a forum and opportunity to brainstorm and consult with their colleagues. By working on the newsletter together and reporting to one another on the feedback from students and investigators, working group members were able to identify and share common challenges in clearly articulating PS-OC science to the broader research community. These meetings have helped develop plans for meeting sessions and have even led to the Northwestern PS-OC hosting the Pulitzer Prize-winning author of *Emperor of All Maladies* for a public lecture. Additionally, this exchange of ideas has been stimulating and helpful, creating a sense of collegiality and professionalism that is often missing from outreach efforts tied to isolated grants. Once this initial foundation was laid, the Outreach and Dissemination Working Group began to build relationships and grow conversations with relevant partners outside the Centers and the PS-OC Network, particularly with patient advocates, external advisors, relevant scientists, and prospective trainees.

The PS-OC *Perspectives* Newsletter

One effective way of disseminating the goals and highlighting the accomplishments of the PS-OC Network is the bi-annual newsletter *Perspectives* (Figure 7.6). A collaborative production by the members of the PS-OC Outreach and Dissemination

Working Group, *Perspectives* is designed to be accessible to scientists from various disciplines and interested members of the general public. *Perspectives* has featured all 12 Centers from the PS-OC Network and has described their unique scientific contributions. It provides a vehicle to disseminate new research ideas and also serves to create a sense of shared mission among the Centers.

Because one of the roles of *Perspectives* is to encourage junior researchers to become familiar with the PS-OC concept, each edition also includes articles by young investigators describing their experiences working at the confluence of cancer and physical sciences. Another role is to use the newsletter to promote the activities of the PS-OC Network. It is distributed by university press officers, given to visiting journalists, displayed at Homecoming events, and shared with academic visitors to workshops and lectures. The patient advocates also report that they value the newsletter and they frequently contribute stories that describe the value of the novel PS-OC approach to cancer research.

Perspectives appeared as a hard copy in the first years of the PS-OCs and is now produced in electronic format to be printed as required.

PS-OC Workshops

While the major focus of the PS-OC Outreach and Dissemination Working Group and the individual Center Units is sharing the accomplishments of the PS-OC Network, the working group has developed a strong network of outreach and dissemination coordinators and has directly facilitated Trans-Network collaborations. The TMHRI PS-OC hosted researchers from the Cornell PS-OC in January 2012 for a

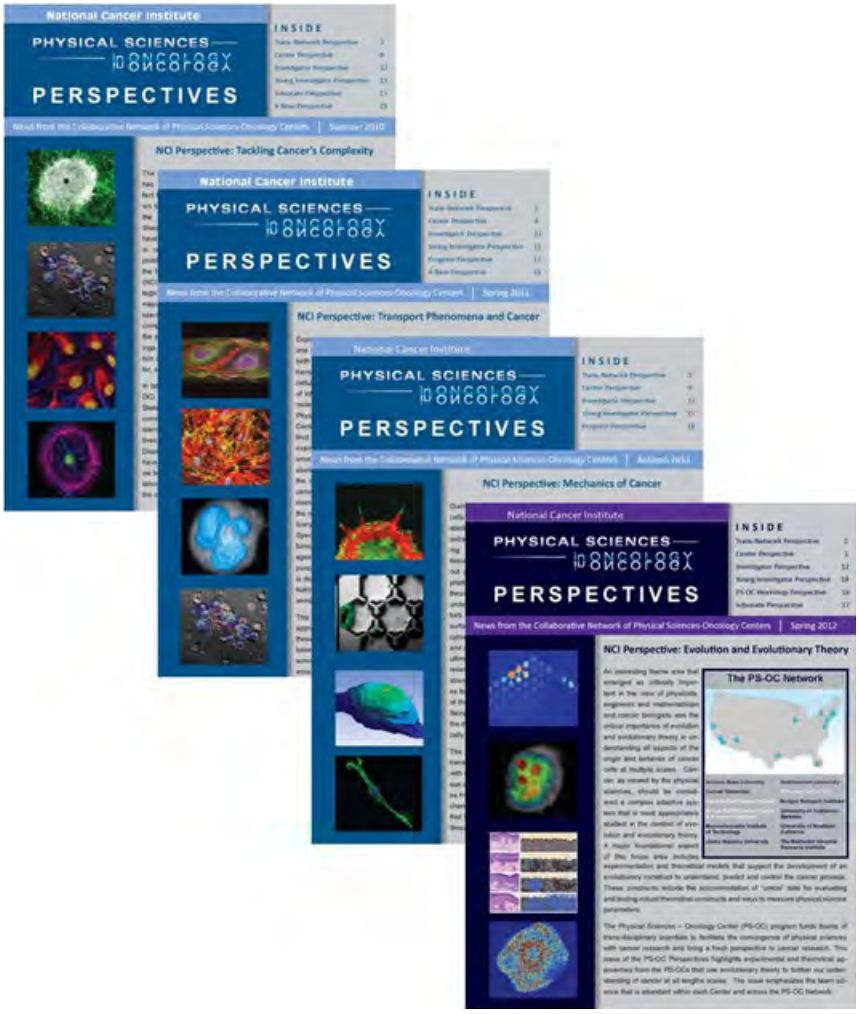
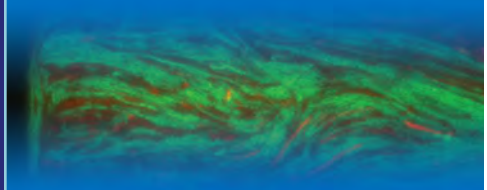


Figure 7.6. PS-OC Perspectives newsletter.

One effective way of disseminating the goals and highlighting the accomplishments of the PS-OC Network has proved to be the bi-annual newsletter *Perspectives*. A collaborative production by the members of the PS-OC Outreach and Dissemination Working Group, *Perspectives* is designed to be accessible to scientists from various disciplines and interested members of the general public.



workshop on Translational Oncophysics. The workshop itself was a direct extension of previous collaborative activities between the Cornell PS-OC and TMHRI PS-OC Outreach and Dissemination Units, including Cornell's hosting of TMHRI PS-OC PI Mauro Ferrari as a seminar speaker and the joint Translational Cancer Research Symposium. The Translational Oncophysics Workshop has in turn fostered new collaborations between the two Centers. For example, Mingming Wu of the Cornell PS-OC has established collaboration with Melissa Landis and Jenny Chang from the TMHRI PS-OC to investigate the motility and chemotaxis of tumor initiating cells using a microfluidic model developed at Cornell. Additionally, Cornell PS-OC undergraduate student, Beatriz Ascencio, is working with Melissa Landis at the Methodist Cancer Center throughout the summer of 2012. Without the PS-OC Network or the Outreach and Dissemination Units therein, natural opportunities for collaboration such as these would not be available.

Table 7.2. Members of the PS-OC Outreach and Dissemination Working Group

PS-OC	Science Outreach and Dissemination Working Group Members
ASU	Pauline Davies
Cornell	Teresa Porri
DFCI	Sara Payton
JHU	Mary Spiro
MIT	Lori Spindler
Moffitt	Robert Gillies
Northwestern	Benette Phillips
Northwestern	Michael G. Sara
Princeton	Melissa R. Aranzamendez
Scripps	Elvia Nunez
Scripps	Katya Kadyshevskaya
TMHRI	Jason Sakamoto
TMHRI	Nina Neil
UCB	Hope Rugo
UCB	Saheli Datta
UCB	Susan Samson
USC	Parag Mallick
USC	Yvonne Suarez

7.3. PS-OC Investigators in the Media/Conferences

PS-OC investigators are reaching out to the broader research community and the general public through interactions with the media. In the first three years, there have been more than 25 articles, radio broadcasts, television broadcasts, or books that highlight PS-OC investigators and the PS-OC Program (Figure 7.7). Additionally, five PS-OC investigators have presented their ideas to the broader community and public at Technology, Entertainment, Design (TED) conferences highlighting "ideas worth spreading." (Figure 7.8) PS-OC research is also being disseminated widely through the respective University and Institute websites, newsletters, and press releases. A complete list of the PS-OC interactions with the media is listed in the Appendix. A selected list of media highlights is below.

Selected News Articles on PS-OC Investigators

Valerie Weaver (UCSF, UCB PS-OC)
"Overcoming Cancer's Stiff Resistance"
Journal of Cell Biology (May 2011)

Peter Kuhn (Scripps PS-OC)
"Circulating Tumor Cells: The Ultimate Assay?"
Journal of Clinical Oncology News (January 2012)

Cynthia Reinhart-King (Cornell PS-OC)
"Let's Get Physical: Mechanical Forces Drive a New Field of Study"
Nature Medicine (March 2011)

Paul Davies (ASU PS-OC)
"Physics Meets Cancer: The Disruptor"
Nature (June 2011)

"Physics Could Help Fight Cancer"
Scientific American Magazine (April 2011)

Mina Bissell (LBL, UCB PS-OC)
Integrative Biology Journal Issue in Honor of Mina Bissell (April 2011)

PS-OC Investigators Presenting at TED Conferences

David Agus, USC PS-OC
TEDMED 2009 "Can a computer analyze proteins as well as the human body?"

TEDMED 2010 "What do proteomics have to do with cancer survival?"

TEDMED 2011 "Can we beat cancer by redefining it?"

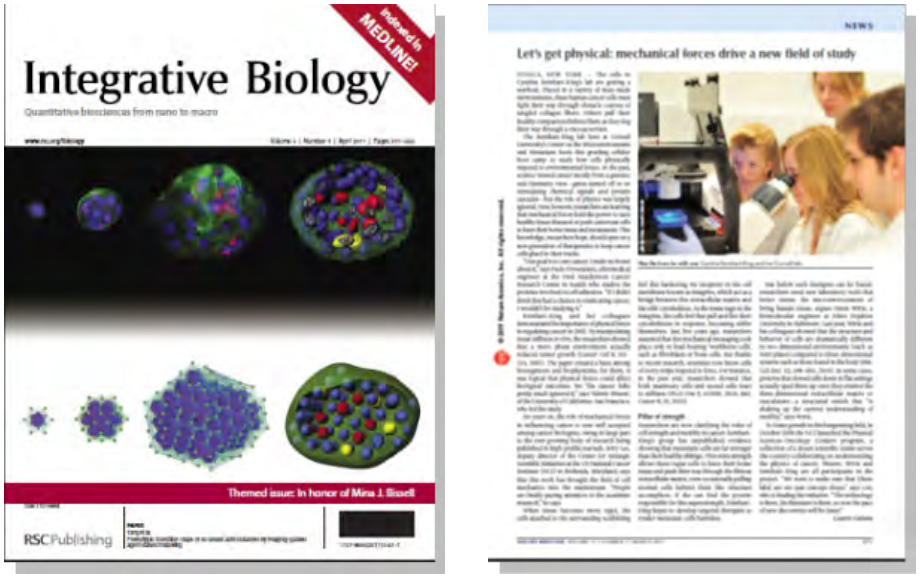


Figure 7.7. Images of PS-OC publications and commentaries.

PS-OC Investigators at Technology, Entertainment, Design (TED) Conferences:

David Agus, USC PS-OC
TEDMED 2009 "Can a computer analyze proteins as well as the human body?"

