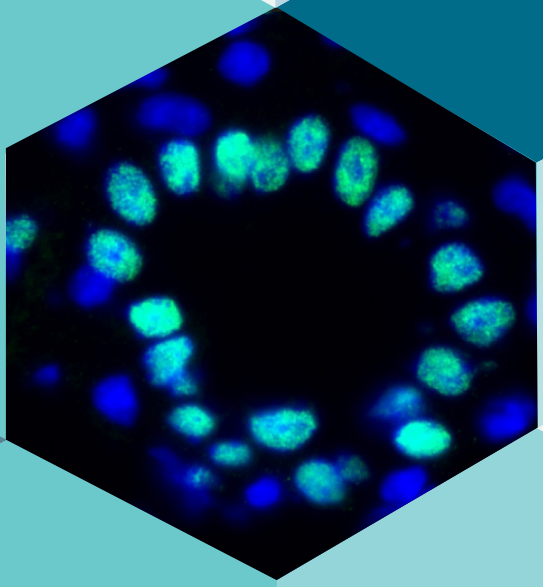

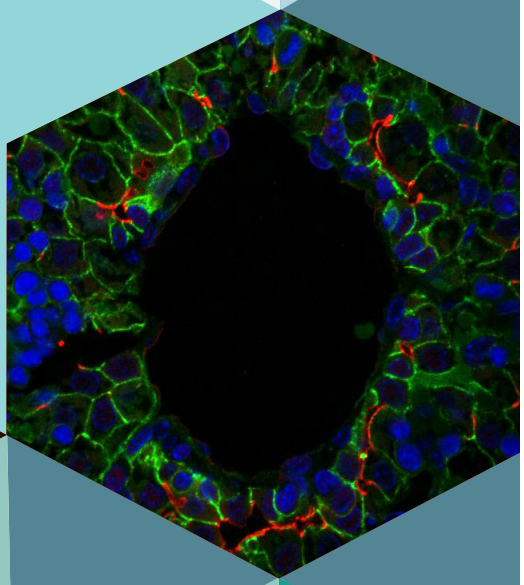

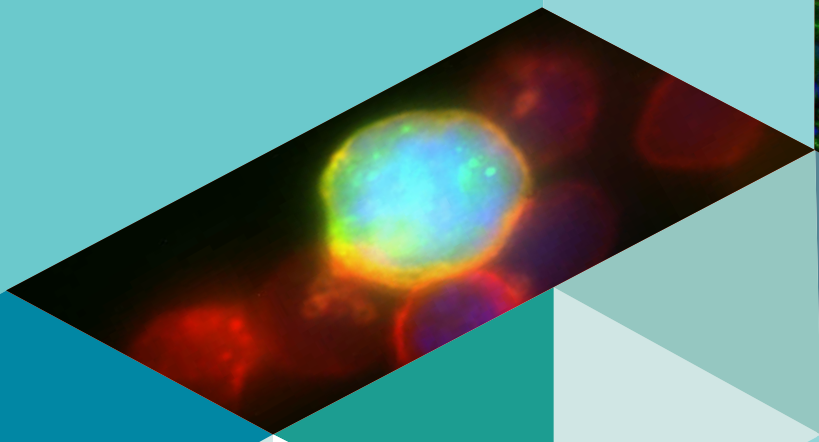

CENTER FOR CANCER RESEARCH

Annual Report 2014

MASSACHUSETTS GENERAL HOSPITAL
CANCER CENTER

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Reports from the Principal Investigators

Message from the Director

The Massachusetts General Hospital (MGH) Center for Cancer Research (CCR) serves as the engine for discovery for the Massachusetts General Hospital (MGH) Cancer Center. Our mission is to build an innovative and cutting edge cancer research program that transcends the boundaries of laboratories, fosters collaborations among investigators in different fields of research and bridges the translational divide to the clinic so that discoveries made at a bench side can impact the way cancer is diagnosed, treated and prevented.

The CCR includes 41 independent, primary and affiliated investigators with Harvard Medical School appointments across multiple clinical and basic science departments including the Departments of Medicine, Pathology, Cell Biology, Surgery and Pediatrics. We occupy over 60,000 sq ft of laboratory space in three MGH research facilities (Charlestown Navy Yard, Simches Research Bldg, Jackson Bldg) and our laboratories have access to state-of-the-art tools from genetics, genomics, developmental biology, advanced microscopy, computational biology, proteomics, and bioengineering that advance the science of cancer biology.

This Annual Report presents a brief overview of research in each CCR laboratory, followed by a complete listing of publications for 2013-2014. As the report demonstrates, the strength of the CCR is based on our outstanding team of principal investigators and the extraordinary dedication and commitment from each CCR member to our goals and our mission. During the past year, CCR investigators have published almost 300 peer-reviewed papers in leading scientific journals and made significant progress in advancing our understanding of cancer biology through basic research. Examples of discoveries from 2014 include (1) the role of mutant IDH expression in modulating HNF-4α silencing of hepatocyte differentiation, cell proliferation and liver cancer. [Bardeesy lab, *Nature*] (2) using ex vivo culture

of circulating breast tumor cells to test for drug susceptibility in individual patients [Haber and Maheswaran lab, *Science*] (3) reconstructing and programming the tumor propagating potential of glioblastoma stem-like cells [Bernstein lab, *Cell*] (4) the role of basal epithelia as a central coordinator of lactogenesis. [Ellisen lab, *Developmental Cell*], (5) dissection of novel DNA damage-serving mechanisms [Zou lab, *Molecular Cell*], and (6) potent new combinations to circumvent targeted cancer drug resistance [Engelman lab, *Cancer Cell*].

Our goal for the upcoming year is to further expand innovative research in basic and translational cancer biology. We are hopeful that our discoveries will help to further define cancer characteristics and create new and innovative treatments that improve clinical outcomes and standard of care for patients treated at the Massachusetts General Hospital Cancer Center and around the world.

Daniel A. Haber, MD, PhD

Director, Massachusetts General Hospital Cancer Center

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The Annual MGH Award In Cancer Research

In memory of Nathan and Grace Shiff

2013

James Allison, PhD
Chair, Department of Immunology
MD Anderson Cancer Center, Houston, TX

2012

Craig Thompson, MD
President and Chief Executive Officer
Memorial Sloan-Kettering Cancer Center, New York

2011

Michael Stratton, MD, FRS
Director, Wellcome Trust Sanger Institute, Cambridge, UK

2010

Charles Sawyers, MD
Chairman of the Human Oncology and Pathogenesis Program
Memorial Sloan-Kettering Cancer Center, New York

2009

Bert Vogelstein, MD
Director of the Ludwig Center for Cancer Genetics & Therapeutics
Sidney Kimmel Comprehensive Cancer Center
Johns Hopkins University, Maryland

2008

Titia de Lange, PhD
Associate Director of the Anderson Cancer Center
Rockefeller University, New York

2007

Joan Massague, PhD
Chairman of the Cancer Biology and Genetics Program
Memorial Sloan-Kettering Cancer Center, New York

2006

Anton Berns, PhD
Director of Research and Chairman of the Board of Directors,
Netherlands Cancer Institute and Antoni van Leewenhoek Hospital
The Netherlands

Center for Cancer Research Faculty

Daniel A. Haber, MD, PhD
Director, Massachusetts General Hospital Cancer Center
Kurt J. Isselbacher/Peter S. Schwartz Professor of Oncology

Kurt J. Isselbacher, MD
Director Emeritus, Massachusetts General Hospital Cancer Center
Mallinckrodt Distinguished Professor of Medicine

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Professor of Medicine

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Associate Scientific Director
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Assistant Professor of Medicine

André Bernards, PhD
Associate Professor of Medicine

Ryan Corcoran, MD, PhD
Assistant Professor of Medicine†

Jeffrey Engelman, MD, PhD
Associate Professor of Medicine

David Fisher, MD, PhD
Professor and Chief of Dermatology

Gad Getz, PhD
*Associate Professor of Pathology**

Timothy Graubert, MD
*Professor of Medicine**

Wilhelm Haas, PhD
Assistant Professor of Medicine†

Othon Iliopoulos, MD
Associate Professor of Medicine

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Associate Professor of Pathology

*David Langenau, PhD**
Assistant Professor of Pathology

*David Louis, MD**
Professor and Chief of Pathology

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Professor of Medicine

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Assistant Professor of Pathology

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Associate Professor of Pathology

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Assistant Professor of Medicine

Jackson Laboratories

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*A. John Iafrate, MD, PhD**
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James W. Rocco, MD, PhD
Associate Professor of Otology and Laryngology

Simches Laboratories

Nabeel Bardeesy, PhD
Associate Professor of Medicine

*Bradley Bernstein, MD, PhD**
Associate Professor of Pathology

*Konrad Hochedlinger, PhD***
Professor of Medicine

*Hanno Hock, MD, PhD***
Assistant Professor of Medicine

Raul Mostoslavsky, MD, PhD
Associate Professor of Medicine

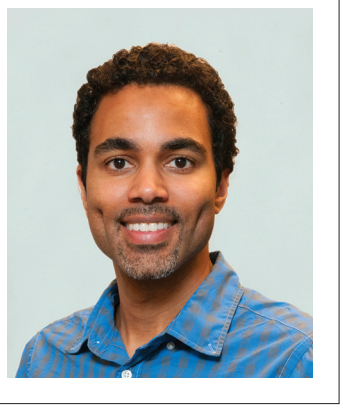
Sridhar Ramaswamy, MD
Associate Professor of Medicine

David Sweetser, MD, PhD
Assistant Professor of Pediatrics

Shobha Vasudevan, PhD
Assistant Professor of Medicine

* Joint appointment, Massachusetts General Hospital Cancer Center and Molecular Pathology Unit
** Joint appointment, Massachusetts General Hospital Cancer Center and Center for Regenerative Medicine and Technology
† Appointment process initiated

Reports from the Principal Investigators



Martin Aryee, PhD

All of the diverse cells in a human body share a virtually identical genome. However, each individual cell only turns on a subset of the genes in this genome, and the precise combination of active and inactive genes defines the resulting cell type. Chemical tags along the genome, termed epigenetic marks, signal the specific genes that will be active or silenced in a given cell. Genetic mutations and environmental exposures can perturb these finely tuned epigenetic patterns, leading to aberrant gene activity that push cells into abnormal states associated with cancer. **The Aryee lab** develops computational algorithms to analyze large cancer genomic and epigenomic datasets. We create statistical tools to extract signals from vast amounts of noisy data, creating maps of epigenetic marks in normal and cancer cells. We use these maps to study the role of epigenetic aberration in cancer.

Aryee Laboratory

Martin Aryee, PhD
Vishal Thapar, PhD

Tumor heterogeneity

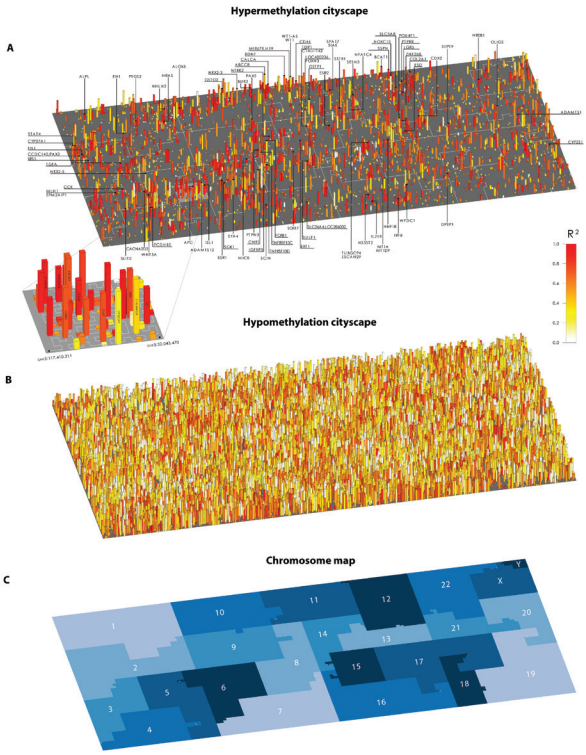
We develop statistical methods to improve our understanding of cell-to-cell variability and its relationship to cancer-related phenotypes. Much of this work relates to the computational and statistical challenges posed by single-cell transcriptome and epigenome data. The goal of these methods is to characterize the somatic changes that occur during tumor development and that are ultimately responsible for disease progression and resistance to therapy.

Different tumors, even of the same type, can harbor extremely heterogeneous epigenetic alterations. To investigate the role of epigenetic stochasticity in cancer, we recently applied a statistical model to study patterns of inter- and intra-individual tumor heterogeneity during metastasis. We established that metastatic prostate cancer patients develop distinctly unique DNA methylation signatures that are subsequently maintained across metastatic dissemination. Further, by quantifying the stability of these individualized DNA methylation profiles we showed that they were strikingly similar to that of copy number alterations, a finding with implications for the promise of epigenetic alterations as diagnostic and therapeutic targets in cancer.

Epigenome mapping

Unlike genome sequencing which has well established experimental and analytical protocols, epigenome mapping strategies are still in their infancy and, like other high-throughput techniques, are plagued by technical artifacts. A central theme of our research involves the development of methods for extracting signal from noisy high-throughput genomic assays. The goal of such preprocessing methods is transform raw data from high-throughput assays into reliable measures of the underlying biological process.

Until recently, studies of DNA methylation in cancer had focused almost exclusively on CpG dense regions in gene promoters. We helped develop the statistical tools used to analyze the first genome-scale DNA methylation assays designed without bias towards CpG islands. These tools enabled the discovery



DNA methylation "Cityscape" plots of lethal metastatic prostate cancer highlight inter-tumor epigenetic heterogeneity.

Genomic cityscapes of somatic (A) hypermethylation and (B) hypomethylation. Each chromosome is folded into neighborhoods as shown in (C). Each structure represents a genomic region showing a somatic methylation alteration. The height of each structure indicates the number of tumors showing an alteration at this site. The color scale represents the degree of stability of these alterations across metastases within individuals. The magnified region in (A) illustrates a representative chromosomal segment showing clustering of frequently hypermethylated regions (skyscrapers).

that the majority of both tissue-specific and cancer-associated variation occurs in regions outside of CpG islands. We showed that there is a strong overlap between genomic regions involved in normal tissue differentiation, reprogramming during induced pluripotency, and cancer.