TEDMED 2010 "What do proteomics have to do with cancer survival?"

TEDMED 2011 "Can we beat cancer by redefining it?"

Mina Bissell, UCB PS-OC
TEDGlobal 2012 "Taking a new look at cancer"

Danny Hillis, USC PS-OC
TEDMED 2010 "What's behind my curtain"

Franziska Michor, DFCI PS-OC
TEDMED 2012 "When does 2+2 = life?"

Jacob Scott, Moffitt PS-OC
TEDMED 2012 "Can we stop the imaginectomies?"

Mina Bissell, LBNL, UCB PS-OC
TEDGlobal 2012 "Taking a new look at cancer"

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Franziska Michor, DFCI PS-OC
TEDMED 2012 "When does 2+2 = life?"

Jacob Scott, Moffitt PS-OC
TEDMED 2012 "Can we stop the imaginectomies?"

Figure 7.8. PS-OC investigators speaking at TED conferences. (top) Mina Bissell (bottom, left to right) Franziska Michor, Jacob Scott.

7.4. OPSO Outreach Activities

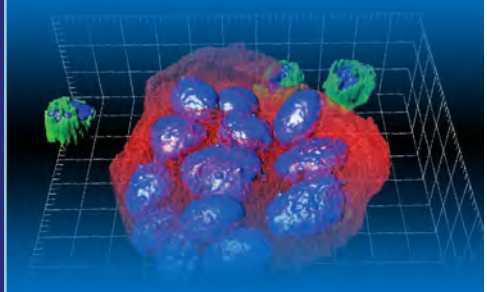
7.4.1 OPSO Website

The OPSO website serves as the public's primary information source on the work of the Office in promoting the convergence between the physical sciences and cancer biology and oncology in general and the PS-OC Program specifically. The site is organized around tabs that provide general information into the mission and goals of the OPSO and the PS-OC Program, descriptions of research conducted by the 12 Centers and links to each Center's own website, funding opportunities for research at the interface between the life and physical sciences, news on upcoming events and the latest research from investigators inside and outside of the PS-OC Network, and, finally, reports from past workshops. The pages most often viewed are those relating to general information about the Program, the individual Centers, funding opportunities, and information on OPSO program officials. The website attracts approximately 15,000 visitors each year; of these, 35 percent are returning visitors, while 65 percent are new. Since the initiation of the website, there have been users from 131 countries, and the site has been used to highlight more than 150 publications, 72 funding opportunities, and 46 events or news stories relevant to the mission of the OPSO (Figure 7.9). Traffic to the website is on course for an almost 10 percent increase in 2012. Soon, the website will also start to facilitate the ability of visitors to recommend the site to their contacts on social media.

Starting in 2013, the website will also be used increasingly to coordinate team efforts and data sharing within the PS-OC Network. One section, for example, will be dedicated to providing information on the PS-OC Bioresource Core Facility (PBCF). The PBCF provides PS-OC Network researchers with a panel of authenticated and characterized cell lines through American Type Culture Collection (ATCC), as well as DNA, RNA, and protein isolates. This section will have links to key protocols, information on the cell lines, and a link to the ATCC for ordering information. Additionally, the website will be used as a portal to disseminate information for collaborations within the Network via the PS-OC Data Coordinating Center (DCC).

Figure 7.9. Source: Google Analytics. *Last year is the period from May 25, 2011, to May 24 2012. Countries in the map showing countries of visitors are shaded according to the fraction of new visitors (dark green = more new visitors, light green = more returning visitors).

"The OPSO website serves as the public's primary information source on the work of the Office in promoting the convergence between the physical sciences and cancer biology and oncology in general and the PS-OC Program specifically."



Finally, in order to further facilitate the dissemination of information, ideas and events throughout and beyond the PS-OC Network, OPSO created a LinkedIn group and Twitter account in July 2012.

7.4.2 OPSO Publications and Collections

Merging physical sciences with cancer research is an emerging field of which neither the broader cancer nor physical sciences communities are fully aware. As an effort to inform these two scientific communities about this important new endeavor and share some of the early success of the PS-OC Network, the OPSO has been coordinating the development of special issue publications in a range of prominent scientific journals aimed at either specific fields or the broader scientific community. These special issues and collections include *Nature Physics Insight, Nature Reprint Collection, Nature Outlook, Physical Biology, Molecular Pharmaceutics,* and *Cellular and Molecular Bioengineering*.

Nature Physics Insight 2010 and Nature Reprint Collection 2011

In 2010, the OPSO partnered with *Nature Physics* to highlight how using quantitative and material-characterization approaches and physical science perspectives are emerging as useful approaches to biological problems. The *Nature Physics Insight – Physics and the Cell* special issue contained commissioned commentaries and reviews addressing these topics. The content focused on how looking at cells from a mechanistic perspective can provide insight into their behavior and function that is not available through more empirical approaches. The issue also included an overview of the PS-OC Program touching on some of the goals and early work of the Network. This special issue was released in October 2010, one year into the PS-OC Program, and served as an introduction of the Program to the broader physics and physical sciences communities.

Following the success of the *Nature Physics Insight* issue, the OPSO partnered with *Nature* in 2011 to develop a reprint collection highlighting some of the key work done over the first two years of the PS-OC Program. The *Nature Reprint Collection – Physical Sciences in Oncology* included both reviews and research articles published by PS-OC investigators in the *Nature* family of journals (Figure 7.10). The review by Michor and colleagues explore examples of how physical science principles have been used to address each of the themes of the PS-OC Program. Wirtz and colleagues provide a more in depth look at the role of motility, physical forces, and mechanics during metastasis while Lambert and colleagues and Gatenby and colleagues explore perspectives on the role of evolution in the development and progression of cancer. Subhajyoti De and Franziska Michor show that evolution of the cancer genome is not solely the result of random genomic instability, but is governed by the very structure of DNA and Shankar Mukherji and demonstrate a radically different view of how microRNAs control gene expression in a non-linear fashion that varies from cell to cell. These articles highlight some of the early accomplishments of the PS-OC Program and demonstrate that when brought to the crossroads of cancer biology and oncology, the physical sciences

perspectives will yield novel insights in cancer research with promise in therapy, diagnosis, and prevention.

Other Publications and Editorials

OPSO scientific staff often write editorials and commentaries for journals and special issues to highlight the accomplishments and aims of the PS-OC Program. In 2010, OPSO program officials contributed an editorial to *Cellular and Molecular Bioengineering* highlighting some of the tools and methodologies engineers from the PS-OC Program are using to understand tumor initiation, progression, and metastasis. In 2011, JHU PS-OC PI Denis Wirtz guest edited the *Physical Oncology* special issue for the journal *Physical Biology*. This issue contained 17 research papers from investigators inside and outside of the PS-OC Network that examine various aspects of cancer from a physical sciences perspective. This special issue contains a preface article written by OPSO program officials that highlights the complexity of cancer, the different approaches employed to simplify and understand this system, and the importance of bringing physical sciences perspective to cancer research. As described in the article, powerful temporal and spatial computational physics-based models are needed to enable a comprehensive integration of large datasets measured at different lengths and time scales to detect and more closely follow multiple emergent trends.

Also in 2011, the Evolution of Drug Resistance Working Group organized a special issue of *Molecular Pharmaceutics* (see section 5.4.1 for more details). Members of the OPSO scientific staff contributed an editorial to this issue detailing several examples of the research being done within the PS-OC Network that employs a physics perspective of evolutionary theory to understand the development of drug resistance. The OPSO will continue to reach out to the broader scientific community through contributions of editorials and commentaries to share the accomplishments and promise of the PS-OC Program.

Moore, N.M., A.M. Calcagno, S.E. Hanlon, L.A. Nagahara, and N.Z. Kuhn. Physical Sciences-Oncology Centers: Bridging Engineers and Oncologists for a New Perspective on Fighting Cancer; *Cellular and Molecular Bioengineering* **3**(4), Dec 2010

Moore, N.M., N.Z. Kuhn, S.E. Hanlon, J.S. Lee and L.A. Nagahara, De-convoluting cancer's complexity: using a 'physical sciences lens' to provide a different (clearer) perspective of cancer. Preface. *Journal/Phys Biol,* 2011. **8**(1): 010302

Kuhn, N.Z., L.A. Nagahara. *A Physical Sciences Perspective of the Evolution of Drug Resistance in Cancer. Molecular Pharmaceutics* 8, Dec 5, 2011

Figure 7.10. Images of the *Nature* covers for Physics Insight and Reprint Collection.

OPSO Publications and Special Issues Highlighting PS-OC Promise and Accomplishments

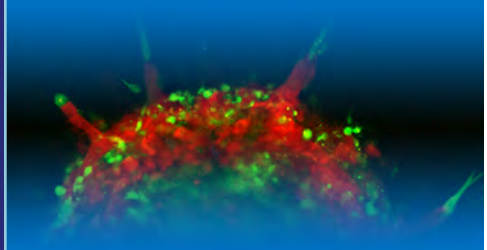
Nature Physics Insights - Physics and the Cell (2010)

Cellular and Molecular Bioengineering, Editorial (2010)

Physical Oncology special issue of *Physical Biology*, Preface (2011)

Molecular Pharmaceutics special issue, Editorial (2011)

Nature Collections - Physical Sciences in Oncology (2011)



7.4.3 Scientific Conferences

Over the first three years of the PS-OC Program, OPSO program officials in collaboration with PS-OC investigators have organized 26 conferences, symposiums, or workshops related to the field of physical sciences in oncology at various scientific meetings. These sessions have been integrated into 10 different scientific society meetings, ranging in diversity from the Electrochemical Society Meeting (ECM) to the American Society of Cell Biology (ASCB). Each session typically highlights a series of invited or contributed presentations from inside and outside the PS-OC Network in a thematic area related to the PS-OC Program. The interest in these sessions has grown with the maturation of the PS-OC Program, and many of these themes are becoming more permanent topic areas at these scientific meetings. A select list of these sessions is presented below and a full list can be found in Appendix.

OPSO program officials, generally the OPSO Director Dr. Nagahara, have also disseminated information about the Program through invited presentations at scientific conferences and workshops. To date, the office has given 38 invited talks related to the PS-OC Program. A select list of these invited presentations is provided below, and a full list can be found in the Appendix.

Selected International Conferences, Symposiums, and Workshops Organized by the OPSO

1. "The Physics and Engineering of Cancer Cells and Their Microenvironment," The Biomedical Engineering Society (BMES) Annual Meeting, October 9, 2010, Austin, TX
2. "Symposium MM: Nanofunctional Materials, Structures, and Devices for Biomedical Applications - II," 2010 Materials Research Society (MRS) Fall Meeting, November 30 - December 2, 2010, Boston, MA
3. "Symposium J6: Sensor Based Fluorescence, SERS, SPR, and Photoelectrochemistry," 220th Electrochemical Society (ECS) Meeting, October 9-14, 2011, Boston, MA
4. "Special Interest Subgroup: 3D Architecture: From Genome to Tissue and Back," The American Society for Cell Biology (ASCB) Annual Meeting, December 3, 2011, Denver, CO
5. "High Content Biophysical Data for Dynamic Studies in Cancer," American Physical Society (APS) March Meeting, February 27-March 2, 2012, Boston, MA
6. "Surface Chemistry in Oncology," 243rd American Chemical Society (ACS) National Meeting, March 25-29, 2012, San Diego, CA
7. "MS3: Converging Clinical Oncology with Physical Sciences Based Mathematical Modeling," 2012 Society of Industrial and Applied Mathematics (SIAM) Conference on the Life Sciences, August 7-10, 2012, San Diego, CA

Selected Invited Talks by OPSO Scientific Staff

1. "Convergence of Physical and Life Sciences Perspectives: Cell Mechanics and Cancer," American Society of Mechanical Engineers (ASME) 2010 International Mechanical Engineering Congress & Exposition, November 12-18, 2010, Vancouver, Canada

2. "The PS-OC Network: Unique Elements and Evaluation," Science of Team Science Annual Conference, April 14-15, 2011, Chicago, IL

3. "Biomedical Innovation & Health Policy: China – US Computational Physics and Cancer," National Academies of Sciences Biomedical Innovation and Health Policy in China and the United States Innovation Summit, June 30, 2011, Peking University, Beijing, China

4. "Convergence: The Death of Disciplinary Science?" Partnering for Cures, November 6-8, 2011, New York, NY

5. "Blending Materials Science, Nanotechnology, and Oncology through a Physical Sciences Perspective," 2011 Fall Materials Research Society Meeting, November 28 - December 2, 2011, Boston, MA

6. "Unconventional Innovative Approaches in Oncology: Physical Sciences Perspectives," National Academies of Sciences: Building the Illinois Innovation Economy, June 28-29, 2012, Evanston, IL

7. "Turning Cancer on its Side: Unconventional Approaches in Physical Sciences – Oncology Centers (PS-OC Network)," Physics and Mathematics of Cancer, July 10, 2012, Kavli Institute for Theoretical Physics (KITP), UC Santa Barbara, CA

7.4.4 NIH Symposiums

Dr. Leroy Hood, "Systems Approaches to Medicine and Cancer"

Figure 7.11. Dr. Leroy Hood Presenting at the NIH symposium on November 2, 2010.

The OPSO sponsored a seminar by Leroy Hood, Co-founder of the Institute for Systems Biology and inventor of the DNA sequencer, addressing the application of systems approaches to cancer research, interpretation of signal versus noise, the use of single cell measurements, data integration, and the current and future states of systems biology (Figures 7.11 and 7.12). Hood's seminar addressed three main topics: systems biology, emerging technology, and the concept of "P4 medicine." He is pioneering the idea that the development of the first two topics, systems approach to disease and emerging technologies and powerful new computational and mathematical tools, will move medicine from its current reactive mode to a predictive, preventive, personalized, and participatory mode (P4 medicine) over the next 5 to 20 years.

"The real grand challenge for all of science and technology is complexity" he said, highlighting one of the four themes from the PS-OC Program, "How do we deal with it?"

Hood noted that systems biology seeks "a holistic, global view toward analyzing data," and indicated a need to embrace a new transdisciplinary culture in which engineering, mathematics, physics, and computer science are as essential as biology and chemistry. "We've got to learn each other's languages," Hood said, and make team science the new norm.

"I have infinite faith that a deep understanding of biology can deconvolute the complexity we are encountering," Hood said. "As a wise man once observed, you have to go through complexity to get to simplicity."

"The real grand challenge for all of science and technology is complexity" Dr. Hood said, highlighting one of the four themes from the PS-OC Program. "How do we deal with it?"

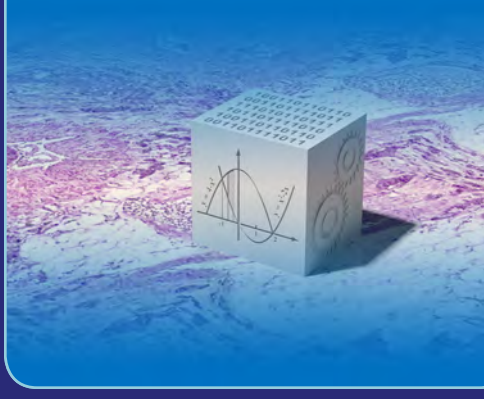


Figure 7.12. Poster advertising the Dr. Leroy Hood symposium on November 2, 2010.

The seminar was well received and attended by more than 100 staff members from across the NIH, including both intramural investigators and extramural program staff. A front page feature on the seminar was included in the November 26, 2010 issue of the NIH Record (http://nihrecord.od.nih.gov/newsletters/2010/11_26_2010/story1.htm).

7.5. Intra-NCI Activities

7.5.1 High Content Screening Approaches for Advancing Biospecimen Research

The OPSO has teamed with the NCI Biorepositories and Biospecimens Research Branch (BBRB; previously OBBR) to explore new approaches in biospecimen science using high content screening (HCS) techniques. HCS techniques are unique in that they bridge the gap between depth and throughput of biological experiments allowing both the capture of large quantities of data and multiple types of data in a single assay. HCS techniques yield rich datasets that have the potential to contribute important information for understanding the impact of cancer biospecimen collection, handling, and processing (CHP) variables on analytical outcomes. By using thoroughly annotated biospecimens, HCS techniques can be used to characterize and evaluate the impact of altering select biospecimen CHP variables on analytical outcomes. This newly formed joint effort is expected to provide new knowledge in biospecimen research that has the potential to establish best practices and define new standards for cancer biospecimen analysis by cutting edge HCS techniques.

The first joint BBRB/OPSO project focused on using HCS methods to evaluate the impact of CHP variables on the analytical outcomes of circulating tumor cells (CTCs). The identification and measurement of CTCs in peripheral blood presents a major challenge due to the low number of CTCs relative to the other cells in the blood and the heterogeneity of CTCs between patients and tumor types. The impact of alterations in CHP variables on the analysis of CTC count and quality has been difficult to assess due to limited non-HCS technologies. HCS techniques developed to identify CTCs provide additional parameters to analyze such as morphology, nuclear size, number and size of cell clusters, microtubule configuration, surface antigen concentration, and proteomic and genomic information. These additional variables available through HCS approaches can be used to assess CTC quality in response to altered CHP variables. As the development and use of HCS techniques for CTC analysis becomes more common, there is also a need for development of standard operating procedures for blood CHP. Little is known about how these pre-analytical variables impact analytical outcomes from HCS analysis of CTCs. A Request for Proposals (RFPs) was recently released to explore the impact of two CHP variables on the analysis of patient CTCs using an HCS method.

As the collaboration between the BBRB and the OPSO continues, more RFPs will be released targeting complex biospecimen science challenges that may be resolved with high content screening methods.

7.5.2 OPSO Collaborations with NCI Office of Science Planning Assessment (OSPA)

The OPSO has interacted on a regular basis with the NCI Office of Science Planning and Assessment (OSPA) to build the resources necessary for a prospective evaluation of the PS-OC Network and to subsequently disseminate information about the PS-OC Program evaluation to other NCI offices. In 2010, the OPSO was granted NIH evaluation set-aside funds through OSPA to perform a prospective study of PS-OC performance through the analysis of data collected from PS-OC Program progress reports. The initiation of this evaluation program has led to increased interest from other programs at NCI and other NIH institutes. The OSPA invited members of the OPSO to present at the trans-NCI evaluation special interest group to disseminate information about the prospective evaluation to interested programs. As a result of this presentation, several NCI programs are designing templates for prospective program evaluation and are applying for NIH evaluation set-aside funds to support similar evaluation projects.

Presentation at the Trans-NCI Evaluation Special Interest Group (SIG)

December 13, 2011

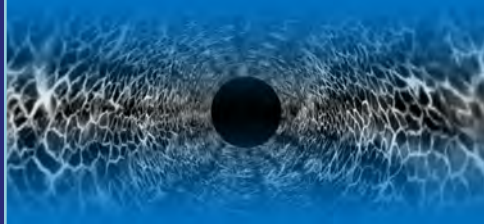
"A Prospective Evaluation of Transdisciplinary Collaborations and Scientific Progress in the NCI's Physical Sciences – Oncology Center Program" - Nicole M. Moore, OPSO

7.5.3 Diversifying the Physics-Cancer Research Community and Addressing Cancer Health Disparities

The OPSO, through active collaborations with the NCI Center to Reduce Cancer Health Disparities (CRCHD) and the use of PS-OC Education and Training and Pilot Project funds, has been actively engaged in promoting a diverse research community at the intersection of physics and cancer and utilizing physical science perspectives to investigate cancer health disparities.

The PS-OC Network has utilized the "Research Supplements to Promote Diversity in Health-Related Research" (Diversity Supplements) supported by CRCHD that aim to improve the diversity of the research workforce by supporting and recruiting students, post-doctoral fellows, and eligible investigators from groups that have been shown to be underrepresented in health-related research. The Northwestern PS-OC utilized a CRCHD Diversity Supplement to bring a physics post-doctoral fellow into the cancer research field to work on a PS-OC project modeling physical properties encoded within DNA. Support for this post-doctoral fellow was recently renewed for another two years. Additionally the JHU PS-OC, Scripps PS-OC, and UCB PS-OC have each recently been awarded a CRCHD Diversity Supplement, contributing to the training of a diverse research team at the physics-cancer interface. The Northwestern PS-OC, which has served as a leader in this area, has allocated a portion of its own Education and Training resources to promoting research opportunities for underserved trainees. Each summer, the Northwestern PS-OC brings undergraduate

"The OPSO, through active collaborations with the NCI Center to Reduce Cancer Health Disparities (CRCHD) and the use of PS-OC Education and Training and Pilot Project funds, has been actively engaged in promoting a diverse research community at the intersection of physics and cancer and utilizing physical science perspectives to investigate cancer health disparities."



students from underrepresented groups into Center labs for eight weeks to work on PS-OC projects (see section 7.1 for more information). This opportunity exposes students to new research fields and immerses them in a transdisciplinary research environment. Together, these opportunities help to diversify the research community, provide a new personal perspective that may lead to asking new questions, and train a new generation of transdisciplinary researchers.

The PS-OC Network has also invested funds in applying a physical sciences perspective to understanding cancer health disparities. Most notable is a Center Pilot Project funded by UCB PS-OC entitled "Investigating clinically silent breast cancer in African American women." The aim of this Pilot Project is to investigate the biological and biophysical properties that determine whether a breast cancer in young African American women forms a palpable mass or remains clinically "silent." The long-term goal of these studies is to develop biophysical tools and imaging strategies for early detection of clinically "silent" (non-palpable) breast cancer in premenopausal African American women.

The OPSO has maintained an active collaboration with the CRCHD as a part of these efforts. OPSO program officials provide reviews of the diversity supplement applications and how these will impact the PS-OC research program to help CRCHD make funding decisions. The CRCHD and the OPSO have an ongoing dialogue, with OPSO program officials and PS-OC investigators providing regular updates to the CRCHD about PS-OC projects and funds that are being used to promote diversity in the research workforce and address disparities issues. The conversation between the OPSO and the CRCHD helps focus the OPSO's efforts in these areas and facilitates integrating advice from experts in the field.