Epigenomic studies of complex disease

Despite the discovery of numerous disease-associated genetic variants, the majority of phenotypic variance remains unexplained for most diseases, suggesting that non-genetic factors play a significant role. Part of the explanation will lie in a better understanding of epigenetic mechanisms. These mechanisms are influenced by both genetic and environmental effects and, as downstream effectors of these factors, may be more directly related to phenotype. There is hope that epigenetic alterations may provide therapeutic targets for pharmacological intervention, due to their reversible nature. However, the broad extent of epigenetic dysregulation in cancer and many other diseases complicates the search for the small subset of alterations with a causal role in pathogenesis. We are developing computational methods to integrate genome-wide genetic and epigenetic data with the goal of identifying the subset of functionally important epigenetic alterations.

Selected Publications:

Aryee MJ, Jaffe AE, Corrada-Bravo H, Ladd-Acosta C, Feinberg AP, Hansen KD, Irizarry RA. Minfi: a flexible and comprehensive Bioconductor package for the analysis of Infinium DNA methylation microarrays. *Bioinformatics*. 2014 May 15;30(10):1363-9.

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Liu Y*, **Aryee MJ***, Padyukov L, Fallin MD, Hesselberg E, Runarsson A, Reinius L, Acevedo N, Taub M, Ronninger M, Shchetynsky K, Scheynius A, Kere J, Alfredsson L, Klareskog L, Ekström TJ, Feinberg AP. Epigenome-wide association data implicate DNA methylation as an intermediary of genetic risk in rheumatoid arthritis. *Nat Biotechnol*. 2013.

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Haffner MC, **Aryee MJ**, Toubaji A, Esopi DM, Albadine R, Gurel B, Isaacs WB, Bova GS, Liu W, Xu J, Meeker AK, Netto G, De Marzo AM, Nelson WG, Yegnasubramanian S. Androgen-induced TOP2B-mediated double-strand breaks and prostate cancer gene rearrangements. *Nat Genet*. 2010;42(8):668-75

Wu Z, **Aryee MJ**. Subset quantile normalization using negative control features. *J Comput Biol*. 2010;17(10):1385-95

*co-authors

Nabeel Bardeesy, PhD

Pancreatic cancer and biliary cancer are among the most lethal types of human cancers. **The Bardeesy laboratory** has developed a series of genetically engineered mouse models to define the role of key gene mutations in driving these cancer types. Current projects focus on defining roles for cancer genes in controlling the way cells modulate their growth and utilize energy in response to available nutrients, and on identifying epigenetic regulators responsible for changes in cellular differentiation state that lead to cancer initiation and maintenance.

Bardeesy Laboratory

Nabeel Bardeesy, PhD
Julian Fitamant, PhD
Filippos Kotakis, PhD
Yusuke Mizukami, MD
Julia Nagle
Mortada Najem
Christine Parachoniak, PhD
Rushika Perera, PhD
Supriya Saha, MD, PhD
Svetlana Stoykova, PhD

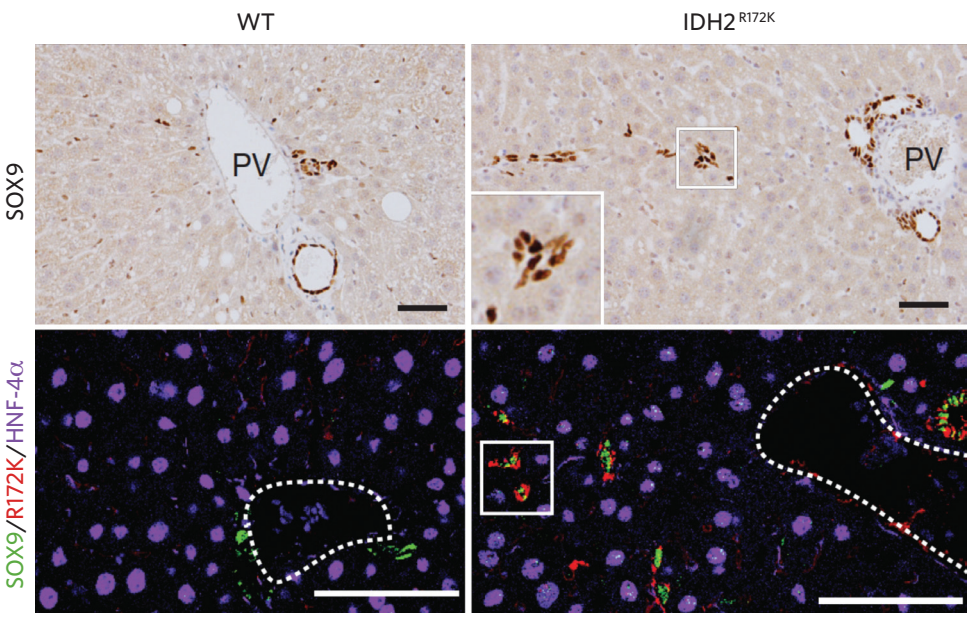
The Bardeesy lab focuses on defining the pathways driving the pathogenesis of pancreatic and biliary cancers. Our lab has developed a series of genetically engineered mouse models that have elucidated the functional interactions of major gene mutations associated with these diseases in humans. Specifically, we have characterized the roles of key cancer genes in the control of cellular differentiation states and in metabolic regulation.

Epigenetic drivers of pancreatic and biliary cancer

An important area of current focus in our lab is to elucidate the epigenetic regulators of pancreatic cancer and biliary cancers, with particular attention paid to factors that subvert normal differentiation pathways and that reprogram cancer cell metabolism. As part of these efforts, we defined the tumorigenic role of a number of chromatin-modifying enzymes that are deregulated in pancreatic cancer progression, KDM2B. This histone demethylase is a major regulator both of polycomb repressor complexes that override cancer cell differentiation states and of a distinct program controlling metabolic homeostasis. In biliary cancer, there are recurrent mutations in the IDH1 and IDH2 genes. Mutant IDH proteins in IHCC and other malignancies acquire a novel enzymatic activity allowing them to convert alpha-ketoglutarate (αKG) to 2-hydroxyglutarate (2HG), which inhibits the activity of multiple αKG-dependent dioxygenases, including the JmjC family histone demethylases. We are focusing on how IDH mutations affect epigenetic programs and regulation of cellular identity in the liver.

Targeting master regulators of metabolic reprogramming in PDAC

In order to couple rapid growth with available nutrients, cancers employ profoundly altered networks of biosynthetic and catabolic pathways. This requirement for metabolic reprogramming is particularly acute in PDAC, which is characterized by hypoxia and limited nutrient availability, and activates anti-oxidant gene expression and autophagy (cellular self-catabolism) as necessary adaptive metabolic changes. While these pathways offer attractive new therapeutic targets, the underlying mechanisms driving altered PDAC metabolism are unclear. We have focused on identifying master transcriptional regulators that broadly orchestrate metabolic reprogramming in PDAC.



Mutant IDH causes expansion and impaired differentiation of liver progenitor cells leading to biliary cancer. Immunohistochemistry (top) and immunofluorescence (bottom) of livers from wild type (WT) and transgenic mice expressing mutant IDH2R172K. Sox9 (top, brown stain; bottom, green) normally marks bile duct cells adjacent to the portal vein (PV), whereas there is aberrant accumulation of Sox9-expressing cells progenitor/stem cells in IDH mutant livers. These cells are highly prone to progression to biliary cancer (cholangiocarcinoma). Image from Saha, Parachoniak et al., Nature 2014.

Mouse models of biliary cancer

Recent genetic studies have identified multiple recurrent mutations in biliary cancers and have indicated considerable genetic heterogeneity between individual tumors. A key limitation in the field includes a paucity of experimental systems with which to define the contributions of the lesions to biliary cancer progression. We have established a series of genetically engineered mouse models that incorporate combinations of the major mutations found in the human disease. In addition, our ongoing efforts include the development of a human biliary cancer cell line bank for the use of genetic and small-molecule screening in genetically defined subtypes of this cancer.

Control of liver progenitor cells and biliary cancer development

The Hippo pathway is a conserved regulator of organ size. Our lab has shown that this pathway is central for controlling the quiescence of liver progenitor cells, and that its loss leads to massive liver overgrowth and development of both major types of liver cancer (hepatocellular carcinoma and cholangiocarcinoma). The lab is studying the circuitry of the Hippo pathway in liver progenitor cells and the key mediators of tumorigenesis downstream of this pathway.

Selected Publications:

Tzatsos A, Paskaleva P, Ferrari F, Deshpande V, Stoykova S, Contino G, Wong KK, Lan F, Trojer P, Park PJ, **Bardeesy N**. KDM2B promotes pancreatic cancer via Polycomb-dependent and -independent transcriptional programs. *J Clin Invest*. 2013 Feb 1;123(2):727-39.

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Zhou D, Conrad C, Xia F, Park JS, Payer B, Yin Y, Lauwers GY, Thasler W, Lee JT, Avruch J, **Bardeesy N**. Mst1 and Mst2 maintain hepatocyte quiescence and suppress hepatocellular carcinoma development through inactivation of the Yap1 oncogene. *Cancer Cell*. 16(5):425-38, 2009 Nov 6.

Cyril Benes, PhD

The Benes laboratory, known as The Center for Molecular Therapeutics, is engaged in the design and application of personalized therapies for cancer. Targeted cancer treatments have emerged from research studies showing that the biology of cancer cells differs from that of healthy cells, and that each person's cancer has a unique genetic signature. Our goal is to pinpoint the cancer cells' biological weak points and then to attack those weak points with smart drugs that are specifically designed for such an attack. Using a large collection of human cancer cell lines, we have made major advances in identifying molecular genetic features, or biomarkers, of a tumor that predict responsiveness to targeted therapies. We are focused on developing molecular diagnostics that will reveal the best treatment course for each patient and at discovering how gene mutations in cancer can be exploited to develop new treatments.

Benes Laboratory

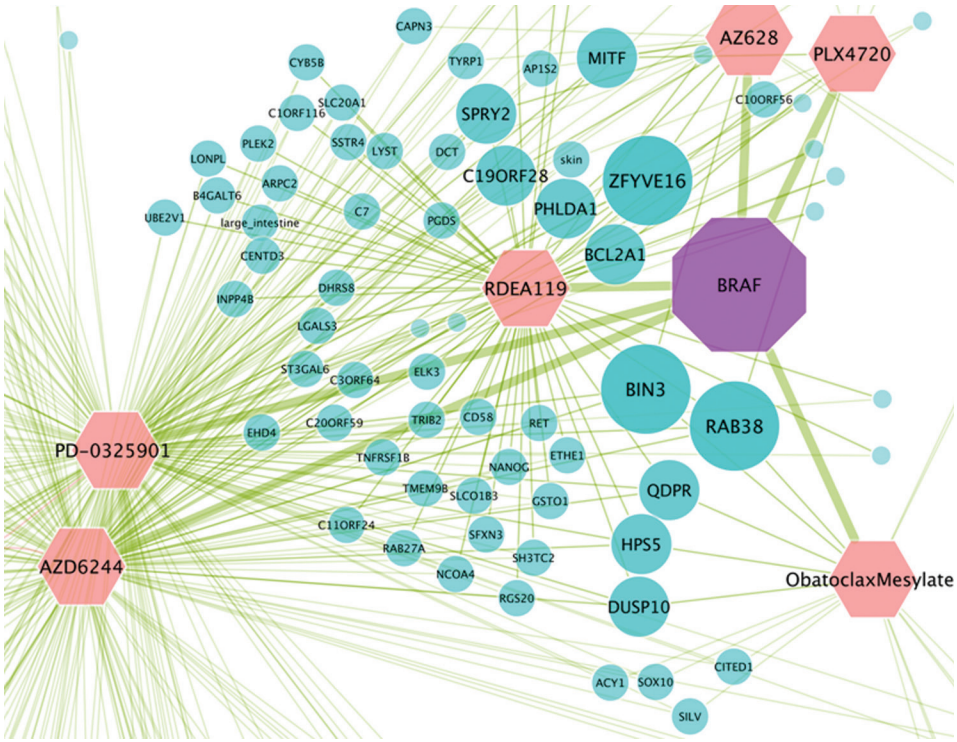
Arnaud Amzallag, PhD
Cyril Benes, PhD
Jessica Boisvert
Li Chen, PhD
Anahita Dastur, PhD
Leah Damon
Regina Egan
Adam Friedman, MD, PhD
Patricia Greninger
Matthew Held, PhD
Ryan March
Xeni Mitropoulos
Iulian Pruteanu-Malinici, PhD

We are studying the molecular basis of response to anticancer agents.

Genetics of Cancer Therapeutic Response

Clinical responses to anticancer therapeutics are often restricted to a subset of cases treated. In some instances, clear evidence is available that correlates clinical responses with specific tumor genotypes. Our goal is to identify tumor cell states (i.e., genotypes, gene expression) that predict sensitivity to anticancer agents. To accomplish this goal, we use high-throughput screening and expose 1,000 cell lines derived from a broad spectrum of cancers to known and potential anticancer therapeutic agents. For each compound, we characterize the variation in response across the cell line collection and correlate response to genomic and transcriptomic information, including cancer gene mutations, genome-wide copy number information, and mRNA expression data. We then translate these findings to clinical use by analyzing clinical specimens to possibly inform the design of clinical studies.

The use of a very large cell line collection allows us to capture some mutational events that—although relatively rare—are very important for therapeutic response. In addition, while some patient selection strategies have proven quite successful, a wide range of variation in response to treatment exists in almost all cases. Similar to this clinical observation—and perhaps related mechanistically—our large cell line collection allows us to observe important variation in drug response within a given sensitizing genotype. For example, among BRAF-mutant cell lines which are, as a group, remarkably sensitive to BRAF inhibitors, some lines do not respond significantly. Based on these observations, we aim to identify additional biomarkers that will permit more accurate prediction of drug response in the clinic.