7.6. Inter-Agency Activities

7.6.1 NCI/NSF "PLIER" — Physical/Life Sciences Early Research Awards Program

An interagency partnership was formed between the NCI and the National Science Foundation (NSF) to promote continued investment in research at the intersection of physical sciences, engineering, and oncology. In recent years, several NSF workshops, including "The Cell as a Machine," "Cell and Molecular Biomechanics," and "Physics of Cancer Metastasis," have highlighted emerging and promising opportunities for collaborative research at the intersection of the physical/engineering sciences and the life sciences. The joint interest of the NSF and the NCI in the converging field of physical and life sciences sparked discussions leading to the Physical/Life Sciences Early Research (PLIER) award program. PLIER serves to promote the trans-disciplinary research being cultivated within the PS-OC Program by seeding new innovative, individual investigator-led Pilot Projects using NSF and NCI funds. The goal of the partnership fits ideally with the OPSO mission to support innovative concepts from potential "new" NCI grantees more likely to apply to the NSF for support.

Table 7.3. Proposals Funded by 2010-2011 PLIER Award Program

PI Last Name	Institution	Title
Betenbaugh	Johns Hopkins University	Collaborative Research: Engineering Approaches to Cancer Metabolism to Interpret and Develop Improved Treatment Modalities
Shuler	Cornell University	Targeting Cancer Stem Cell Self-Renewal and Proliferation Mechanisms Using In Vitro Microscale Models
Champion	GA Tech Res Corp - GIT	Engineering Effector Protein Nanoclusters for Breast Cancer Therapy
Burdick	Ohio University	Cancer Stem Cell Phenotyping: Establishing Correlations and Regulatory Crosstalk Between Molecular Markers and Mechanical/Rheological Properties
Bleris	U of Texas Dallas	Detecting Cancer at the Single-Cell Level Using Endogenous Signal Biomolecular Sensors
Bonin	Wake Forest University	Cell Mechanics and Protein Mobility during Neoplastic Transformation

PLIER Award Program (2010-2011)

In 2010, a NSF Dear Colleague letter was issued, requesting proposals on innovative transdisciplinary research at the intersection of physical sciences, engineering, and oncology. In response to the PLIER program call, 54 proposals were received by 3 different divisions at the NSF (Division of Civil, Mechanical, and Manufacturing Innovation [CMMI], Division of Chemical, Bioengineering, and Technology [CBET], Division of Materials Research [DMR]). Of the 54 proposals, 62 percent were submitted by investigators considered to be new or early-stage NIH investigators. The proposals were diverse, but several themes were prevalent across multiple proposals. These included (1) biosensors and tools for understanding the role of mechanical properties in metastasis; (2) novel transport systems for understanding therapeutic delivery to tumors; and (3) computational simulations and devices to understand subcellular/cellular dynamics. By NSF procedures, all 54 proposals were reviewed by a special panel composed of physical scientists, cancer biologists, and clinicians. Based on suggestions from the review panel, the NSF funded six projects for three years at $175,000 per year on average (Table 7.3). NCI funds were matched three-fold by NSF divisions, for a combined total investment of $2.7 million to support these projects.

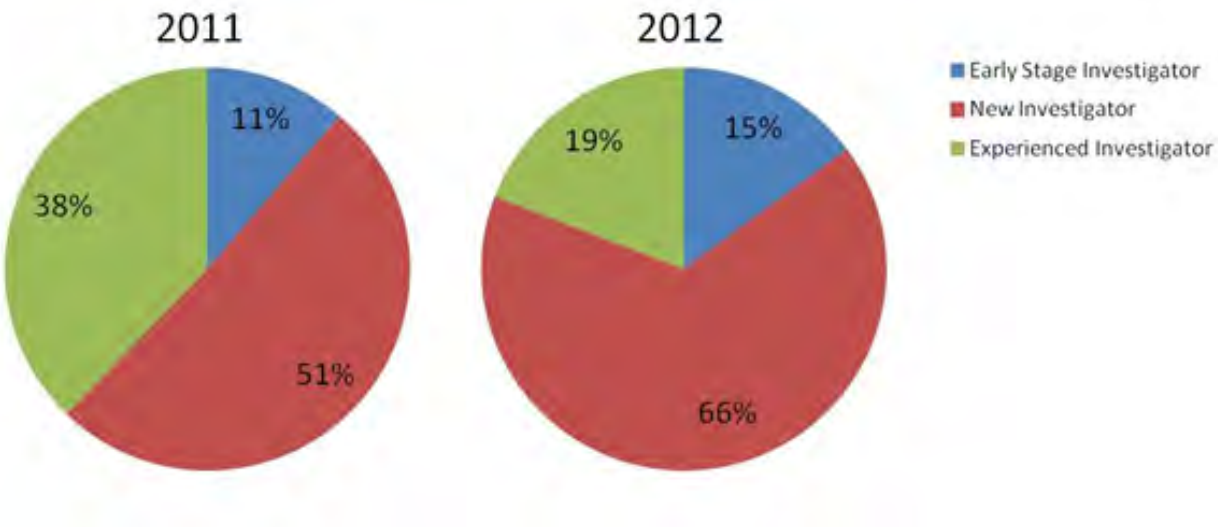
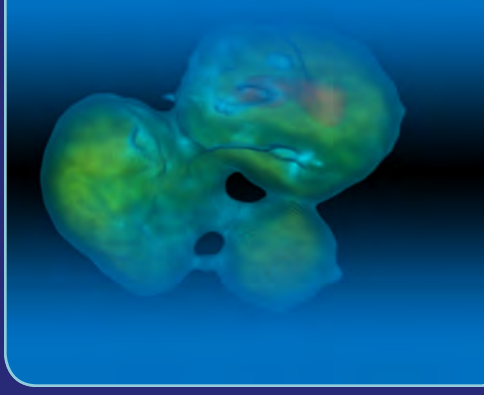


Figure 7.13. Breakdown of investigators that submitted proposals to the PLIER award program by NIH experience level.

"The joint interest of the NSF and the NCI in the converging field of physical and life sciences sparked discussions leading to the Physical/Life Sciences Early Research (PLIER) award program. PLIER serves to promote the trans-disciplinary research being cultivated within the PS-OC Program by seeding new innovative, individual investigator-led pilot projects using NSF and NCI funds."



The proposals funded by the first year of the PLIER award program met all of the objectives set forth by the NCI and the NSF. The program resulted in the support of four new investigators in the area of biomedical research and three early-stage investigators, representing both engineering and physics departments. The proposals aim to apply physical science and engineering approaches and perspectives, such as atomic force microscopy, synthetic fluorescent sensors, microfluidic platforms, mathematical simulations, and nanoparticle fabrication, toward advancing the fundamental understanding of cancer biology and clinical oncology promoting the prevention, detection, and treatment of cancer diseases.

PLIER Award Program (2011-2012)

Because of the success of the first year of the PLIER award program, the NSF and the NCI partnered again to release a program announcement requesting applications for Physical Sciences and Engineering in Oncology (PESO). The announcement was released in November 2011, and 96 proposals, a 78 percent increase from the first year of the program, were received by 3 different divisions at the NSF (CMMI, CBET, and DMR). More than 80 percent of these proposals were submitted by new or early-stage NIH investigators and represented a diverse background of training. Interestingly, a large portion of the proposals aimed to understand cancer initiation, progression, and treatment with biomaterial interfaces and tissue engineering approaches (Figure 7.13). Another major theme included the development of predictive modeling tools and biosensors.

Based on suggestions from a diverse scientific panel of investigators, the NSF will fund six proposals with $3.3 million joint NSF and NCI funds. NCI funds were matched almost four to one as a result of the enthusiasm for these proposals by the NSF. The funded proposals represent diverse topic areas, including biomaterials, biosensors, and computational simulations. Half of these funded proposals are led by early-stage investigators new to cancer research. Additionally, one funded proposal stemmed from collaborations on a PS-OC Pilot Project of a new NIH investigator with a cancer biologist representing a successful outcome of the PS-OC Pilot Project Program.

Summary

Overall, the collaborative NCI/NSF PLIER award program is supporting 12 highly meritorious proposals at the intersection of physical sciences, engineering, and oncology to further scientists' understanding of cancer. In line with the mission of the OPSO, the PLIER award program appealed to new investigators from the physical sciences transitioning to cancer research and will fund seven proposals from new or early-stage investigators. The program is anticipating a third year due to increased interest from the research community and continued interest from both the NSF divisions and the OPSO in supporting new innovative ideas in this area.

7.6.2 World Wide Evaluation Studies Final Reports

Stem Cell Engineering

In 2011, the OPSO partnered with the NSF's Biomedical Engineering Program to support a global assessment of research in the area of stem cell engineering (SCE). SCE is a field at the intersection of engineering and biology that is accelerating progress toward innovative solutions to basic and translation problems in regenerative medicine. Research topics in SCE emphasize how quantitative and physical sciences approaches can yield an increased understanding of the biological mechanisms that underlie stem cell fate choices, cancer stem cells, induced pluripotent stem (iPS) cells, technologies to study stem cell function, and the development of bioprocesses to culture stem cells for commercial applications. Similar physical sciences approaches and tools are applicable to understanding questions in cancer biology and are the main focus of the PS-OC Program.

The assessment used the World Technology Evaluation Center's (WTEC's) methodology of employing an expert panel to conduct site visits to overseas laboratories where the best work in SCE is done. The expert panel structured its assessment into four key areas in the SCE field:

- Physical and Engineering Principles in Stem Cell Research
- High-Throughput Screening, Mircofluidics, and Real-time Phenotyping
- Computational Stem Cell Engineering
- Bioprocessing and Biomanufacturing

The findings of this study as well as the expert panel's knowledge of the field were presented in a public workshop on May 24, 2012, at the NSF. The presentations are available online (http://www.tvworldwide.com/events/nsf/120524), and the final report is expected in the fall of 2012. A few of the conclusions and future opportunities highlighted in the workshop and expected to be described in the final report are listed next.

Key Study Conclusions

- Physical sciences and engineering principles play major regulatory roles for stem cells.
- Tissue mechanics exerts effect that are in many situations as potent as soluble protein factors.
- Materials and technology development has played an important role in the investigation of physical science principles on stem cell biology.
- Computational modeling is a foundation to understanding complex systems such as stem cells.
- Strong mechanistic progress is often made when engineers and physical scientists collaborate with biologists.

Future Opportunities

- Analysis identifying the components of highly complex cellular systems and providing an understanding of how these work together
- In vitro systems benefiting from being engineered to be more physiologic, including organ on a chip models
- Engineered in vitro tumor models leading to a better understanding of cancer

APHELION

In 2012, the OPSO coordinated an international study with the WTEC to determine the global status of research and development at the interface of physical sciences and oncology. The study, titled Assessment of PHysical sciences and Engineering advances in LIfe sciences and ONcology (APHELION), was co-sponsored by the NCI OPSO, the NSF (Directorate of Engineering; Directorate of Mathematical & Physical Sciences), and the NIBIB. An expert panel was formed to identify leading laboratories overseas, conduct site visits, and generate a report of its findings. The initial phase of APHELION was to determine the status and trends of applying physical sciences and engineering principles to oncology research and development in leading laboratories

Key Conclusions of the WTEC Stem Cell Engineering Study

- Physical sciences and engineering principles play major regulatory roles for stem cells.
- Tissue mechanics exert effects that are in many situations as potent as soluble protein factors.
- Materials and technology development has played an important role in the investigation of physical science principles on stem cell biology.
- Computational modeling is a foundation to understanding complex systems such as stem cells.
- Strong mechanistic progress is often made when engineers and physical scientists collaborate with biologists.

and organizations in Europe via an on-site peer review process. More details on the study are available at www.wtec.org/aphelion.

The scientific panel members and the expert advisors are listed below.

Scientific Panel Members

- Paul Janmey, Ph.D. (study chair). Professor of Physiology, Physics, and Bioengineering at the Institute of Medicine and Engineering at the University of Pennsylvania
- Daniel Fletcher, Ph.D., D.Phil. Professor of Bioengineering and Biophysics at the University of California, Berkeley
- Sharon Gerecht, Ph.D. Assistant Professor of Chemical and Biomolecular Engineering at Johns Hopkins University
- Parag Mallick, Ph.D. Assistant Professor of Radiology, Bio-X Program, at the Canary Center for Cancer Early Detection, Stanford University
- Owen McCarty, Ph.D. Associate Professor of Biomedical Engineering at the Oregon Health and Science University
- Lance Munn, Ph.D. Associate Professor of Radiation Oncology at the Massachusetts General Hospital/Harvard Medical School
- Cynthia Reinhart-King, Ph.D. Assistant Professor of Biomedical Engineering at Cornell University

Expert Advisors to the Study Panel

- Antonio Tito Fojo, M.D., Ph.D. Head, Experimental Therapeutics Section Medical Oncology Branch and Affiliates, National Institutes of Health
- Denis Wirtz, Ph.D. Theophilus H. Smoot Professor, Department of Chemical and Biomolecular Engineering, Johns Hopkins University

On January 18, 2012, the sponsors/chair meeting of the WTEC APHELION study was held at the NSF headquarters in Arlington, Virginia. The main goals of the sponsors/chair meeting were to provide an overview of the plans for the study, to solicit interest and participation of other U.S. government agencies, and to coordinate the study design with WTEC. As a result of the sponsor meeting, the NCI OPSO was joined in sponsorship by the NSF Directorates of Engineering (ENG) and Mathematical & Physical Sciences (MPS) and the NIBIB.

On February 1, 2012, the kickoff meeting of the WTEC APHELION study was held at the NIH campus in Bethesda, Maryland, where the scientific panel and advisors met with the sponsors. The outcome of the kickoff meeting was the development of an outline for the APHELION report.

The following topics were selected to form the basis for each of the chapters in the report:

1. Information and complexity: New methods for dealing with the enormous datasets generated by modern imaging methods and integrating methods developed by the physics community to understand complex, non-linear systems and emergent properties that cannot be predicted by traditional biological models

2. Microenvironment: The influence of chemical composition, spatial patterning, nutrient supply, oxygen stress, and other features of the tissue and extracellular environment on the growth and homeostasis of normal tissues and tumors

3. Cell and tissue mechanics: How the forces generated by cells and the viscoelasticity of the cell and extracellular matrix affect cell growth, survival, differentiation, and movement

4. Transport: How the movement of cancer cells, nutrients, growth factors, drugs, and fluids affect cell survival and tissue mechanics. How the removal of metabolic waste products and cell debris are controlled and how they are altered in the tumor environment

5. Dynamics: How the rates and patterns of cell shape change, migration, and division can be measured, understood, and integrated with biochemical and genetic information

6. Devices and new diagnostic principles: New technologies based on physical principles, especially those in which the physical properties of tissues are exploited for cancer diagnosis or treatment

The panel members made visits to laboratories in France, Italy, Israel, Germany, the Netherlands, Spain, Sweden, and Switzerland, typically meeting with representatives of multiple institutions at each stop (Figure 7.14, Table 7.4).

Figure 7.14. Countries visited by APHELION panel members.

Selected Key Conclusions of the WTEC APHELION Study

- The interface of physical and biomedical sciences is a growth area with potential for both scientific discovery and medical applications.
- At all length scales, cancer can be identified as an emergent phenomenon that can be modeled as a complex adaptive system using computational physics approaches.
- There is a frequent need for close integration between experimentalists, modelers, and bioinformaticians, often with the need to integrate experimental results with those from larger published datasets.
- Information transfer, evolution, and complex adaptive systems research is rapidly progressing and critically important for impacting cancer and more generally understanding biology.
- Here is a need for a more complex approach for mimicking the cellular microenvironment in vitro.

On June 12, 2012, a final workshop presented by the panel members was held at the NIH campus in Bethesda, Maryland, to report the APHELION study findings and discuss these findings with the sponsors and the public. The details presented at this workshop (www.tvworldwide.com/events/nih/120612/) and the final report (http://www.wtec.org/aphelion/AphelionFinalReport09.23.12.pdf) are available online. Highlighted below are key conclusions and future directions as identified by the APHELION panel members.

Key Study Conclusions and Future Challenges and Opportunities

- The interface of physical and biomedical sciences is a growth area with potential for both scientific discovery and medical applications.
- The European funding agencies are embracing the concept of interdisciplinary science and also bringing physical sciences perspectives to cancer research and other biomedical research areas.
- A combination of top-down and bottom-up approaches by the funding agencies helps to maintain a balance that is vital for major advancements in science and technology.
- Throughout Europe, national boundaries are blurred when it comes to interdisciplinary projects, where nearly all large groups include partners from other European countries and often collaborators in North America or Asia.
- At all length scales, cancer can be identified as an emergent phenomenon that can be modeled as a complex adaptive system using computational physics approaches.
- There is a frequent need for close integration between experimentalists, modelers, and bioinformaticians, often with the need to integrate experimental results with those from larger published datasets.
- Information transfer, evolution, and complex adaptive systems research is rapidly progressing and critically important for impacting cancer and more generally understanding biology.
- The use of biologically accurate biomaterials is a growing area in Europe.
- There is a need for a more complex approach for mimicking the cellular microenvironment in vitro, which will take into account the presentation of adhesions at the microscale and how this can relate to mechanics. The generation of cue gradients in vitro such as cytokines and oxygen tension is also a necessity. Decoupling paramerters in 3D presents a major challenge and world wide advances in biomaterial synthesis are needed to enable progress.

Table 7.4. APHELION Sites Visited in Europe

Site	Host(s)	Date
FRANCE		
Institute Curie, Paris	Daniel Louvard et al.	9-May-12
University of Mons, Belgium (presented at Institute Curie, Paris)	Sylvain Gabriele	9-May-12
University of Paris Diderot	François Gallet et al.	10-May-12
GERMANY		
Max Planck Institute, Dresden	Guillaume Salbreux	11-May-12
Max Planck Institute, Gottingen	Oskar Hallatschek	11-May-12
Technical University of Munich	Andreas Bausch et al.	9-May-12
University of Heidelberg and the German Cancer Research Center	Joachim Spatz et al., and Evgeny Gladilin et al.	10-May-12
University of Leipzig	Josef Käs	11-May-12
University of Rostock	Adelinde Uhrmacher	11-May-12
University of Freiburg	Jens Timmer	10-May-12
University of Nurnberg-Erlangen	Ana Sun-ana Smith	9-May-12
ISRAEL		
Weizmann Institute	Ronen Alon et al.	14-May-12
NovoCure/Technion University	Yoram Palti	14-May-12
ITALY		
University of Padua	Nicola Elvassore et al.	11-May-12
University of Milan	Stefano Zapperi	11-May-12
European Institute of Oncology	Alberto d'Onofrio	11-May-12
NETHERLANDS		
The Hubrecht Institute, Utrecht	Johan de Rooij	7-May-12
Radboud University Nijmegen	Peter Friedl et al.	7-May-12
The University of Leiden	Helmut Schiessel	7-May-12
University Medical Center Utrecht	Philip de Groot	7-May-12
SPAIN		
University of Barcelona	Josep A. Planell et al.	7-May-12
University of Basque Country	José M.G. Vilar	7-May-12
SWEDEN		
Uppsala University	Karin Forsberg Nilsson et al.	8-May-12
The Royal Institute of Technology	Wouter van der Wijngaart et al.	8-May-12
SWITZERLAND		
École Polytechnique Fédérale de Lausanne (EPFL)	Jeffrey Hubbell et al.	8-May-12
University of Basel	Cora-Ann Schonenberger	8-May-12

Sites Visited by WTEC APHELION European Study

FRANCE
Institute Curie, Paris
University of Mons, Belgium
University of Paris Diderot

GERMANY
Max Planck Institute, Dresden
Max Planck Institute, Gottingen
Technical University of Munich
University of Heidelberg and the German Cancer Research Center
University of Leipzig
University of Rostock
University of Freiburg
University of Nurnberg-Erlangen

ISRAEL
Weizmann Institute
NovoCure/Technion University

ITALY
University of Padua
University of Milan
European Institute of Oncology

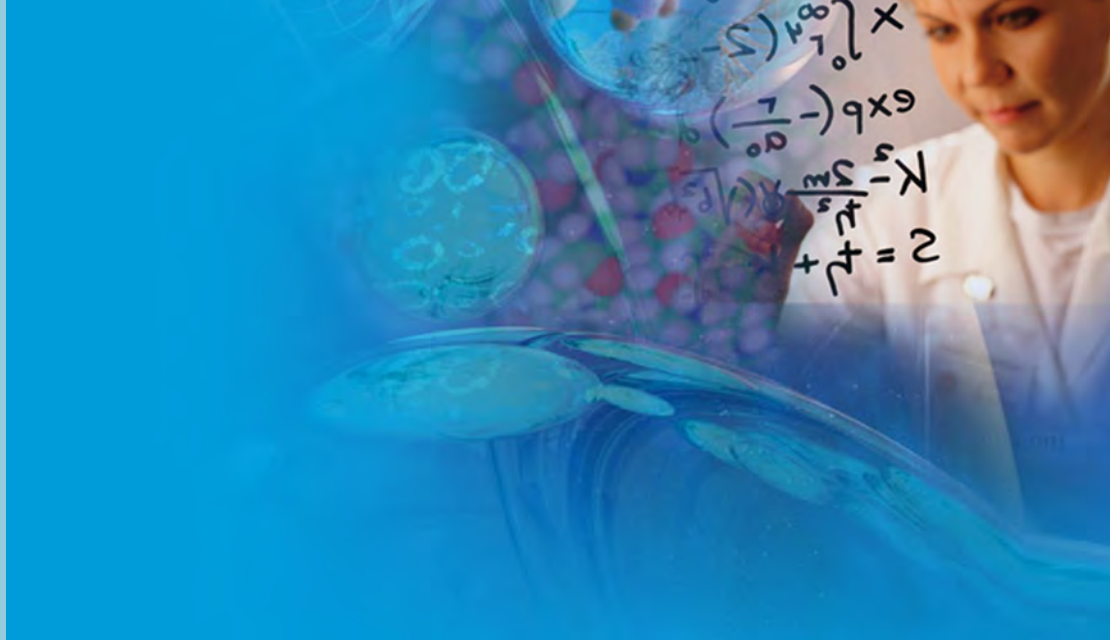
NETHERLANDS
The Hubrecht Institute, Utrecht
Radboud University Nijmegen
The University of Leiden
University Medical Center Utrecht