Network representation of genomic correlates shared by MEK1/2 and RAF inhibitors indentified by linear regression using drug sensitivity data across hundreds of cancer cell lines.

Resistance to Cancer Therapies

Even for the most successful anticancer therapies, drug resistance invariably emerges and limits the impact on patient lives. The molecular mechanisms underlying acquired resistance to cancer therapeutics are not well defined but are likely to be different for each therapy and cancer. We are investigating how drug combinations could overcome resistance, and within this context, studying how changes in intracellular signaling pathways affect drug response.

We are tackling the problem of therapeutic resistance using cell lines made resistant in the laboratory or isolated from resistant tumors. Previous results have shown that these cell line models do recapitulate at least some of the mechanisms of resistance at play in patients. We interrogate combinations of a panel of clinically relevant anticancer drugs as a way to quickly identify candidate therapeutic strategies and to jumpstart mechanistic studies that will help characterize the molecular basis of acquired resistance.

We are also approaching the problem of resistance using a very different and complementary approach. We systematically identify genes that can cause resistance to a given drug in a given context using a transposon-based genetic screen. Transposons are mobile genetic elements that can insert into a host genome—in our case, the genome of cancer cells. We use an engineered version of a transposon so we can control its mobility and identify genes with expressions that are modified by its insertion, leading to drug resistance.

Selected Publications:

Kwiatkowski N, Zhang T, Rahl PB, Abraham BJ, Reddy J, Ficarro SB, Dastur A, Amzallag A, Ramaswamy S, Tesar B, Jenkins CE, Hannett NM, McMillin D, Sanda T, Sim T, Kim ND, Look T, Mitsiades CS, Weng AP, Brown JR, **Benes CH**, Marto JA, Young RA, Gray NS. Targeting transcription regulation in cancer with a covalent CDK7 inhibitor. *Nature*. 2014 Jul 31;511(7511):616-20.

Yu M, Bardia A, Aceto N, Bersani F, Madden MW, Donaldson MC, Desai R, Zhu H, Comaills V, Zheng Z, Wittner BS, Stojanov P, Brachtel E, Sgroi D, Kapur R, Shioda T, Ting DT, Ramaswamy S, Getz G, Iafrate AJ, **Benes C**, Toner M, Maheswaran S, Haber DA. Cancer therapy. Ex vivo culture of circulating breast tumor cells for individualized testing of drug susceptibility. *Science*. 2014 Jul 11;345(6193):216-20.

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Chen L, Stuart L, Ohsumi TK, Burgess S, Varshney GK, Dastur A, Borowsky M, **Benes C**, Lacy-Hulbert A, Schmidt EV. Transposon activation mutagenesis as a screening tool for identifying resistance to cancer therapeutics. *BMC Cancer*. 2013 Feb 27.

André Bernards, PhD

Neurofibromatosis type 1 (NF1) is a genetic disease that affects an estimated two million people worldwide. It is caused by mutations in a gene on human chromosome 17. Patients with this genetic defect are at increased risk of developing a long list of symptoms, including skin and skeletal abnormalities; learning disabilities; and both noncancerous and cancerous tumors, many of which arise from the peripheral nervous system. The NF1 protein functions as a negative regulator of the Ras oncogene. Thus, scientists believe most NF1 symptoms are caused by excessive Ras activity. However, Ras is a central player in many molecular pathways, and the main goal of **the Bernards laboratory** is to identify the specific pathways responsible for different NF1 symptoms. Among our most significant recent findings is the identification of the ALK growth factor receptor as a critical component of pathways responsible for learning and other defects in a fruit fly model of NF1 and as a candidate therapeutic target in human NF1.

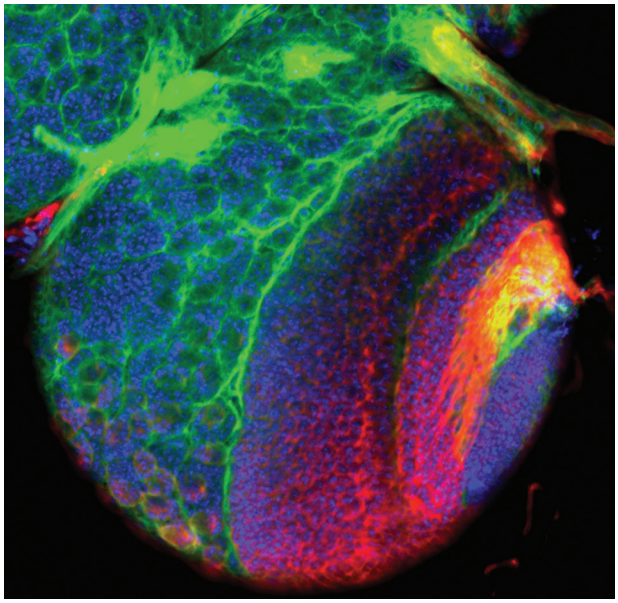
Bernards Laboratory

André Bernards, PhD
Prasanna Parasuraman, PhD

Neurofibromatosis-1 (NF1) affects 1 in ~3000 people worldwide. Highly variable expressivity is among the hallmarks of NF1, with common symptoms including benign and malignant tumors, abnormal skin pigmentation, skeletal defects, and learning deficiencies. NF1 is caused by loss of neurofibromin, which functions as a GTPase activating protein for Ras family members. Our NF1-related research focuses on answering three questions: 1) what are the in vivo functions of neurofibromin; 2) to what extent does defective Ras regulation explain the diverse symptoms of NF1; and 3) what is the identify of modifier genes that affect NF1 symptom development?

We have sought to answer these questions through a genetic study of a 60% identical *Drosophila NF1* ortholog. Homozygous *dNf1*-null mutants are viable and normally patterned but reduced in size. Mutants also have electrophysiological, circadian rhythm, learning/memory, and synaptic overgrowth defects. The size and cognitive deficits resemble human NF1 symptoms, and while manipulating Ras signaling readily modifies neither, both are rescued by increasing—or mimicked by decreasing—signaling through the cAMP/PKA pathway.

While others have reported that *dNf1* has physically separable Ras and cAMP related functions, our work identified a neuronal Ras signaling defect as the proximal cause of *dNf1* phenotypes. To shed further light on how excess neuronal Ras/ERK signaling causes various cAMP/PKA-sensitive phenotypes, we performed affinity capture mass spectroscopic identification of dNf1 protein complexes, determined gene expression profiles of *dNf1* expressing brain and non-expressing peripheral tissues, performed metabolomic profiling, and analyzed the spatial requirement of PKA expression. We also screened 486 1st and 2nd chromosome deficiencies for dominant modifiers of the cAMP/PKA-sensitive *dNf1*



Confocal immunofluorescence image of Drosophila larval central nervous system with glial cells (green), actin (red) and nuclei (blue).

growth defect. Responsible suppressor genes, identified through the use of mutant alleles or through RNAi-mediated knockdown, can be grouped into three functional categories. The first consists of the neuronal *dAlk* receptor tyrosine kinase and its activating ligand, *jellybelly*. Further collaborative work implicated *dAlk* as a rate-limiting upstream activator of *dNf1*-regulated Ras/ERK signals responsible for both growth and learning defects. Importantly, NF1-regulated ALK/RAS signaling appears conserved in man, identifying ALK as a potential therapeutic target. A second category of modifiers consists of genes involved in cAMP/PKA signaling, whereas a third includes genes involved in regulating synaptic architecture or functioning. A strong suppressor in the latter category is the *CCKLR-17D1* drosulfakinin receptor, recently implicated as a regulator of neuromuscular junction growth, which is abnormal in *dNf1* mutants. Interestingly, *CCKLR-17D1*, *dAlk* and other size defect suppressors also suppress the neuromuscular junction overgrowth defect, suggesting that abnormal synaptogenesis caused by excessive *dAlk* signaling may be responsible for many *dNf1* defects. Another important finding is that whereas only widespread neuronal *dNf1* expression rescues the mutant size defect, similar rescue can be achieved by increasing cAMP levels or PKA activity in a part of the neuroendocrine ring gland that produces adipokinetic hormone (AKH). Moreover, *AKH* mRNA levels are much reduced in *dNf1* mutants, as are the levels of trehalose and other energy sources mobilized by this hormone. Thus, the underlying cause of the *dNf1* size deficiency may be a metabolic deficiency.

Another project, pursued in collaboration with MGH Cancer Center colleague Dr. Gad Getz, involves the functional analysis of cancer-associated p190A RhoGAP mutations. Our analysis suggests that beyond its well-established function as a negative regulator of Rho GTPases, p190A may have a role in the initiation of mRNA translation. We are exploring whether this novel function explains why among the 60 or so human RhoGAP genes, p190A RhoGAP appears unique in carrying frequent loss-of-function mutations in specific human cancers.

Selected Publications:

Walker JA, **Bernards A**. A Drosophila screen identifies neurofibromatosis-1 genetic modifiers involved in systemic and synaptic growth. *Rare Diseases* 2014; 2:e28341.

Walker JA, Gouzi JY, Long JB, Huang S, Maher RC, Xia H, Khalil K, Ray A, Van Vactor D, Bernards R, **Bernards A**. Genetic and functional studies implicate synaptic overgrowth and ring gland cAMP/PKA signaling defects in the Drosophila melanogaster neurofibromatosis-1 growth deficiency. *PLoS Genet*. 2013; :e1003958.

Walker JA, Gouzi JY, **Bernards A**. Drosophila as an invertebrate model of NF1. In "Molecular and Cellular Biology of Neurofibromatosis type 1" *Springer*. 2012.

Gouzi JY, Moressis A, Walker JA, Apostolopoulou AA, Palmer RH, **Bernards A**, Skoulakis EMC. The receptor tyrosine kinase Alk controls neurofibromin functions in Drosophila growth and learning. *PLoS Genetics*. 7(9):e1002281, 2011.

Bradley Bernstein, MD, PhD

The Bernstein laboratory studies how the DNA in the human genome is packaged by a structure called chromatin. A central question in human biology is how the one genome we inherit at birth can give rise to the hundreds of cell types in the body. The genome consists of genes that code for the protein machines in our cells as well as regulatory elements that control those genes. A liver cell is different from an immune cell or a neuron because it makes different proteins. The way a gene is organized into chromatin predicts whether it will be turned on or off—and thus make protein—in a particular cell type. Our lab has identified specific types of chromatin that help determine when certain genes are on or off or that keep a gene poised to be turned on later in development. We leverage emerging technologies in genomics and computation to study chromatin organization across the genome. We use this information to better understand chromatin regulatory processes and how their failure contributes to cancer.

Bernstein Laboratory

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A central question in human biology is how a single genome sequence can give rise to the hundreds of different cell types in the body. Scientists understand that differential patterns of gene expression underlie the many different cellular phenotypes seen in multicellular organisms. However, our understanding of how these gene expression patterns arise during development and how they are subsequently maintained in the adult organism remains poor. A number of studies have indicated that these different expression patterns and phenotypes are intimately related to the way in which genomic DNA is organized into chromatin in the cell. This organizational structure of proteins and DNA, sometimes referred to as the epigenome, helps control which genes are expressed in a given cell type and is critical to the function of normal cells. Moreover, a large body of evidence suggests that the epigenome is inappropriately altered in most—if not all—human cancers.

The long-term goal of our research is to achieve a comprehensive understanding of how the human genome is organized into chromatin. Our group is further focused on understanding how dynamic alterations in chromatin structure contribute to mammalian development and how aberrant chromatin regulation contributes to cancer progression, heterogeneity and therapeutic resistance. We are taking a multifaceted approach involving stem cell biology, biochemistry genetics, genomics and computational biology. The specific areas of research activity in the lab are explained below.

Technologies for mapping histone modifications and chromatin proteins

We are combining tools in cell biology, biochemistry and molecular biology with next-generation sequencing to achieve increasingly precise, genome-wide views of chromatin structure, chromatin regulator binding and genome organization. Integrative analysis of

The machinery of chromatin regulation

The Bernstein group is focused on understanding the genome-wide regulation and control of chromatin — DNA and its associated proteins. Studies in this group provide views into the 'machinery' that regulates chromatin in mammalian cells, demonstrating that Chromatin Regulators (CRs) act in a similar manner to the way gears function in a machine. In the illustration, the gears represent CRs that may act in concert or alone to control different genomic environments.

Artwork by Lauren Solomon, Alon Goren and Leslie Gaffney, MGH and The Broad Institute. Original photograph from iStockphoto (Maksim Toome, photographer).

such chromatin state maps yields detailed annotations of the locations and dynamics of functional elements in the human genome, including promoters, transcripts, silencers, insulators and enhancers. Ongoing projects are applying these annotations to understanding cell circuits and how they vary across cell types during development and in cancer.

Epigenetic regulation of stem cell differentiation

Chromatin regulators, such as the Polycomb and trithorax complexes, play critical roles in controlling the expression and potential of genes during development. We identified a novel chromatin structure, termed bivalent domains, that is subject to simultaneous regulation by Polycomb repressors and trithorax activators. Bivalent domains appear to keep developmental regulator genes poised in pluripotent embryonic stem cells and may also serve similar functions in multipotent progenitor cells. Current studies are leveraging a new generation of experimental assays to characterize the functions of bivalent domains and to understand the mechanisms that underlie their establishment and function.

Chromatin regulation in cancer cells

Genes encoding chromatin regulators are frequently mutated in human cancer. In specific cases, these alterations appear to be major drivers of the malignant state. Ongoing studies in the lab seek to apply epigenomic technologies to characterize the transcriptional and epigenetic landscapes of cancer stem cells and to identify mechanisms by which epigenetic changes contribute to therapeutic resistance.

Selected Publications:

Suva ML, Rheinbay E, Gillespie SM, Wakimoto H, Cahill DP, Nashed BV, Curry WT, Martuza RL, Louis DN, Rozenblatt-Rosen O, Suva ML, Regev A, **Bernstein BE**. Reconstructing and programming the tumor propagating potential of glioblastoma stem-like cells. *Cell*. 157: 580-594, 2014.