SPAIN
University of Barcelona
University of Basque Country

SWEDEN
Uppsala University
The Royal Institute of Technology

SWITZERLAND
École Polytechnique Fédérale de Lausanne (EPFL)
University of Basel

- Advances in miniaturization technologies will allow the high-resolution analysis of the engineered microenvironments and the resulting cellular behaviors.
- Cancer cells react with the chemical, spatial, and physical features of their tissue microenvironments.
- "Cell mechanics" is a growing field that encompasses not only cell-scale behaviors and properties, but also the mechanical forces and mechanical properties at the molecular and tissue scales.
- Multiscale contributions of subcellular structures and supracellular tissue properties cannot be overlooked when studying cell mechanics.
- Fluid dynamics in blood and lymphatic vessels in and around tumors plays an important role in disease progression and treatment as they can guide cell migration, facilitate the immune response, angiogenesis, and metastasis.
- Recent advances in microfluidics and tissue analogs can provide solutions to the historical difficulty in studying fluid mechanobiology in tissues or in vitro, helping to create a more firm foundation for others to enter into the area to identify flow-activated gene pathways and to increase the appreciation in the physical sciences for the importance of intra- and extra-vascular fluid flow in tumors.
- Tumor growth and metastasis is dependent on cancer cell dynamics, and cytoskeletal shape and tension is critical for cell movement.
- The different modes of cell migration (such as collective migration versus single-cell migration) must be understood to gain a clear understanding of tumor metastasis. Studies in three-dimensional culture systems are proving to be important as well.
- Fostering the development of novel devices and new diagnostic principles is critical for the advancement of cancer diagnosis and treatment.
- It is imperative that advanced technologies that are developed to measure physical properties of cells be relatively simple to use so that they will be adopted by multiple laboratories in the physical sciences and in biomedicine.



8. Education and Training Activities

The PS-OC Program has placed an emphasis on eyeing the future generation of young scientists with continuous access to training and education in transdisciplinary research. Hence, training a new generation of transdisciplinary scientists in the area of physical sciences in oncology was established as one of the five initial objectives of the PS-OC Program.

To facilitate the unique training of PS-OC Network students, postdocs, and investigators, each PS-OC was required to establish an Education and Training Unit that would be funded explicitly to foster the development of transdisciplinary researchers. The set-aside funds for the Education and Training Units are to establish both the development of training programs and to support student exchanges. To further foster collaborations and encourage transdisciplinary training and innovation, the PS-OC Network organized programatic activities with the coordinators of the Education and Training Units. These included the following:

- PS-OC Education and Training Working Group
- PS-OC Network Young Investigators' Meeting
- ICBP/PS-OC Joint Junior Investigators' Meeting
- PS-OC Workshops

Highlights of the accomplishments of the Education and Training Working Group, the individual Education and Training Units, and additional OPSO education programs are described below.

8.1. Education and Training Working Group

The goal of the PS-OC Education and Training Working Group is to facilitate future collaborations between physical scientists, cancer biologists, and oncologists by enhancing the cross-training of young investigators. The working group serves as a resource to help coordinate and share ideas, best practices, and insights for running the PS-OC Education and Training Units and is made up of Education and Training coordinators from each PS-OC and the OPSO program officials. The working group meets quarterly, including a face-to-face meeting at the Annual PS-OC Network Investigators' Meetings. During meetings, group members present successful programs that they have run or courses that they have developed, discuss ideas for new programs or courses, and solicit advice on addressing problems or implementing programs.

The working group has developed a strong network of PS-OC Education and Training coordinators that are beginning to share educational materials and programs across the PS-OC Network and establish plans for coordinating the development of new educational programs within the Network.

For example, at the working group meeting during the 2011 Annual PS-OC Network Investigators' Meeting, members of the Cornell PS-OC Education and Training Unit presented their "Cancer Brainstorming Club" program that brings together trainees from the physical sciences and cancer biology to discuss current relevant research issues and generate ideas and proposals for addressing emerging research questions. Over the past year, Cornell's program has expanded to include trainees from the Moffitt PS-OC, and at the 2012 Annual Investigators' Meeting, trainees from Cornell and Moffitt led a session of the "Brainstorming Club" for all interested PS-OC trainees. Furthermore, based on the presentation at the 2011 Annual Investigators' Meeting, the ASU PS-OC Education and Training Unit developed a similar program for ASU trainees. The ideas generated from the

From the beginning of the initiative, the PS-OC Program has eyed the future. Training a new generation of transdisciplinary scientists in the area of physical sciences in oncology is one of the five initial objectives of the PS-OC Program.

Education and Training activities are coordinated by the Education and Training Working Group with representatives from each Center.

- Selected Education and Outreach Activities
- PS-OC Network Young Investigators' Meeting
- Joint ICBP/PS-OC Junior Investigators' Meeting
- PS-OC Boot Camps/ Workshops
- Student Exchanges
- Internship Programs
- Development of New Courses

Cornell-Moffitt "Brainstorming Club" have led to proposal submissions to both Cornell PS-OC Young Investigator awards funded by Cornell University and the PS-OC Young Investigator Trans-Network Program.

Table 8.1. Members of the Education and Training Working Group by PS-OC.

PS-OC	Education and Training Working Group Members
ASU	Pauline Davies
Cornell	Peter Doerschuk
Cornell	Michael Shuler
DFCI	Sara Payton
JHU	Ashanti Edwards
MIT	Lori Spindler
Moffitt	Sandy Anderson
NIH/NCI	Ming Lei
NIH/NCI	LeeAnn Bailey
Northwestern	Benette Phillips
Northwestern	Michael Sara
Northwestern	Will Kazmier
Princeton	Melissa Aranzamendez
Princeton	Robert Austin
TMHRI	Amy Wright
Scripps	Anand Kolatkar
Scripps	Kelly Bethel
Scripps	Owen McCarty
UCB	Hope Rugo
UCB	Saheli Datta
USC	Yvonne Suarez

8.2. PS-OC Education and Training Activities

8.2.1 Workshops: Hands-On and Interactive Training Opportunities

A number of Centers have developed hands-on or interactive training programs as part of their PS-OC Education and Training Units. These programs provide unique immersion opportunities for trainees and other members of the PS-OC Network to experience first-hand aspects of physical sciences in oncology research that are outside of their previous experiences and expertise. Over the first three years of the PS-OC Program, 15 immersive training programs have been developed by the Network. The hands-on and interactive programs implemented by the Network represent a variety of training approaches. At the Cornell PS-OC, all Biomedical Engineering graduate students spend seven weeks at the Weill Cornell Medical College participating in a variety of activities that provide a connection to the medical implications of their research. The Scripps PS-OC developed a Pathology Boot Camp that provides Center

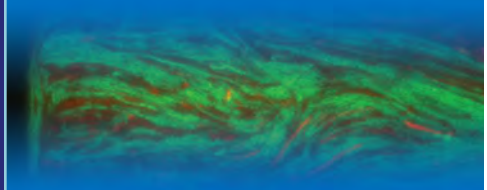
investigators with an up-close clinical perspective of cancer, allowing many participants their first view of cancer outside of an in vitro or even in silico setting. At the Princeton PS-OC, a Microfluidics Boot Camp has been implemented to provide researchers from the Center, PS-OC Network, and even the broader scientific community a way to gain hands-on experience applying state-of-the-art microfluidics approaches. Both the Northwestern and TMHRI PS-OCs have developed summer research programs that provide undergraduate and medical students with the opportunity to directly contribute to PS-OC research projects. These five example programs are detailed below.

Cornell Summer Immersion Program

Every Ph.D. student in the Biomedical Engineering graduate field, as well as selected Ph.D. students from other graduate fields, spends seven weeks at Weill Cornell Medical College, usually during the summer after their first year of graduate school. The goal of the Immersion Term is to provide each Ph.D. student with the opportunity to see first-hand how the results of engineering impact patient diagnosis and care through the entire spectrum of medical practice from the first presentation of a patient in a physician's office or clinic with a vague complaint through diagnosis, treatment, and rehabilitation. The students engage in a wide range of activities. Facilitated by individually assigned mentors, each student observes the practice of medicine follow-up in a wide range of settings from private offices to operating rooms. Each student performs a small-scale clinical research project and presents the results during a poster session. As a group, the students participate in a weekly multihour didactic session where each student presents compelling portions of activities, and the moderators present material and lead discussions of the clinical meaning underlying the activities. The Immersion Term often has unplanned but not unanticipated outcomes for the students. These outcomes include changes in thesis research directions, for example by adding work with primary tumor cells to a thesis project; addition of clinical experts to thesis committees; and new collaborations.

Figure 8.1. Participants in the Cornell Summer Immersion Program. Biomedical engineering graduate students spend the summer at Cornell Weill Medical School. Several participants each year are supported by the PS-OC Program.

"Over the first 3 years of the PS-OC Program, 15 immersive training programs have been developed by the Network."



Scripps Pathology Boot Camp

The Scripps PS-OC has developed a pathology boot camp to introduce fellows and graduate students to the process of clinical pathology. The boot camp shadows the steps of tissue collection, sample processing, fixation, staining, and imaging. The boot camp consists of a series of microscope tutorials to familiarize both biological scientists and physical scientists with the features of human cancers as they actually exist in the clinic and hospital. From demonstrations of actual biopsy needles, to whole tumor slice microanatomy, to microscopic features of tumors, their blood supply, and their cellular morphologies. For many, this is the first exposure to cancer outside of an in vitro or in silico setting. This boot camp has made a profound impact on the participants, as they were able to visualize the invasiveness and aggressive growth of cancer within tissues. Also, the participants gained an appreciation of the fact that cancer is a disease affecting a patient, a fact that was treated with respect and was moving for the participants. A shorter version of the pathology boot camp was offered to participants during the Second Annual PS-OC Investigator's meeting in La Jolla, CA (Figure 8.2).

Princeton Microfluidics Boot Camp

The goal of the Princeton PS-OC Microfluidic Core Facility is to make microfluidic and microfabrication technologies accessible for research and education. The Microfluidics Boot Camp caters to researchers and students with little or no experience in the field with the aim of lowering the threshold to make and use these microfluidic tools and approaches. The inaugural Microfluidic Boot Camps were held on August 1-5 and

Figure 8.2. Scripps SI, Dr. Kelly Bethel, leads a group at the Scripps Pathology Boot Camp.

August 8-12, 2011, at Princeton University (Figure 8.3). Sixteen participants—eight each week—from six institutions took part in the course, which was taught by Saurabh Vyawahare, Director of the Princeton University Physics Department Microfluidic Laboratory, with assistance from Megan McClean of the Lewis Sigler Institute and Temple Douglas from the Department of Electrical Engineering at Princeton. The Microfluidics Boot Camp provided a broad introduction to microfluidics with lectures and hands-on experiments. The participants had a chance to make devices from start to finish and then use their devices to perform experiments. All materials from the Boot Camp are posted online and are available to participants and the broader research community (http://www.princeton.edu/microfluidics/training/).

Participants of the course at Princeton have continued to use the microfluidics laboratory for their research. Additionally, many of the lab groups that had one or more participants in the course and have had additional researchers from the group join and use the facility.

Below are some of the comments received:

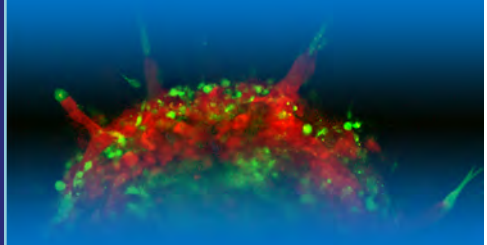
- "The course was absolutely exceptional. I cannot think of a more productive use of one week since I came to graduate school. I was giving daily updates to my lab, and they are so excited about these technologies that they have asked me to give a lab meeting summarizing what I've learned."
- "It has been a truly valuable learning experience for someone like me who comes from a computational sciences background. This workshop met and exceeded all my expectations…Many thanks for organizing this."
- "Very nice experience. Know how to make chips and the principle to use the chips. Expect more application."

Northwestern Summer Program for Undergraduates

The summer research program for Northwestern undergraduates is attracting a large and outstanding pool of applicants; 40 students applied for 4 slots in the second year of the program. The four accepted students, all with previous research experience, were placed in labs under the direction of PS-OC faculty investigators. For eight weeks, from late June through mid-August, the students conducted research related to PS-OC projects under the supervision of a graduate student or post-doctoral fellow. While the bulk of the students' time was spent gaining hands-on laboratory experience, program participants also received a broad introduction to major issues in cancer research through attendance at weekly seminars on tumor biology, held at the Robert H. Lurie Comprehensive Cancer Center in conjunction with its Continuing Umbrella of Research Experience (CURE) Program, and a half-day PS-OC workshop on cancer therapy. At the conclusion of the summer,

Figure 8.3. Students participating at the Princeton PS-OC Microfluidics Boot Camp.

"Very nice experience. Know how to make (microfluidic) chips and the principle to use the (microfluidic) chips. Expect more application."



students presented their results at a research symposium attended by approximately 25 PS-OC investigators and trainees. Both student and faculty reaction to the program was extremely positive. In their anonymous evaluations of the research experience, all participants stated they would strongly recommend it to their peers while also unanimously agreeing that it stimulated their interest in participating in future cancer research projects. Faculty members were equally enthusiastic, praising their summer trainees for their intellectual ability and work ethic. Perhaps the greatest indicator of the program's success is that two of the summer participants are currently continuing their PS-OC research and have indicated that they plan to increase their participation in these research projects as their academic careers progress.

TMHRI Summer Internship Program

Each year, TMHRI PS-OC organizes a summer internship program to provide undergraduate students the opportunity to perform hands-on research at the interface of physical sciences and oncology. For the 2012 summer internship program, the TMHRI PS-OC sponsored 10 undergraduate summer interns for the 10-week period, June 4 to August 10, 2012. The interns studied under PS-OC investigators and completed projects in the realm of oncophysics. The interns then presented their findings at the retreat that was held at the end of the summer. This opportunity provided undergraduates with a broad transdisciplinary training environment and exposed them to the emerging field of oncophysics (Figure 8.5).

8.2.2 PS-OC Course Development and Support

A second major focus of the Education and Training Units across the PS-OC Network has been the development of courses that provide structured education and training at the interface of physical sciences and cancer. To date, a total of 12 courses have been developed or supported using PS-OC Education and Training Unit funding. The courses being developed and supported by the PS-OC Network are a mix of undergraduate and graduate classes and include standard classroom, seminar-style, and laboratory courses. The ASU PS-OC has designed an undergraduate course addressing the broad theme of physical sciences and cancer. Over the first two years, more than 70 students from the ASU Barrett's Honors College have taken the course, and several of the students have gone on to research opportunities within the ASU

Figure 8.4. Students from the Northwestern PS-OC summer internship program.

PS-OC. At the UCB PS-OC, the Education and Training Unit has provided support to expand the hands-on UCB Microscopy Course and focus student projects on cancer problems. The UCB PS-OC has also helped develop a new undergraduate mathematics course that has the potential to revolutionize the way that life science-focused undergraduates are taught mathematics at UCB. These three examples of PS-OC courses are detailed below.

The ASU PS-OC Physical Sciences and Cancer Course

The ASU PS-OC "Physical Sciences and Cancer" is a new course designed for excelling undergraduate students in the ASU Barrett's Honors College. Only students with previous course work in both physical and life sciences are admitted to the course. Classes include presentations from key PS-OC members and from relevant visiting researchers. In accordance with Honors College requirements, student participation and group discussion are emphasized. In addition, students investigate and formulate novel research proposals and present them both in class and in a written report. Other activities include tours of the laboratories and attendance at cancer-related public lectures and PS-OC seminars.

Students indicate that the Physical Sciences and Cancer course is unlike any course they have previously taken and that their experiences are extremely positive. In several cases, the course has provided a stepping stone to a more extensive involvement in physical sciences-oncology research and the PS-OC Program. Last year four students from the course obtained research internships with the ASU Biodesign Institute, and so far this year one student has been accepted in the Harvard-MIT Photo-Dynamic Cancer Therapy Program.

Following is feedback from students:

- "Taking the Physical Sciences and Cancer course in Spring 2011 has had a major impact on my life. Not only has it increased my knowledge in many of the fields involved in cancer research, it has enabled me to get personally involved in cancer research.…"
- "As a Biological Sciences major, I was especially interested in approaching this topic from the physical sciences angle.…I now work on a project to improve the culture and study of single cancer cells. I intend to pursue joint careers in clinical and medical research."

UCB Microscopy Course

The UCB Microscopy Course is a cancer-research themed laboratory class training upper division undergraduates and graduate students in the design, construction, and operation of cutting edge optical microscopy. The microscopy course, taught by PS-OC Investigator Dan Fletcher, is designed to attract students with a physical sciences background and introduce them to deep and beautiful problems in the life and biomedical sciences, especially cancer biology. The microscopy class helps students with a math/engineering/physics background to realize how important and sought after their skills are in the contemporary life sciences and biomedical research.

Figure 8.5. Students from the TMHRI Summer Internship program.

Scripps Pathology Boot Camp
Introduces fellows and graduate students to the current techniques used in clinical pathology

Princeton Microfluidics Boot Camp
Teaches researchers and students introductory design and manufacture of microfluidic devices

Northwestern Summer Program for Undergraduates
A highly competitive summer placement for four undergraduate students per year to work in labs that are part of the Northwestern PS-OC

TMHRI Summer Internship Program
A summer internship for 10 students per year to study under PS-OC investigators and work on cutting-edge projects

University of California, Berkeley Math for Biologists Course

The "Math for Biology" course addresses another long-standing problem in the way young life scientists are trained. In the traditional math class required of all biologists at UCB, there is not a single example of how mathematics is being applied in biochemistry, genetics, physiology, bioinformatics, biology, or medicine. The course content has not changed significantly since 1868, when the university was founded. This lack of change is puzzling, since literally every aspect of contemporary biology and medicine now involves increasingly advanced tools and concepts from mathematics. Examples are the trend to "evidence based medicine," where primary care physicians are being asked to statistically rank potential treatments according to proven benefits from trials and studies, and contemporary genomics, which involves hundreds of algorithms and theorems from discrete mathematics. Many math departments are missing a tremendous opportunity to teach mathematics in a way that fascinates non-math majors and is highly relevant to their future careers in research or medicine. Math 91ab: Math for Biologists: Statistics & Discrete Math is cancer themed and integrates statistics, discrete math, and computation with an innovative, inverted approach to calculus. The class is currently on track to become a requirement for all biology majors at the UCB in the next five years and will allow every other class that biology majors take to be redesigned around quantitative thinking and statistical and computational methods. If the course's current trajectory of gaining support as the base math class for biology majors continues, it could eventually be taught to more than 900 students a year. The course has proven extremely popular during its initial trial run, with a lower than average drop rate and a higher than average course rating, and the math faculty are enthusiastic about supporting its growth. This is not surprising to us: Undergraduates are smart to realize when material is being taught in a way that makes it clear why they are required to take the class. Contemporary biological research and medicine are

Figure 8.6. Prof. Daniel Fletcher (upper right of image) instructs graduate students at the UCB PS-OC Microscopy course.

packed with tools and ideas from mathematics, and this trend is only accelerating.

8.2.3 Student Exchanges and Immersion Programs

Each PS-OC has funds dedicated to supporting trainee exchange programs. These funds provide trainees the opportunity to spend a few days or a few weeks at another lab within the Center or at another Center within the PS-OC Network. The exchanges allow trainees to bring approaches and perspectives back to their labs and advance their research. With the Education Program funds, the Centers were given wide latitude on how to implement and utilize the trainee exchange funds, with an emphasis on establishing a program that best support the goals of the Center. Both Scripps and USC PS-OCs have utilized trainee exchanges to bring computational modelers and data analyzers to the labs that produce the data that they utilize, thereby strengthening the collaboration and the understanding of the experiments, the data, and their implications. The Moffitt and Princeton PS-OCs have utilized trainee exchanges to facilitate a cross-Center collaboration. At Northwestern PS-OC, trainee exchange funds were used to bring young investigators from across the PS-OC Network to attend a symposium and workshop, network with Northwestern trainees, and meet with Northwestern investigators. Each of these examples is detailed below.

The Scripps Research Institute PS-OC Exchange [within Center]

The Owen McCarty lab (Biomedical Engineering, Oregon Health and Science University, Scripps PS-OC) hosted a laboratory boot camp for the members of the Paul Newton lab (Aerospace Engineering, USC, Scripps PS-OC). This was the first time that the graduate students from the Newton lab worked in a wet lab environment, as their research is focused on developing mathematical models of cell interactions. Together, this experience formed a basis for a project between these groups to characterize the response of cancer cells to shear stress, resulting in the release of cancer cells into a fluid phase. This work has been submitted for publication to the journal *Physics of Fluids*.