Patel AP, Tirosh I, Trombetta JJ, Shalek AK, Gillespie SM, Wakimoto H, Cahill DP, Nahed BV, Curry WT, Martuza RL, Louis DN, Rozenblatt-Rosen O, Suva ML, Regev A, **Bernstein BE**. Single Cell RNA-seq highlights intratumoral heterogeneity in primary glioblastoma. *Science*. 344:1396-1401, 2014.

Knoechel B, Roderick JE, Williamson KE, Zhu J, Lohr JG, Cotton MJ, Gillespie SM, Fernandez D, Ku M, Wang H, Piccioni F, Silver SJ, Jain M, Pearson D, Kluk MJ, Ott CJ, Shultz LD, Brehm MA, Greiner DL, Gutierrez A, Stegmaier K, Kung AL, Root DE, Bradner JE, Aster JC, Kelliher MA, **Bernstein BE**. An epigenetic mechanism of resistance to targeted therapy in T-cell acute lymphoblastic leukemia. *Nat Genet*. 46: 364-70, 2014.

Suva ML, Riggi N, **Bernstein BE**. Epigenetic reprogramming in cancer. *Science*. 339:1567-70, 2013.

Rheinbay E, Suva ML, Gillespie SM, Wakimoto H, Patel AP, Oksuz O, Rabkin SD, Martuza RL, Rivera MN, Louis DN, Kasif S, Chi AS, **Bernstein BE**. Chromatin profiles reveal an aberrant transcription factor network connected to Wnt signaling and essential for glioblastoma stem cell maintenance. *Cell Reports*. 3:1567–79, 2013.

Zhu J, Adli M, Zou JY, Verstappen G, Coyne Michael, Zhang X, Durham T, Miri M, Deshpande V, De Jager PL, Bennett DA, Houmard JA, Muoio DM, Onder TT, Camahort R, Cowan CA, Meissner A, Epstein CB, Shoresh N, **Bernstein BE**. Genome-wide chromatin state transitions associated with developmental and environmental cues. *Cell*. 152:642–654, 2013.

Michael J. Birrer, MD, PhD

The Birrer laboratory has had a long-term interest in characterizing the molecular origins of gynecologic cancers. This interest includes the identification and characterization of mutations in oncogenes and tumor suppressor genes within cancers of the ovary, endometrial and cervix. In addition, we have extensively characterized the differential gene expression in these tumors. The role of these genes in the development of these cancers has been tested using in vitro and in vivo model systems. Our laboratory is focused on using the genomic events characterized in these cancers to produce translational science endeavors, which will result in clinically important discoveries. These genomic abnormalities form the basis for early detection assays, prevention strategies, and novel therapeutic approaches. Our laboratory focuses on bench-to-bedside-and-back-again approaches to produce clinically relevant strategies to improve the outcome of women with these types of cancers.

Birrer Laboratory

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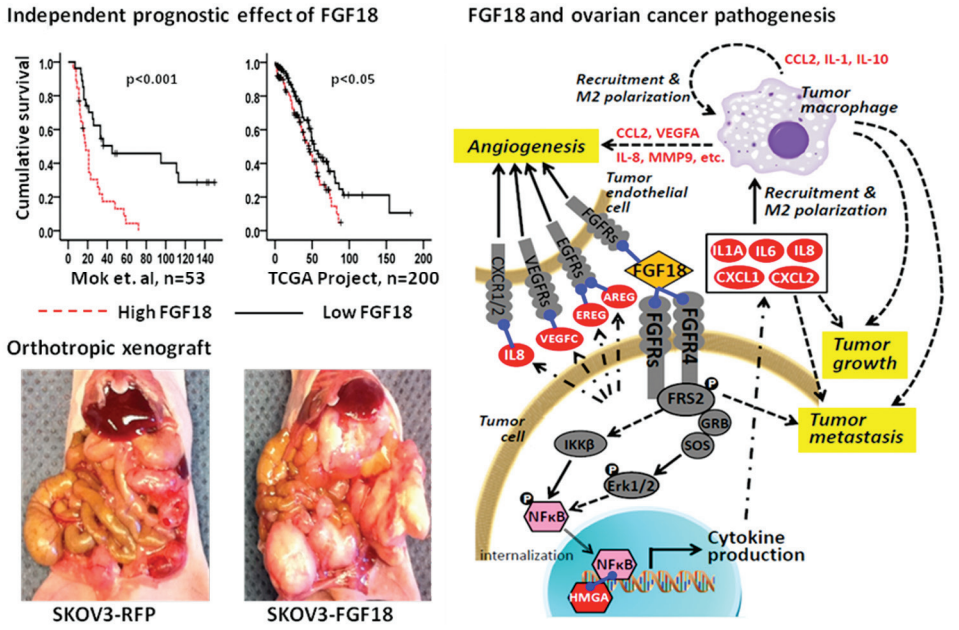
Young Jeong Na, MD, PhD
Tsun Yee Tsang, PhD
Wei Wei, PhD

Our laboratory focuses on characterizing the function and clinicopathologic impact of key genes and pathways in gynecologic cancers. The majority of our work is focused on ovarian cancer. In 2000, the laboratory was awarded an NCI Director's Challenge grant for the genomic analysis of ovarian cancer. The laboratory—in collaboration with Memorial Sloan Kettering Cancer Center, University of Pennsylvania, Fox Chase Cancer Center, and the Australia Ovarian Cancer Study—has conducted a large-scale study of expression profiling. These efforts have systematically characterized differential gene expression on the whole-genome level between ovarian tumors of different histology and tumor grade. The study led to the identification of activated biochemical pathways, which underlie the clinical pathologic characteristics of these tumors. Subsequently, our findings made possible the identification of clear cell and mucinous tumors of the ovaries as unique tumors essentially unrelated to the majority of ovarian cancers. This discovery has led to a change in clinical trial structure in the gynecologic cancer group, establishing for the first time unique trials for patients with these cancers.

We are presently testing activated pathways within these tumors, utilizing in vivo models for the discovery of novel therapeutic approaches. Our laboratory has validated the co-amplification and overexpression of FGF18 and its receptor FGFR4 as predictive of poor clinical outcome in patients with advanced stage, high-grade serous ovarian cancer. An NIH R-01 grant has been awarded to investigate the role of FGF18/FGFR4 signaling in the pathogenesis of serous ovarian cancer. Large-scale prospective validation and pharmaceutical targeting studies are currently underway.

Recently, the laboratory completed two large profiling studies on advanced-stage papillary serous tumors of the ovary. These studies generated differential gene expression signatures which classify patients into good versus poor prognosis and identify new and novel targets for therapy and prevention. The laboratory has been awarded an RC4 grant (in collaboration with Giovanni Parmigiani, PhD, of the Dana-Farber Cancer Institute) to extend these studies into a validation study, utilizing 1600 clinical trial specimens from the recently completed GOG clinical trial 218. The results



Co-amplification and overexpression of FGF18 and its receptor FGFR4 (on chromosome 5q31.3-qTER) have been validated as predictive of poor clinical outcome in this patient with advanced stage, high-grade serous ovarian cancer. Using cell culture and xenograft models, we show that FGF18/FGFR4 signaling activated NF-kB signaling and promoted tumor progression by modulating the ovarian tumor aggressiveness and microenvironment.

will be rapidly integrated into prospective clinical trials of patients with advanced-stage ovarian cancer.

The laboratory has also shown that low malignant potential tumors of the ovary (Grade 0) are a unique form of serous tumors and require specific therapeutic approaches. As a result, the laboratory has been instrumental in testing the MEK inhibitor AZD6244 in clinical trials with women diagnosed with low-grade cancers.

This genomic database will be extended to identify early detection biomarkers for ovarian cancer. Through collaboration with Steven Skates, PhD, the laboratory has successfully received an Early Detection Research Network UO1 grant to identify new and novel early detection approaches to this disease. We will systematically compare the gene expression profiles of ovarian cancer with its normal counterparts found on the surface of the ovary and fallopian tube. Through a collaborative effort with Ronny Drapkin, MD, PhD, of DFCI and Steven Carr, PhD of the Broad Institute, we will identify the early genomic abnormalities in ovarian cancer. We will validate these findings using specimens from Massachusetts General Hospital, Brigham and Women's Hospital and DFCI and ultimately translate our work into serum-based early detection assays.

Research direction for the future will focus on four major directions: 1) Characterizing the function of key genes which dysregulation are associated with clinicopathologic characteristics of ovarian cancer; 2) characterizing new tumor cellular subsets of ovarian cancer for the clinical features and the role in tumor formation; 3) utilizing the genomic characteristics of ovarian cancer to identify new and novel early detection, prevention and therapeutic targets; and 4) utilizing the genomic abnormalities found in ovarian cancer as targets for novel imaging techniques. Our laboratory efforts remain highly translational and collaborative in nature. We are committed to bring laboratory-based and scientifically rational concepts into the clinic to improve the lives of women with these cancers.

Selected Publications:

Riester M, Wei W, Waldron L, Culhane AC, Trippa L, Oliva E, Kim S, Michor F, Hutterenhower C, Parmigiani G, **Birrer MJ**. Risk prediction for Late-Stage Ovarian Cancer by Meta-Analysis of 1525 patient samples. *J Natl Cancer Instit*. 2014 106(5).

Wei W, Mok SC, Oliva E, Kim SH, Mohapatra G, **Birrer MJ**. FGF18 as a prognostic and therapeutic biomarker in ovarian cancer. *J Clin Invest*. 2013 Oct 1;123(10):4435-48.

Zaid TM, Yeung TL, Thompson MS, Leung CS, Harding T, Co NN, Schmandt RS, Kwan SY, Rodriguez-Aguay C, Lopez-Berestein G, Sood AK, Wong KK, **Birrer MJ**, Mok SC. Identification of FGFR4 as a potential therapeutic target for advanced-stage, high-grade serous ovarian cancer. *Clin Cancer Res*. 2013 Feb 15;19(4):809-20.

Farley J, Brady WE, Vathipadiekal V, Lankes HA, Coleman R, Morgan MA, Mannel R, Yamada SD, Mutch D, Rodgers WH, **Birrer M**, Gershenson DM. Selumetinib in women with recurrent low-grade serous carcinoma of the ovary or peritoneum: an open-label, single-arm, phase 2 study. *Lancet Oncol*. 2013 Feb;14(2):134-40.

Burger RA, Brady MF, Bookman MA, Fleming GF, Monk BJ, Huang H, Mannel RS, Homesley HD, Fowler J, Greer BE, Boente M, **Birrer MJ**, Liang SX; Gynecologic Oncology Group. Incorporation of bevacizumab in the primary treatment of ovarian cancer. *N Engl J Med*. 365(26):2473-83, 2011 Dec 29.

Cancer Genome Atlas Research Network. Integrated genomic analyses of ovarian carcinoma. *Nature*. 474(7353):609-15, 2011 Jun 29.

Ryan Corcoran, MD, PhD

The Corcoran laboratory focuses on developing new and effective therapies for gastrointestinal cancers, such as colorectal and pancreatic cancers, by targeting the specific survival signals that are active in a given patient's cancer. Our research utilizes targeted therapies, which are drugs that inhibit signaling pathways activated by the specific mutations that drive individual tumors. Since cancer cells often become resistant to these targeted therapies by activating alternative signaling pathways, we focus on identifying these key resistance signals in cancer cells. We utilize this information to devise effective combinations of targeted therapies that anticipate and ultimately overcome these mechanisms of drug resistance. Overall, our goal is to develop promising therapeutic strategies that can be evaluated in clinical trials for patients whose cancers are driven by specific mutations.

Corcoran Laboratory

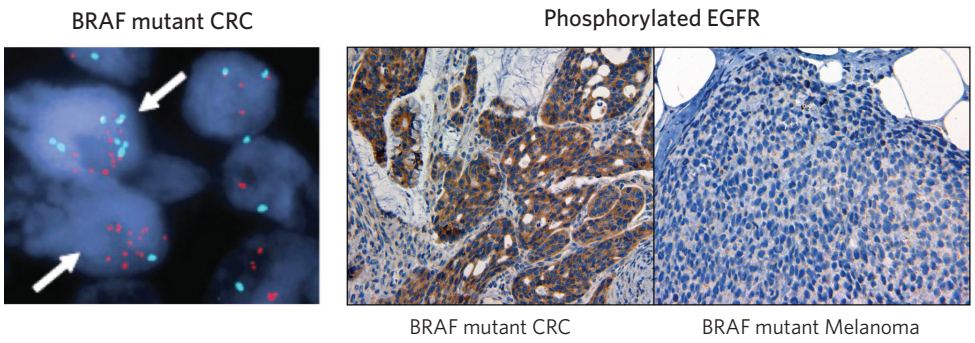
Leanne Ahronian, PhD
Ryan Corcoran, MD, PhD
Jason Godfrey, MS
Anisa Khadraoui
Koki Nishimura

Targeted therapy strategies for gastrointestinal cancers

Our laboratory focuses on targeted therapies directed against specific mutations commonly found in human gastrointestinal cancers, with a focus on BRAF and KRAS mutant cancers. Our work explores the hypothesis that the optimal therapy for individual tumors may vary widely based on the genetic alterations present, and that prior knowledge of these genetic changes and an understanding of the signaling pathways involved will allow us to select an optimal targeted agent or combination of agents capable of inhibiting the critical survival signals within a given tumor. Knowing that cancers often activate parallel or redundant signaling pathways to become resistant to specific targeted therapies, our laboratory also focuses on identifying these critical resistance signals and using this information to devise combinations of targeted agents that can overcome or even prevent resistance.

BRAF mutant colorectal cancer

BRAF mutations occur in 10-15% of colorectal cancers and confer poor prognosis. Interestingly, while BRAF inhibitors have shown dramatic anti-tumor activity in the ~50% of melanomas that harbor BRAF mutations, these agents have been largely ineffective in BRAF mutant colorectal cancers. Therefore, our laboratory has focused on determinants of resistance to BRAF inhibitors in BRAF mutant colorectal cancers. We have identified BRAF amplification as a potential cause of acquired and de novo resistance in BRAF mutant colorectal cancers (*Science Signaling*, 2010), and have shown that combined BRAF and MEK inhibition can overcome resistance. Additionally, we have found that EGFR-mediated reactivation of MAPK signaling contributes to the relative insensitivity of BRAF mutant colorectal cancers to BRAF inhibition, compared to BRAF mutant melanomas, and that combined BRAF and EGFR inhibition can overcome resistance, leading to tumor regressions in BRAF mutant colorectal cancer models in mice (*Cancer Discovery*, 2012). We are also focused on identifying additional causes of de novo resistance in BRAF mutant cancers using a combination of preclinical models and



Resistance mechanisms in BRAF mutant colorectal cancers. BRAF amplification (left, red probes) and increased activation of EGFR (right) can lead to BRAF inhibitor resistance in BRAF mutant colorectal cancer (CRC).

patient tumor specimens. Simultaneously, we are developing biomarkers to predict response to therapy (*Cancer Discovery*, 2011), including real-time pharmacodynamic assessment of signaling changes in on-treatment patient tumor biopsies (*Science Translational Medicine*, 2013), and combination therapy strategies to overcome resistance.

KRAS mutant cancers

KRAS is the most commonly mutated oncogene in human cancer and is mutated in ~20% of all cancers, with particularly high frequency in pancreatic (~90%) and colorectal cancers (~40%). However, currently no effective therapies exist for KRAS mutant cancers, likely because KRAS itself has proven difficult to target directly with small molecules. Our current work focuses on identifying novel target pathways in KRAS mutant cancers though hypothesis-based and large-scale pooled RNA interference screening approaches, with the goal of developing new targeted therapy combination approaches for KRAS mutant cancers. Recently, through a pooled RNA interference drug screen, we identified combined targeting of BCL-XL and MEK as a promising therapeutic strategy that leads to dramatic tumor regressions in KRAS mutant mouse tumor models (*Cancer Cell*, 2013). We are currently expanding these approaches to identify other potentially effective targets and therapeutic strategies in KRAS mutant cancers.

Translational Oncology

The overall goal of our research is to develop improved treatments for patients with gastrointestinal cancers and to identify molecular markers that may help us identify those patients most likely to respond to a given therapy. As such, our laboratory takes a highly translational approach with a central focus bringing new therapeutic strategies into the clinic for evaluation in novel clinical trials. Based on our observation that combined BRAF and MEK inhibition can overcome certain resistance mechanisms in leads BRAF mutant colorectal cancer models, we developed and completed a clinical trial assessing combined BRAF and MEK inhibition in patients with BRAF mutant colorectal cancer, which showed promising activity in a subset of patients. (*ASCO abstract*, *J ClinOncol*, 2013). Based on our observations that EGFR may contribute to resistance in many BRAF mutant colorectal cancers, we are currently enrolling patients to clinical trials evaluating the combination of BRAF and EGFR inhibitors. Finally, we are developing a clinical trial combining the BCL-XL/BCL-2 inhibitor navitoclax with the MEK inhibitor trametinib in KRAS mutant cancers.

Selected Publications:

Corcoran RB*, Rothenberg SM*, Hata A, Piris A, Nazarian R, Brown RD, Godfrey JT, Winokur D, Walsh J, Mino-Kenudson M, Maheswaran S, Settleman J, Wargo JA, Flaherty KT, Haber DA, Engelman JA. TORC1 suppression predicts responsiveness to vemurafenib in BRAF mutant melanoma. *Science Translational Medicine*. 5: 196ra98 (2013).

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Faber AC*, **Corcoran RB***, Ebi, H*, Sequist LV, Waltman BA, Chung E, Incio J, Digumarthy S, Pollock S, Song Y, Lifshits E, Roberge S, Coffman EJ, Benes CH, Gomez H, Baselga J, Arteaga C, Rivera MN, Dias-Santagata D, Jain R,Engelman JA.BIM expression in treatment naïve cancers predicts responsiveness to kinase inhibitors. *Cancer Discovery*. 1: 352-65 (2011). PMCID:3229203.

* denotes equal contribution

Nick Dyson, PhD

The Dyson Laboratory studies the role of the retinoblastoma tumor suppressor gene (RB). RB is found in most cell types and it enables cells to stop dividing. RB is inactivated in many types of cancer; a change that is thought to be an important step in tumor progression. We have three main goals. First, we want to understand the molecular details of how RB acts. Second, we want to understand how the inactivation of RB changes the cell. Third, we want to be able to use these insights to target tumor cells. There are three general opportunities. If we can identify the specific defects that promote tumor development it may be possible to suppress these changes. Alternatively, the properties of RB-deficient cells may represent points of weakness that are specific to tumor cells and that can be enhanced. Another possibility stems from the knowledge that RB is actually expressed in many tumor cells but it has insufficient activity to stop cell division. For these tumors, the challenge is to find ways to enhance the activity of RB.

Dyson Laboratory

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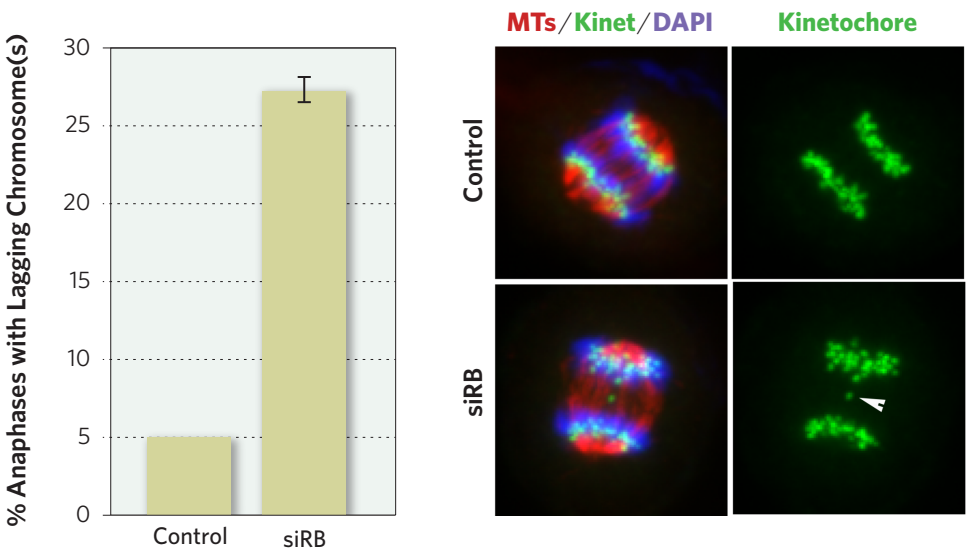
Ioannis Sanidas, PhD
Michael Korenjak, PhD
Amity Manning, PhD
Wayne Miles, PhD
Brandon Nicolay, PhD
Katrin Tschoep, PhD

We investigate the mechanisms that limit cell proliferation in normal cells and the ways that these controls are eroded in cancer cells. Our research focuses on the retinoblastoma tumor suppressor (RB) and its target, the E2F transcription factor. E2F controls the expression of a large number of target genes that are needed for cell proliferation. This transcriptional program is activated when normal cells are instructed to divide, but it is deregulated in tumor cells, providing a cellular environment that is permissive for uncontrolled proliferation. RB has multiple functions, but one of its most important roles is to limit the activity of E2F. As a result, most tumor cells select for changes that compromise RB function. Publications in the past year include progress in three different aspects of E2F/RB biology.

The original discovery of the RB1 gene was made possible by the fact that its mutation is a causal and rate-limiting event in the development of retinoblastoma. An exciting development in this area of research has been the discovery that the loss of pRB undermines genomic stability. A careful analysis of pRB-deficient cells revealed that pRB loss leads to centromere dysfunction, reduced cohesion, and whole chromosome instability (CIN). CIN is a common feature in tumor cells. High levels of CIN correlate with poor prognosis and promote tumor relapse after seemingly effective anticancer treatments. The finding that pRB loss causes CIN raised the possibility that the functional inactivation of pRB may be an underlying source of much of the aneuploidy seen in tumor cells. We discovered that pRB-loss reduces the level of both condensin II complexes and cohesin complexes on chromosomes. Our recent results (Manning et al 2014) show that reducing the levels of Wapl, a negative regulator cohesin loading, remarkably corrects the centromeric defects of RB-depleted cells and strongly suppresses CIN. Wapl-depletion also suppressed CIN in a panel of tumor cells in which pRB was functionally compromised. Reducing CIN may limit the ability of tumor cells to evolve, and these observations will make it possible to test whether reducing CIN can improve the effectiveness of targeted therapies in RB-mutant tumors.



The depletion of pRB results in a high frequency of lagging chromosomes. pRB was selectively depleted from RPE1 cells, a non-tranformed cell line, and the appearance of lagging chromosomes during anaphase was scored in cells stained to show the kinetochore (green), microtubules (red) or DNA (blue).

In many tumors the regulation of RB is altered by mutations, such as loss-of-function mutations in p16INK4A (CDKN2A), that elevate the activity of CDK4 or CDK6). CDK/6 inhibitors are currently in clinical trials but these often have only modest or transient effects. We searched for targeted therapies that might enhance the effects of CDK4/6 inhibitors and found a strong synergy between CDK4/6 inhibitors and the IGF1R/IR inhibitor BMS-754807. This combination blocked proliferation of p16INK4-deficient pancreatic ductal adenocarcinoma (PDAC) cells that are inherently resistant to CDK4/6 inhibitors (Heilmann et al 2014). Sensitivity to this drug combination was seen in vitro and in vivo and correlated with reduced activity of the master growth regulator mechanistic target of rapamycin complex 1 (mTORC1). Accordingly, replacing the IGF1R/IR inhibitor with the rapalog inhibitor temsirolimus broadened the sensitivity of PDAC cells to CDK4/6 inhibition. These results establish targeted therapy combinations with robust cytostatic activity in p16INK4-deficient PDAC cells and have potential implications for improving treatment of a broader spectrum of human cancers characterized by p16INK4 loss.

Working with the premise that the key targets of RB/E2F regulation are likely to have been maintained during evolution, we searched genome-wide datasets for groups of evolutionary conserved, functionally-related genes that are directly bound by pRB/E2F proteins. In this way, we discovered that the expression of NANOS, a key facilitator of the Pumilio post-transcriptional repressor complex, is directly repressed by pRB/E2F in flies and humans (Miles et al 2014). In both species, NANOS expression increases following inactivation of pRB/RBF1 and becomes important for tissue homeostasis. Interestingly, analysis of datasets from normal retinal tissue and pRB-null retinoblastomas revealed that there is a strong enrichment for putative PUM substrates among genes de-regulated in tumors. Functional assays indicate that NANOS increases in importance in pRB-deficient cells and helps to maintain homeostasis by repressing the translation of PRE-containing transcripts. We hypothesize that increased translational control helps to limit the consequences of deregulated E2F-dependent transcription in tumor cells.

Selected Publications:

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Leif William Ellisen, MD, PhD

Cancer therapy is being revolutionized through the development of more specific and less toxic treatment approaches that are collectively known as targeted therapeutics. A key to the successful application of targeted cancer therapy is the identification of specific genetic abnormalities within tumor cells that are not present in normal tissues. **The Ellisen laboratory** is broadly interested in identifying these genetic abnormalities, understanding how they influence the biology of cancer cells, and discovering how that biology can inform the selection of the most effective therapy for each patient. We address these questions through basic research studies of key tumor-cell signaling pathways and through genetic analysis of patient tumor samples conducted in partnership with the Massachusetts General Hospital Translational Research Laboratory (TRL). Our discoveries in both the basic laboratory and the TRL have already been translated to clinical trials that seek to identify new predictive markers and new therapeutic strategies for breast and other cancers.

Ellisen Laboratory

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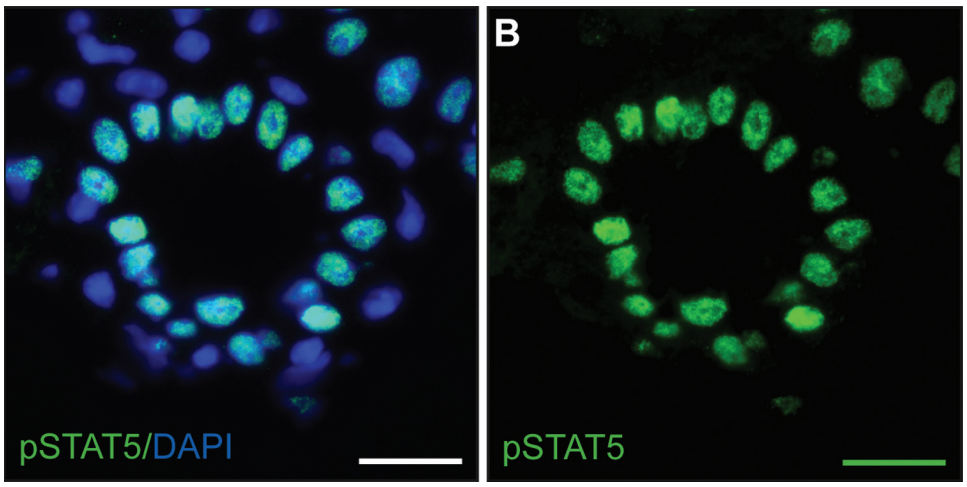
Our group is broadly interested in how genetic abnormalities within cancer cells influence their biology and how that biology can, in turn, be exploited to therapeutic advantage. We address these questions through basic research studies of key tumor cell signaling pathways including p53, mTOR, and BRCA1/2. This work is complemented by genetic analysis of patient tumor samples conducted in partnership with the Massachusetts General Hospital Translational Research Laboratory (TRL). Our discoveries in both the basic laboratory and the TRL are being applied in ongoing clinical trials that seek to identify predictive markers for response to specific therapeuties for breast and other cancers. Our ability to work at the interface of basic tumor biology and therapeutic application is strongly supported by our network of collaborators and by the research and clinical infrastructure of the Mass General Cancer Center.