University of Southern California PS-OC Exchange [within Center]

Members of the USC PS-OC believe that trainee exchange has been a critical component of its transdisciplinary studies. Throughout the Center, a diverse set of unique technologies is being employed. For example, the CyTOF instrument used

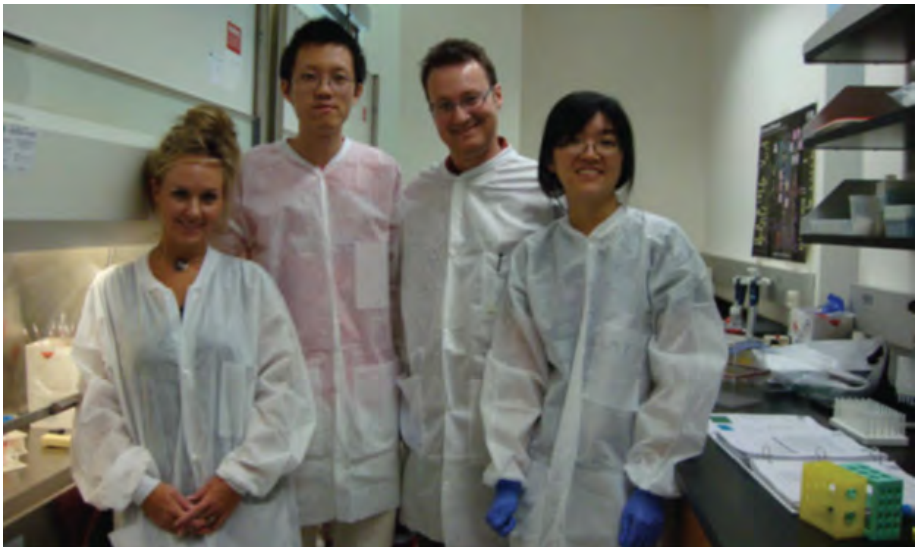


Figure 8.7. Participants in the 2011 PBCF ATCC Cell Culture Workshop. Left to right: Heather Branscome (ATCC instructor), John Qiucen Zhang (Austin lab; Princeton PS-OC), John Foulke (ATCC instructor), and Amy Wu (Austin lab; Princeton PS-OC)

ASU Physical Sciences and Cancer Course
A new course designed for outstanding undergraduate students. Course has been a stepping stone for students to join prestigious institutions such as Harvard, MIT, and the ASU Biodesign Institute

UCB Microscopy Course
Trained graduate students in the design, construction, and operation of cutting edge, bespoke optical microscopes. Course has successfully recruited students with backgrounds in physics and engineering and introduced them to cancer cell biology

UCB Math for Biologists Course
Aims to revolutionize the teaching of math to biology undergraduates by equipping them with the math skills they will need to succeed as 21st-century biologists. Currently, the course is on track to become a requirement for all UCB biology majors

to measure phosphoproteins in single cells is being utilized by the Nolan lab, which is one of a handful of such labs in the world. Consequently, it was of critical educational value to send Alex Greenfield, a computational biologist from the Richard Bonneau lab (New York University, USC PS-OC) to the Gary Nolan lab (Department of Molecular Pharmacology, Stanford University Medical Center, USC PS-OC) to learn more about this technique. His direct immersion with the group has enabled him to have a deeper understanding of the technology, and its benefits and limitations and thus permitted him to develop more accurate and detailed models from the CyTOF data. Furthermore, the fundamental perspectives of the Nolan lab, with regard to single-cell analysis have influenced his modeling approaches, which were previously dominantly predicated on data from multicellular studies.

H. Lee Moffitt Cancer Center PS-OC and Princeton University PS-OC Exchange [between Centers]

Tamir Epstein of the Moffitt PS-OC is currently working with the Princeton PS-OC's Austin lab (Department of Physics) and has participated in trainee exchanges on various occasions, including 2 visits to Princeton. Epstein is a postdoc in the Imaging Department at the Moffitt Cancer Center; together with Robert Gatenby, he is working on quantitative imaging of physical properties in cancer cells using highly accurate nanosensors. The collaboration with the Austin lab entails bringing together specialized approaches of each of the Centers and is greatly facilitated by spending time at the Princeton PS-OC. The trainee exchanges have been critical in two aspects of the collaboration: (1) imaging of oxygen and pH levels inside the Death Galaxy microhabitat device, developed by the Princeton PS-OC ; and (2) training Epstein to design and fabricate microfluidic chips for other projects at the Moffitt PS-OC.

Figure 8.8. PS-OC trainees participate in a speed networking activity during the First Annual Young Investigators' Meeting in Tampa, FL.

Northwestern Bringing Trainees from Other PS-OCs to Northwestern [bringing people to Center]

The Northwestern PS-OC invited PS-OC Network trainees to participate in a Symposium and Workshop in June 2011. In response, 12 students and post-doctoral fellows from other PS-OCs attended, with expenses paid by Northwestern PS-OC Trainee Exchange funds. Many of the Network trainees also requested lab tours and additional individual meetings with PS-OC personnel.

8.3. OPSO Education Activities

8.3.1 PBCF Annual Basic Workshop

The PS-OC Network Bioresource Core Facility (PBCF) held the 1st Annual Basic Workshop on Cell Culture Techniques August 9-12, 2011, at the ATCC facility in Manassas, Virginia. The workshop was designed to provide a comprehensive overview of best practices in cell culture through classroom presentation and laboratory work. This integrated approach, which utilized hands-on training to reinforce lecture material, allowed the PS-OC participants to translate culture techniques into applications in their own laboratories. In addition, the PBCF workshop provides a platform for strengthening collaborations across Centers by standardizing techniques and materials used by each laboratory. This will be necessary for continuation of Network-wide projects similar to the Cell Line Pilot Project (Section 5.2). The workshop comprised seven PS-OC trainees representing six PS-OCs. Their scientific backgrounds were in physics, applied physics, electrical engineering, mathematical biology, bioengineering, biochemistry, and molecular biology (Figure 8.7). Each morning, the four-day workshop began with a lecture, and each afternoon it followed with a lab practicum. Workshop lecture and lab topics included aseptic techniques, media preparation, freezing and thawing cells, setting up cell cultures, determining cell counts, and viability and growth curves of cell lines.

The PBCF held its 2nd Annual Basic Workshop on Biotechniques in Immunochemistry July 17-20, 2012, at ATCC in Manassas, Virginia. Eight trainees representing six PS-OCs and all of distinct scientific training were in attendance. Topics covered in the workshop included the following hands-on laboratory techniques: immunocytochemistry, flow cytometry, Western blot, high-throughput bead array, antibody sources and selection, and probes and methods of detection.

The workshop evaluations indicated that the workshop was well organized and informative, especially for novices in cell culture. In an essay written about the workshop in the PS-OC *Perspectives* Spring 2012 Newsletter, one of the workshop participants, Amy Wu (graduate student, physics department at Princeton University PS-OC, Robert Austin's group) writes, "In four days, we were trained to use the strictest practice to culture both suspension and adherent mammalian cell lines. Most importantly, we learned how to manage the contamination issues… After I went back to my institution, our lab had a revolution to abandon all the sloppy practices… Since then, our lab has been operating very well."

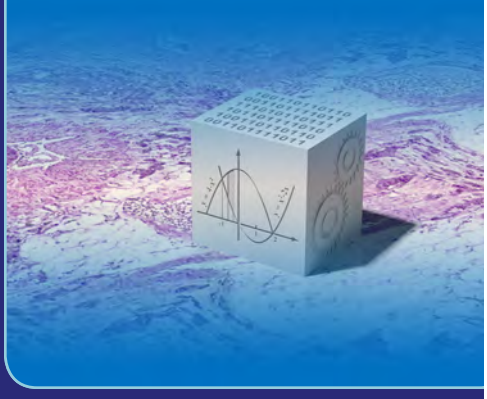
Figure 8.9. Dr. Thomas Peterson from the NIH Center for Scientific Review presented some best practices for grant writing and opportunities for young faculty to participate in the NIH review process.

"As a graduate student trained in cancer biology, the PS-OC has provided me with a new interdisciplinary way to approach cancer research. The Program has broadened my view of what cancer entails and has provided me with new tools to tackle this disease."

— Student, TMHRI PS-OC

"It has been a truly valuable learning experience for someone like me who comes from a computational sciences background. This [microfluidics] workshop met and exceeded all my expectations…Many thanks for organizing this."

— S.H., TMHRI PS-OC



8.3.2 Young Investigators' Annual Meeting

On April 15-16, 2012, the OSPO hosted the first Annual PS-OC Network Young Investigators' Annual Meeting in Tampa Bay, Florida. The meeting brought together over 50 trainees from the PS-OC Network to share research results, build and strengthen trans-Network collaborations, and offer professional development opportunities to students transitioning to other careers. Activities during the meeting included a speed networking session (Figure 8.8) to initiate collaborations, professional development speakers and panel, as well as scientific presentations by trainees (see Appendix). Key speakers are listed below.

- Dr. Ming Lee, NCI Center for Cancer Training, presented an overview of the many NIH funding and training opportunities available to young investigators.
- Dr. Thomas Peterson, NIH Center for Scientific Review, described the grant review process and provided some tips for how to make sure grant applications reach the right study section and receive the best possible reviews (Figure 8.9).
- Dr. Jennifer Couch, NCI's Integrative Cancer Biology Program (ICBP), provided an overview of the ICBP and opportunities for interaction with the PS-OC Network.
- Dr. Sean Hanlon, NCI OPSO, described the AAAS Science & Technology Fellowship Program.

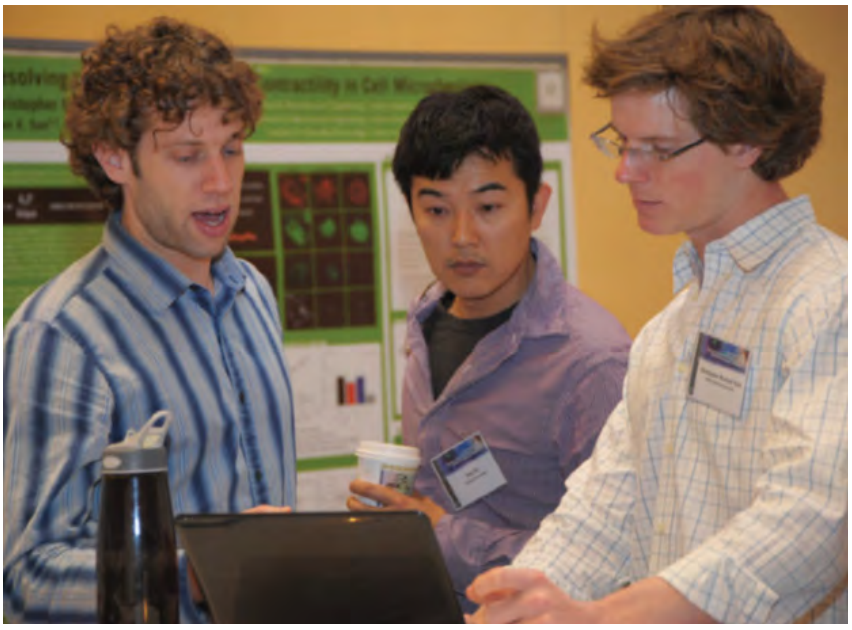


Figure 8.8. PS-OC Young Investigator Trans-Network Award winners Bryan Smith (left) and Chris Hale (right) discuss their collaborative research project with postdoctoral scholar Ken Ito of Stanford University (center)..

8.3.3 Joint ICBP and PS-OC Junior Investigators' Meeting

One of the outcomes from the PS-OC Young Investigators' Meeting was an agreement to organize a joint meeting between the junior investigators of both programs. Program staff had noted the potential synergies between the two programs, and the joint meeting was designed to promote interaction and collaborations. The meeting was held between September 26-28 at the Fred Hutchinson Cancer Research Center in Seattle, Washington.

The participants had the opportunity to share their research interests through short (one-minute presentations), learn about new data resources and tools, as well as discuss and brainstorm ideas on the topics (1) drug response and resistance; (2) personalized medicine; (3) tumor heterogeneity; and (4) epigenetic regulation of tumor progression. Most importantly, however, Junior Investigators were encouraged to develop trans-disciplinary proposals that had at least one representative from both the ICBP and PS-OC programs. The proposals were to address one of the four topics listed previously and were designed to generate preliminary data for traditional mechanisms. Ten teams were formed over the course of the meeting, and two of the proposals were selected for an award of $10,000 in direct costs (pending).

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