The p53 network in cancer biology and therapy

The p53 tumor suppressor is inactivated in more than 50% of sporadic human cancers, and patients carrying heterozygous germline p53 mutations show striking tumor predisposition . P53 encodes a transcription factor that functions as a key nodal point for integrating cellular responses to DNA damage. As such, p53 regulates genes involved in diverse cellular processes including cell cycle progression, apoptosis and angiogenesis. The identification of two p53-related genes, p63 and p73, provided a new paradigm in the study of p53. We and others have defined a functional network through which these factors interact in human tumorigenesis. These findings are likely to explain the observation that p63 is over-expressed in a broad variety of epithelial tumors, particularly squamous cell and breast carcinomas. Our recent work has revealed roles for p63 and



The lactating mammary alveolus (shown) requires activation of STAT5 (pSTAT5, green/aqua) in luminal cells, which is controlled by paracrine hormonal signaling from basal cells (blue). Loss of this signaling may block luminal differentiation and predispose to breast cancer.

p73 in a variety of cancers, including the refractory triple-negative breast cancer subtype which occurs commonly in BRCA1 mutation carriers. Our success in defining novel functional interactions within the p53 family provides new therapeutic possibilities for these treatment-refractory malignancies. We are currently carrying out high-throughput approaches to identify specific therapeutic targets within the critical pathways we have uncovered.

P53 and TOR-associated metabolic reprogramming in tumorigenesis

Our efforts to identify new pathways regulated by p53 family members have yielded surprising insights into the re-wiring of cellular metabolism that drives carcinogenesis. A central player in this effect is REDD1, a p53-regulated gene we identified that functions as a critical negative regulator of the mammalian Target of Rapamycin (mTOR) kinase. Most human tumors exhibit abnormalities of p53 and/or mTOR signaling, and our recent studies have demonstrated the contribution of REDD1 to autophagy and metabolic homeostasis during tumorigenesis. We are currently using animal models, in vitro studies, and biochemical approaches to understand key metabolic dependencies of tumors that can be exploited to therapeutic advantage.

Tumor genotyping to drive personalized cancer therapy

Specific somatic genetic abnormalities—including gene mutation, rearrangement and amplification—are acquired by nascent tumor cells and drive cancer pathogenesis. Activation of diverse oncogenes (e.g., RAS, RAF, EGFR) through such somatic mutation not only causes cancer, but is now known to be an important determinant of the clinical response to targeted therapeutics. Until recently, identifying such abnormalities was restricted to research settings as the technologies required for routine, high-performance tumor genotyping were not available. The Mass General TRL has developed and validated high-throughput clinical diagnostic platforms for broad-based tumor genetic analysis. The availability of tumor genotyping for our large cancer patient population is accelerating the clinical trials process and is providing remarkable new opportunities for translational research.

Selected Publications:

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Jeffrey A. Engelman, MD, PhD

The overarching aim of research in **the Engelman laboratory** is to develop new and more effective therapeutic strategies for the treatment of cancer, with a particular emphasis on lung cancer. Cancer therapies are changing from general chemotherapeutic agents to drugs that target specific proteins and signaling pathways (i.e., targeted therapies). Our laboratory aims to understand the biological underpinnings of cancer sensitivity and resistance to this emerging class of therapies. We are particularly interested in the regulation of the PI3K pathway, a signaling network that is crucial for the growth and survival of many epithelial cancers. The ultimate goal of our research is to develop therapies that are more effective and less toxic for patients with cancer.

Engelman Laboratory

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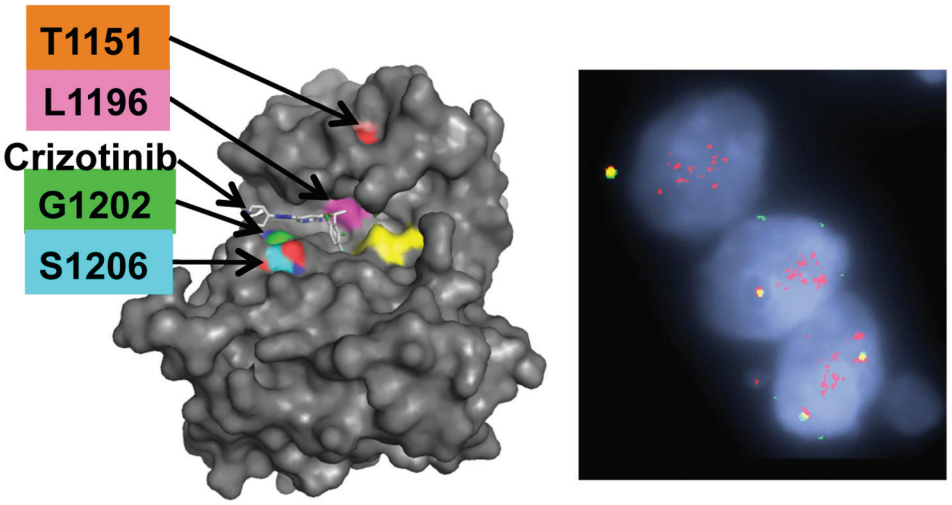
Targeted therapies

The research goal of my laboratory is to advance targeted therapies to benefit patients with cancer. Our research focuses on understanding the biological underpinnings of sensitivity and resistance to specific kinase inhibitor targeted therapies in cancers with specific genetic abnormalities. In particular, we focus on the regulation of key signaling networks that regulate cancer cell growth and survival, such as the PI3K-AKT and MEK-ERK signaling pathways. We study how perturbation of specific signaling pathways (alone or in combination) impairs cell growth and induces cell death in the context of specific genetic abnormalities. More recently, our research has also begun to focus on how one should model responsiveness to therapies in the laboratory to optimally inform what will occur in the clinic. Our studies encompass biochemistry, molecular biology, cell culture models, mouse models and assessment of clinical specimens. Our laboratory studies encompass established targeted paradigms such as EGFR-, ALK-, and ROS-mutant lung cancers as well as cancers for which no effective targeted therapy currently exists, such as KRAS mutant cancers.

Resistance mechanisms to targeted therapies

Unfortunately, clinical experience has taught us that we cannot yet cure even the simple cancers that are addicted to a single kinase. Although targeted therapies are often initially very effective for such cancers, these cancers almost always develop resistance. For example, EGFR-mutant lung cancers that are sensitive to EGFR inhibitors invariably develop resistance, and the same is true for EML4-ALK lung and BRAF-mutant cancers treated with ALK and BRAF inhibitors respectively. Resistance usually develops within one year in each of these cancer paradigms. My laboratory is highly focused on understanding how resistance develops in patients so we can devise strategies to overcome it or thwart its emergence. To understand how resistance to these therapies develops, we culture genetically defined, sensitive cancer cell line models until resistance emerges (in vitro and



ALK resistance mutations and gene amplification (red probes) identified in ALK positive lung cancers with acquired resistance to crizotinib.

in vivo). We then use these models to figure out how resistance develops. Using these methodologies, we have discovered resistance mechanisms that occur in patients, and these findings have led to novel therapeutic strategies that are being explored in the clinic.

These laboratory efforts leverage complementary efforts in the clinic in which we actively biopsy tumors from patients upon the development of resistance. These patient-tumor-derived models have proven invaluable for elucidating results from clinical trials and developing new ideas for future therapeutic strategies. By developing patient-derived models in the laboratory, we are able to determine how cancers become resistant to therapies in the clinical trial and to identify new treatment strategies to overcome resistance.

Selected Publications:

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David E. Fisher, MD, PhD

The Fisher laboratory focuses on mechanistic studies which underlie the biology and pathophysiology of skin and melanoma. Research studies range from molecular analyses of pigment cell biology to risk factors responsible for the formation of melanoma and other skin cancers. The laboratory utilizes deep molecular tools to understand how genes are regulated, how they contribute to cancer formation, and how they may be successfully targeted by drugs in order to improve disease treatments or to prevent disease formation altogether. Several areas of particular focus include 1) the study of redhead, fair skinned pigmentation and the manner in which such individuals are at increased risk for skin cancer; 2) identification and analysis of oncogenes which control melanoma cell survival; 3) discovery of new drugs that affect pigmentation, melanoma survival, and other skin-related effects; and 4) examination of the ways in which a gene called MITF plays a master-regulatory role in specifying the development of pigment-producing cells in the body.

Fisher Laboratory

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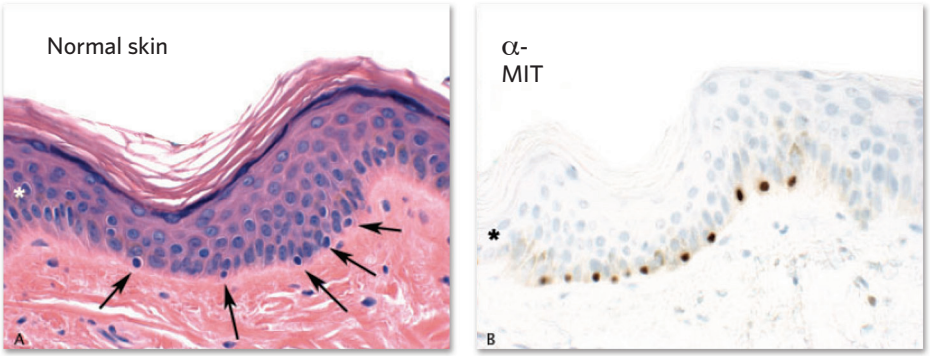
Our group studies cell death/proliferation signals in relation to development and disease, particularly in cancer of pigment cells (melanoma) and tumors of childhood. We attempt to understand critical modes of cell homeostasis with a goal of molecular targeted therapy as well as prevention of melanoma and other human cancers. Areas of particular focus are explained below.

Lessons for malignancy from normal development

We study the biology of melanocytes as a means of identifying pathways which drive human melanoma. This area of research includes examination of the mechanisms underlying the growth/survival of benign moles, most of which contain mutations in either BRAF or N-Ras oncogenes. We also study melanocyte death in hair follicles, a process associated with hair graying. Our work led to the identification of pathways linking graying to melanocyte and melanoma survival, offering potential leads for novel therapies. Other studies focus on pathways modulating melanocytic responses to environmental cues and employ oncogene-transformed melanocytic lines which exhibit growth factor independence, mimicking human melanoma in a genetically controlled manner.

Control of life and death in melanoma

Malignant transformation of melanocytes produces one of the most treatment-resistant malignancies in human cancers. We have identified a transcriptional network that regulates melanoma cell survival and proliferation and melanocyte differentiation during development. Using diverse methods—including mouse models, human tumor expression arrays, and cellular assays—we examine mechanisms through which melanoma cells evade death with the goal of improving therapy. Studies include preclinical



Histologic images of human skin. Left image shows hematoxylin and eosin (H&E) stain. The top layer is Stratum Corneum (consisting of dead cell derivatives) followed by the deeper purple keratinocyte cell layers constituting the epidermis. Beneath the epidermis is the pink, collagen containing dermis. Melanocytes reside at the base of the epidermis and are highlighted by arrows. The image to the right shows antibody staining for the melanocytic transcription factor MITF, which highlights the melanocytes at the dermal-epidermal junction. Histologic images were generated by Dr. Scott Granter.

and clinical analyses of novel melanoma treatments. We also study the role of UV in pigmentation responses and carcinogenesis.

MITF transcription factor family in development and cancer

MITF is a helix-loop-helix factor homologous to the Myc gene which, when mutated in humans, produces absence of melanocytes. MITF acts as a master regulator of melanocyte development and is targeted by several critical signaling pathways. Recently, members of the MITF family have been identified as oncogenes in a variety of human malignancies, particularly sarcomas of childhood. We are currently investigating their roles in cancer as well as strategies to target them therapeutically. Detailed mechanistic studies focus on transcription factor interactions with chromatin, and epigenetic control of gene expression.

Selected Publications:

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Gad Getz, PhD

The Getz Laboratory is focused on cancer genome analysis which includes two major steps: (i) *Characterization* – cataloging of all genomic events and the mechanisms that created them during the clonal evolution of the cancer, including events at the DNA, RNA and protein levels in normal and tumor samples from an individual patient; and (ii) *Interpretation* – analysis of the characterization data across a cohort of patients with the aim of identifying the alterations in genes and pathways that cause cancer or increase its risk as well as identifying molecular subtypes of the disease, their markers and relationship to clinical variables.

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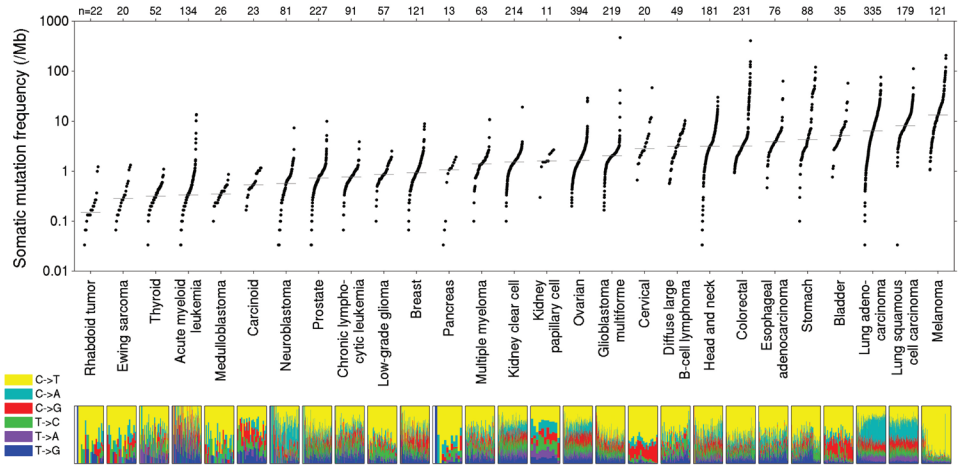
Characterizing the Cancer Genome

Cancer is a disease of the genome that is driven by a combination of possible germline risk-alleles together with a set of 'driver' somatic mutations that are acquired during the clonal expansion of increasingly fitter clones. Mutations occur at all levels and scales, including DNA point mutations, small insertions and deletions, larger genomic rearrangements and copy-number alterations, as well as epigenetic, transcriptional and proteomic changes. In order to generate a comprehensive list of all germline and somatic events that occurred during life and the development of the cancer, we are applying and developing highly sensitive and specific tools for detecting these events in massively-parallel sequencing data (and other high-throughput data). The volume and noise in these data, as well as the complexity of the underlying genomes present in a cancer sample, require developing computational tools using state-of-the-art statistical and machine learning approaches to most efficiently extract the signal from the noise (tools we developed include MuTect, Indelocator, SegSeq, CapSeg, dRanger and BreakPointer). We are also developing rigorous benchmarking approaches to carefully assess the sensitivity and specificity of these tools to detect the various types of events in different experimental and sample conditions to help guide and interpret the experiments.

Detecting Cancer-Associated Genes

Once we detect the events in the cancer genomes, we analyze them across a cohort of samples searching for genes (and pathways) that show significant signals of positive selection, e.g. the number of mutations exceeds what is expected by random chance. In order to do that, we need to construct a detailed statistical model of the background mutational processes and then detect genes that deviate from this model. As part of constructing the background models, we study and infer the mutational processes that affected the samples, such as the types of carcinogens, defects in repair mechanisms and general mutational mechanisms. In some cases we can also infer the order of events.

We have developed tools for detecting genes which are significantly gained or lost in cancer (GISTIC) and genes with increased density or irregular patterns of mutations (MutSig). We recently reported the importance of modeling the heterogeneity of these mutational



Somatic mutation frequencies across cancer.
Each dot represents the total frequency of somatic mutations (in the exome) in each tumor–normal pair. Tumor types are ordered by their median somatic mutation frequency, from haematological and paediatric tumors (left), to tumours induced by carcinogens such as tobacco smoke and ultraviolet light (right). Mutation frequencies vary more than 1,000-fold between lowest and highest across different cancers and also within several tumour types. The bottom panel shows the relative proportions of the six different possible base-pair substitutions. Taken from Lawrence et al. (2013).

processes across patients, sequence contexts and along the genome, when searching for cancer-associated genes. We are continuously improving these methods and working towards generating a unified method that takes into account all types of alterations and incorporates prior knowledge to better detect cancer genes and driver alterations

Heterogeneity and clonal evolution of cancer

Cancer samples are heterogeneous, containing a mixture of normal (i.e. non-cancer) cells and a population of cancer cells that often represents multiple subclones. Keeping in mind that cancer is a dynamic system, these subclones may represent the remaining cells of less-fit clones which have not yet been overtaken by the expanding most-fit clone or they may represent interacting sub-clones that co-evolved to support each other and reached an equilibrium or a combination of these scenarios. Our lab has been developing tools (ABSOLUTE) for characterizing the heterogeneity of cancer samples using copy-number, mutational and other data measured on bulk samples and now also getting into the analysis of single or few cells. Using these tools, we can infer which mutations are clonal (i.e. exist in all cancer cells) or sub-clonal (i.e. exist in subclones), as well as estimate the number of subclones and monitor their evolution over time or space by studying multiple samples from the same patient. In a recent study of chronic lymphocytic leukemia, we demonstrated that sub-clonal driver mutations are associated with shorter time to relapse after treatment. This demonstrates the importance of taking clonal information into account in clinical trials and correlating the clonal/sub-clonal nature of the mutations and in particular driver mutations that are targeted by therapy with clinical outcome. Our aim is to introduce these concepts to various clinical trials.

Selected Publications:

Lawrence MS, Stojanov P, Mermel CH, Robinson JT, Garraway LA, Golub TR, Meyerson M, Gabriel SB, Lander ES*, **Getz G***. Discovery and saturation analysis of cancer genes across 21 tumour types. *Nature*. 2014 Jan 5.

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Mermel CH, Schumacher SE, Hill B, Meyerson ML, Beroukhim R*, **Getz G***.GISTIC2.0 facilitates sensitive and confident localization of the targets of focal somatic copy-number alteration in human cancers. *Genome Biol*. 2011 Apr 28;12(4):R41

*Co-corresponding authors

Timothy Graubert, MD

The Graubert laboratory focuses on the molecular basis of human blood cancers, including acute myeloid leukemia and myelodysplastic syndromes. The laboratory utilizes a variety of genomic platforms to interrogate primary samples from patients with myeloid malignancies to identify inherited and somatic mutations that drive these diseases. The goal of these studies is to gain insight into the biological basis of myeloid leukemias, and to improve strategies for diagnosis, risk stratification, and targeted therapy.

Graubert Laboratory

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Timothy A. Graubert, MD
Emily Silva

Clonal heterogeneity of myelodysplastic syndromes

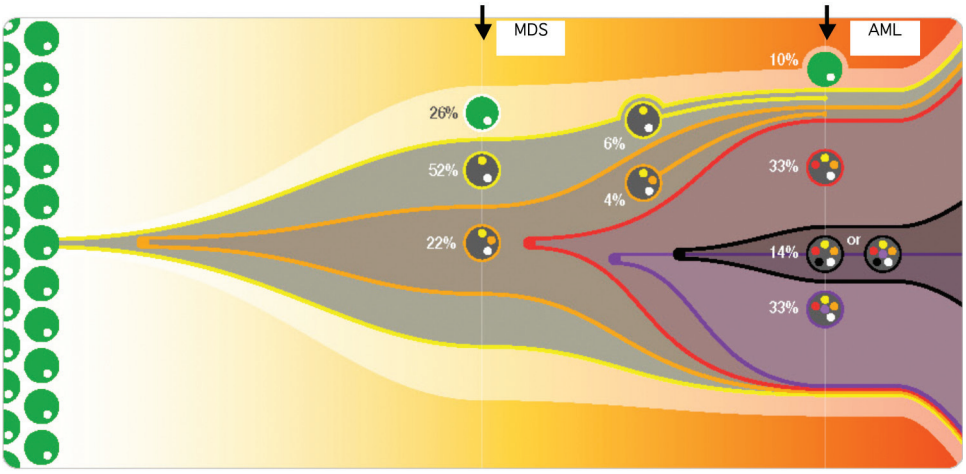
Myelodysplastic syndromes are the most common form of acquired bone marrow failure in adults. Despite the ineffective hematopoiesis that is characteristic of this disease in its early stages, we found through whole genome sequencing that nearly all cells in the bone marrow of these patients are clonally derived (see Figure). When patients evolve to acute myeloid leukemia (which occurs in approximately one third of cases), new subclonal populations emerge that are derived from the original ("founding") clone. These findings raise the possibility that the prognostic value of recurrent mutations in myelodysplastic syndrome and the efficacy of therapies that target these mutations may depend not only on the presence or absence of these mutations, but also on their position within the clonal hierarchy of this disease.

RNA splicing defects at the root of myelodysplastic syndromes

We and several other groups discovered recurrent somatic mutations in genes encoding core components of the RNA splicing complex (the "spliceosome") in patients with myelodysplastic syndrome. Mutations in this pathway tend to be mutually exclusive, suggesting that more than one splicing gene mutation in a cell provides no additional selective advantage, or is deleterious to the clone. We have focused on *U2AF1* which encodes a component of the U2 snRNP that binds to the AG dinucleotide at the 3′ intronic splice acceptor site. Mutations in U2AF1 arise early in the pathogenesis of myelodysplastic syndromes (in the founding clone) and affect almost exclusively two codons in predicted zinc finger domains. We have shown that the most common mutation (S34F) has gain-of-function activity in splicing assays. Current work in the Graubert laboratory is focused on comprehensive analysis of the impact of U2AF1 mutations on splicing, generation of mouse models of these mutations, and testing the hypothesis that splicing gene mutations create a dependency in cells that confers sensitivity to the cytotoxic effects of small molecule modulators of the splicing complex.

Inherited predisposition to myelodysplastic syndrome/acute myeloid leukemia

Acute myeloid leukemia and myelodysplastic syndromes are usually sporadic, late-onset cancers, but in rare instances (<1%) these diseases aggregate in families. In these families, predisposition to acute myeloid leukemia/myelodysplastic syndrome may be a consequence of an inherited bone marrow failure syndrome, but in other cases these are highly penetrant, autosomal dominant,



Clonal evolution from myelodysplastic syndrome (MDS) to acute myeloid leukemia (AML). Whole genome sequencing at the time of MDS diagnosis (left arrow) in a representative patient identified a founding clone comprising ~52% of the bone marrow cellularity and a subclone derived from the founding clone in ~22% of cells. When this patient progressed to AML (right arrow), the original clones were still present and had spawned three new subclones that were dominant in the bone marrow at this time point.

Mendelian disorders. Three genes (*RUNX1, GATA2, CEBPA*) explain roughly half of these Mendelian cases. The genetic basis in the remaining half is not yet known. Furthermore, the latency and incomplete penetrance of acute myeloid leukemia/myelodysplastic syndrome in mutation carriers suggest that acquisition of cooperating somatic mutations is required for malignant transformation. We have accumulated a large panel of samples from affected and unaffected members of these families. Ongoing studies in the Graubert laboratory are focused on identification of novel germline variants in families that lack known predisposing factors and characterization of the landscape of cooperating somatic mutations that arise in these cases. This information is important for genetic counseling in these families, for selection of optimal bone marrow transplant donors, and to increase our understanding of the biological basis of acute myeloid leukemia and myelodysplastic syndromes.

Selected Publications:

Hughes AEO, Magrini V, Demeter R, Miller CA, Fulton R, Fulton LL, Eades WC, Elliott K, Heath S, Westervelt P, Ding L, Conrad DF, White BS, Shao J, Link DC, DiPersio JF, Mardis ER, Wilson RK, Ley TJ, Walter MJ, **Graubert TA**. Clonal architecture of secondary acute myeloid leukemia defined by single-cell sequencing. *PLoS Genet*. 2014 Jul 10;10(7):e1004462.

Klco JM, Spencer DH, Miller CA, Griffith M, Lamprecht TL, O'Laughlin M, Fronick C, Magrini V, Demeter RT, Fulton RS, Eades WC, Link DC, **Graubert TA**, Walter MJ, Mardis ER, Dipersio JF, Wilson RK, Ley TJ. Functional heterogeneity of genetically defined subclones in acute myeloid leukemia. *Cancer Cell*. 2014 Mar 17;25(3):379-92.

Janke MR, Baty JD, **Graubert TA**. SWR/J Mice are Susceptible to Alkylator-Induced Myeloid Leukemia. *Blood Cancer Journal*, 2013;3:e161.

The Cancer Genome Atlas Research Network. The Genomic and Epigenomic Landscape of Adult de novo Acute Myeloid Leukemia. *N Engl J Med*. 368:2059-2074, 2013 May 30.

Walter MJ, Shen D, Shao J, Ding L, White BS, Kandoth C, Miller CA, Niu B, McLellan MD, Dees ND, Fulton R, Elliot K, Heath S, Grillot M, Westervelt P, Link DC, DiPersio JF, Mardis E, Ley TJ, Wilson RK, **Graubert TA**. Clonal diversity of recurrently mutated genes in myelodysplastic syndromes. *Leukemia*. 2013 Jun;27(6):1275-82.

Walter MJ, Shen D, Ding L, Shao J, Koboldt DC, Chen K, Larson DE, McLellan MD, Dooling D, Abbott R, Fulton R, Magrini V, Schmidt H, Kalicki-Veizer J, O'Laughlin M, Fan X, Grillot M, Witowski S, Heath S, Frater JL, Eades W, Tomasson M, Westervelt P, DiPersio JF, Link DC, Mardis ER, Ley TJ, Wilson RK, **Graubert TA**. Clonal architecture of secondary acute myeloid leukemia. *N Engl J Med*. 2012 Mar 22;366(12):1090-8.

Wilhelm Haas, PhD

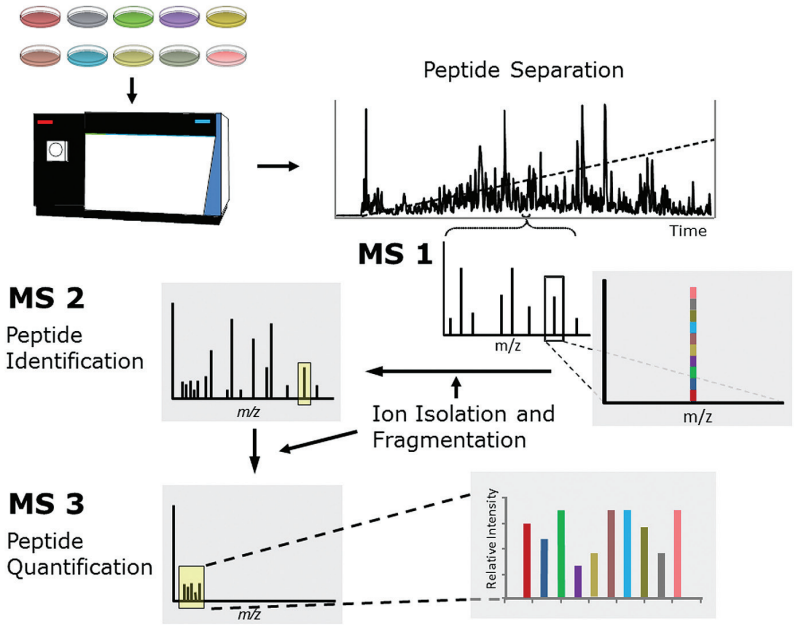
The Haas laboratory uses quantitative mass spectrometry-based proteomics to study the cellular pathways that characterize cancer cells in a comprehensive proteome-wide manner. This is fueled by recent discoveries that have enhanced the depth and throughput of proteomics in quantifying proteins and their post-translational modification. These improvements have put us at a pivotal point in the field of mass spectrometry, where - for the first time – we are able to handle the analysis of the large number of samples that have to be examined to generate the basis for understanding a disease that displays the heterogeneity found in cancer. Beyond trying to understand the global changes that occur in cancer cells, we are applying these methods to expand our understanding of how the proteome is altered when resistance emerges in response to treatment in individual patients. We believe that proteomics has the potential to become a diagnostic tool in cancer by identifying novel protein biomarkers that may be used to diagnose cancer, predict its susceptibility and monitor its progression.

Haas Laboratory

Myriam Boukhali, MS
Amanda Edwards, PhD
Wilhelm Haas, PhD
John Lapek, PhD

Cancer is based on dynamic changes of the genome that ultimately translate into an altered proteome, optimized for uncontrolled cell growth and division. In addition, many pathways initially causing cancer further promote the propagation of altered genetic information, accelerating the adaption of cancer cells to new environments. This dynamic process becomes even more complex if taking into account the dynamic state of the cellular proteome that is regulated by protein synthesis and degradation, posttranslational modifications, protein localization, and the interaction of proteins with other proteins as well as with different classes of biomolecules. While the "cancer genome" can now be easily accessed due to advances in DNA sequencing technology, the information contained in the "cancer proteome" has remained largely untapped due to technical challenges in quantifying the large amount of proteins expressed in mammalian cells. Yet, the proteome holds an enormous potential to improve our understanding of the basic principles underlying cancer to revolutionize early diagnosis of the disease and to improve patient care. Up to date, virtually all targeted therapeutics in cancer treatment are targeting proteins. Understanding how these drugs alter the proteome has the potential to help us refine our approaches to drug design.

Despite the potentials of studying the proteome in order to improve our understanding of cancer, the proteome-contained information is substantially underused in cancer research. This is based on technical limitations of the proteomics technology, which for a long time did not match the capabilities of genetics tools already widely used in studying cancer. However, the past few years brought enormous improvements in all aspects of proteomics but especially in mass spectrometry, the main tool used in studying the proteome.



The Tandem Mass Tag MS3 (TMT-MS3) method allows the accurate quantification of up to ten proteome samples in a single experiment.

The performance gap between genomics and proteomics technologies is closing fast and this provides the optimal basis for my laboratory to start our work at the MGH Cancer Center.

The level of a high comprehensiveness in proteomics, which allows us to quantify almost all proteins and their post-translational modifications in a single experiment, was a first step in increasing the technology's competiveness in comparison to genomics tools. A second and more recent improvement was the enhancement of the technology's throughput, which now enables us to quantify up to 10 different samples in one experiment. In addition to applying these new methodologies to samples from primary tumor and cell culture models, my lab will continue to work on improving both aspects by developing methods that will allow a more efficient monitoring of levels of post-translational modifications but also by increasing the throughput of proteomics through enhancing its multiplexing capacity. Both directions are aimed at improving proteomics as a tool in basic research but also pushing the technology's capacity to enable its use in a clinical environment.

We will apply existing and new methods in two specific areas. By establishing quantitative maps of protein concentration and site specific protein phosphorylation levels from an extensive number of cancer cell lines and primary tumors, we will search for proteome biomarkers in order to direct targeted therapies for individual patients. We will focus these studies on lung cancer and will work in collaboration with the laboratories of Jeffrey Engelman and Cyril Benes. In collaboration with these laboratories, we will study cellular mechanisms that enable cancer cells to develop resistance against treatment by targeted therapeutics. We will work with cell line models and monitor changes in protein and phosphorylation levels while evoking resistance against the treatment with targeted therapeutics. We plan to manipulate levels of proteins or pathways found to be regulated using genetic tools (siRNA) to confirm their role in overcoming the effect of drug treatment.

Selected Publications:

Tolonen AC*, **Haas W***. (2014) Quantitative proteomics using reductive dimethylation for stable isotope labeling. *J. Vis. Exp.* 89, doi: 10.3791/51416.

McAlister GC, Nusinow, DP, Jedrychowski MP, Wühr M, Huttlin EL, Erickson BK, Rad R, **Haas W**, Gygi SP. (2014) MultiNotch MS3 enables accurate, sensitive, and multiplexed detection of differential expression across cancer cell line proteomes. *Anal. Chem.* 86, 7150-8.

Ting L, Rad R, Gygi SP*, **Haas W***. (2011) MS3 eliminates ratio distortion in isobaric multiplexed quantitative proteomics, *Nat. Methods* 8, 937-940.

Wu R, **Haas W**, Dephoure N, Huttlin EL, Zhai B, Sowa ME, Gygi SP. (2011) A large-scale method to measure absolute protein phosphorylation stoichiometries, *Nat. Methods* 8, 677-683.

Tolonen AC*, **Haas W***, Chilaka, AC, Aach J, Gygi SP, Church GM. (2011) Proteome-wide systems analysis of a cellulosic biofuel-producing microbe, *Mol. Syst. Biol.*, 7, 461.

Haas W, Faherty BK, Gerber SA, Elias JE, Beausoleil SA, Bakalarski CE, Li X, Villen J, Gygi SP. (2006) Optimization and use of peptide mass measurement accuracy in shotgun proteomics. *Mol. Cell. Proteomics* 5, 1326-1337.

*Co-corresponding authors

Daniel Haber, MD, PhD

The Haber laboratory focuses on understanding the fundamental genetics of human cancer, from inherited mutations that confer familial predisposition to genetic mutations that are acquired by tumors themselves and may render them susceptible to specific targeted drug therapies. For example, we have identified mutations in the EGFR gene that confer dramatic sensitivity of some lung cancers to drugs that inhibit that pathway, pointing toward the importance of genetic classification of common epithelial cancers in applying novel targeted therapies. We have also collaborated with the bioengineering team led by Dr. Mehmet Toner, the molecular biology group of Dr. Shyamala Maheswaran, and the Massachusetts General Hospital Cancer Center clinical disease centers to develop, characterize and apply a microfluidic device capable of isolating rare circulating tumor cells (CTCs) in the blood of patients with cancer. This new technology offers the promise of 1) noninvasive monitoring of cancers during their treatment for the emergence of drug resistance; 2) early detection of invasive cancers; and ultimately 3) understanding and preventing blood-borne spread of cancer.

Haber Laboratory*

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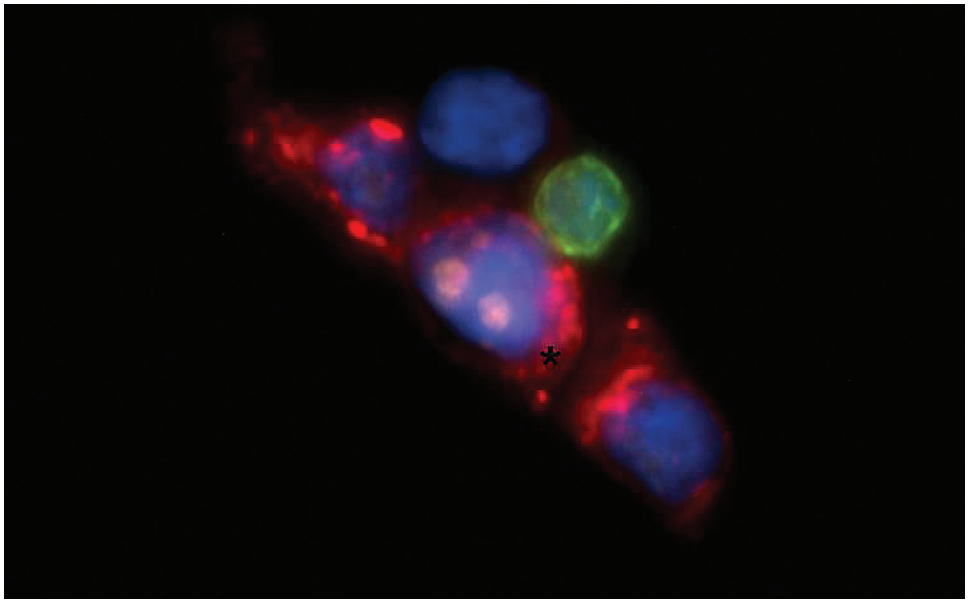
* co-directed with Shyamala Maheswaran, PhD

Our laboratory is interested in the genetics of human cancer. Current projects include the use of a microfluidic device to capture circulating tumor cells (CTCs) and its application in molecular-directed therapy and in the study of human cancer metastasis.

Circulating Tumor Cells and Molecular Genetics Underlying Targeted Cancer Therapeutics

Activating mutations in the epidermal growth factor receptor (*EGFR*) were identified in our laboratory in the subset of non-small cell lung cancer (NSCLC) with dramatic responses to the tyrosine kinase inhibitor gefitinib. We have studied mechanisms underlying such oncogene addiction, as well as the pathways that lead to the acquisition of resistance to targeted therapies, including the application of irreversible kinase inhibitors to circumventing mutations that alter drug binding affinity. Following on our efforts to monitor the emergence of drug resistance mutations, we are now collaborating with the Toner and Maheswaran laboratories to characterize novel microfluidic devices capable of isolating CTCs from the blood of cancer patients. Our most advanced version of these CTC-Chips relies upon blood flow through a specialized chamber, which allows the high efficiency separation of antibody-tagged leukocytes, thereby identifying intact CTCs without selection bias. In a series of CTC studies, we have shown that the number of captured CTCs correlates with clinical evidence of tumor response, and that the cells can be used to define molecular markers characteristic of the underlying malignancy, including *EGFR* mutations and *EML4-ALK* translocations in lung cancer, and measurements of androgen receptor (AR) activity in prostate cancer. We have applied next generation single-molecule RNA sequencing to identify non-canonical Wnt signaling as a suppressor of anoikis



Circulating prostate tumor cell cluster stained for PSA (green) along with Ki67 (orange) and CD45 (red).

pathways in circulating pancreatic cancer cells, while in melanoma and in glioblastoma, we developed tools to isolate and molecularly characterize CTCs.

Our most recent studies have focused on breast cancer, where we demonstrated treatment-associated epithelial-to-mesenchymal transition (EMT) within CTCs. Using a combination of mouse models and patient-derived studies, we observed that tumor-derived fragments generate CTC-Clusters, which have greatly enhanced metastatic propensity compared with single CTCs. CTC-Clusters are held together by plakoglobin, whose knockdown dramatically suppresses CTC-Cluster formation and metastatic spread of breast cancer cells. Finally, we successfully established long-term *in vitro* cultures of CTCs from patients with estrogen-receptor positive breast cancer, identifying treatment-associated mutations in the estrogen receptor (ESR1), as well as acquired mutations in drugable therapeutic targets, such as *PIK3CA* and *FGFR*. The development of such CTC-derived cultures may enable functional predictive drug testing, combined with detailed genetic analysis of tumor cells sampled noninvasively during the course of cancer treatment.

Current efforts are directed at isolating single CTCs to uncover the heterogeneous nature of these rare metatastic precursors. Further technological improvements in CTC capture and detection are under study for potential applications in early detection of cancer, monitoring tumor genotypes over the course of treatment, and biological characterization of CTCs themselves.

Selected Publications:

Aceto N, Bardia A, Miyamoto DT, Donaldson MC, Wittner BS, Spencer JA, Yu M, Pely A, Engstrom A, Zhu H, Brannigan BW, Kapur R, Stott SL, Shioda T, Ramaswamy S, Ting DT, Lin CP, Toner M, **Haber DA***, Maheswaran S*. Circulating tumor cell clusters are oligoclonal precursors of breast cancer metastasis. *Cell*. 158(5):1110-22, 2014.

Yu M, Bardia A, Aceto N, Bersani F, Madden M, Donaldson MC, Desai R, Comaills V, Zheng Z, Wittner BS, Stojanov P, Brachtel E, Sgroi D, Kapur R, Shioda T, Ting, DT, Ramaswamy S, Getz G, Iafrate AJ, Benes C, Toner, M, Maheswaran S* and **Haber DA***. Ex vivo culture of circulating breast tumor cells for individualized testing of drug susceptibility. *Science*. 346(6193): 216-22, 2014.

Luo X, Mitra D, Sullivan RJ, Wittner BS, Kimuar AM, Pan S, Hoang MP, Brannigan BW, Lawrence DP, Flaherty KT, Sequist LV, McMahon M, Bosenberg MW, Stott SL, Ting DT, Ramaswamy S, Toner, M, Fisher DE, Maheswaran S* and **Haber DA*** Isolation and molecular chaccterization of circulating melanoma cells. *Cell Reports*. 7: 645-653, 2014.

Haber DA and Velculescu VE. Blood-based analyses of cancer: Circulating tumor cells and circulating tumor DNA. *Cancer Discovery*. 4: 650-661, 2014.

Yu M*, Bardia A*, Wittner BS, Stott SL, SMas ME, Ting DT, Isakoff SJ, Ciciliano JC, Wells MN, Shah AM, Concannon KE, Donaldson MC, Sequist LV, Bracheter E, Sgroi D, Baselga J, Ramaswamy S, Toner M, **Haber DA*** and Maheswaran S*. Circulating breast tumor cells exhibit dynamic changes in epithelial and mesenchymal composition. Science 339: 580-4, 2013.

Ozkumur E*, Shah AM*, Cicilano JC, Emmink BL, Miyamoto DT, Brachter E, Yu M, Chen PI, Morgan B, Trautwein J, Kimura A, Sengupta S, Stott SL, Karabacak NM, Barber TA, Walsh JR, Smith K, Spuhler PS, Sullivan JP, Lee RJ, Ting DT, Luo X, Shaw AT, Bardia A, Sequist LV, Louis DN, Maheswaran S, Kapur R, Haber DA, Toner M. Inertial focusing for tumor antigen-dependent and –independent sorting of rare circulating tumor cells. Science Transl. Med 3: 179, 2013.

*Co-corresponding authors

Konrad Hochedlinger, PhD

The Hochedlinger laboratory explores the molecular mechanisms underlying cellular reprogramming. Recent groundbreaking discoveries have shown that adult cells can be reprogrammed into cells resembling embryonic stem cells by activating a handful of embryonic genes. The resultant cells, called induced pluripotent stem cells (iPSCs), have tremendous therapeutic potential; they can be derived from any patient's skin or blood cells. In the laboratory, iPSCs can be coaxed into many specialized cell types. Our lab has contributed to a better understanding of the process of cellular reprogramming, which remains elusive. These findings allowed us and other labs to generate iPSCs in safer, better and more efficient ways. Our ultimate goal is to utilize these mechanistic insights for the development of new strategies to treat cancer and other complex diseases.

Hochedlinger Laboratory

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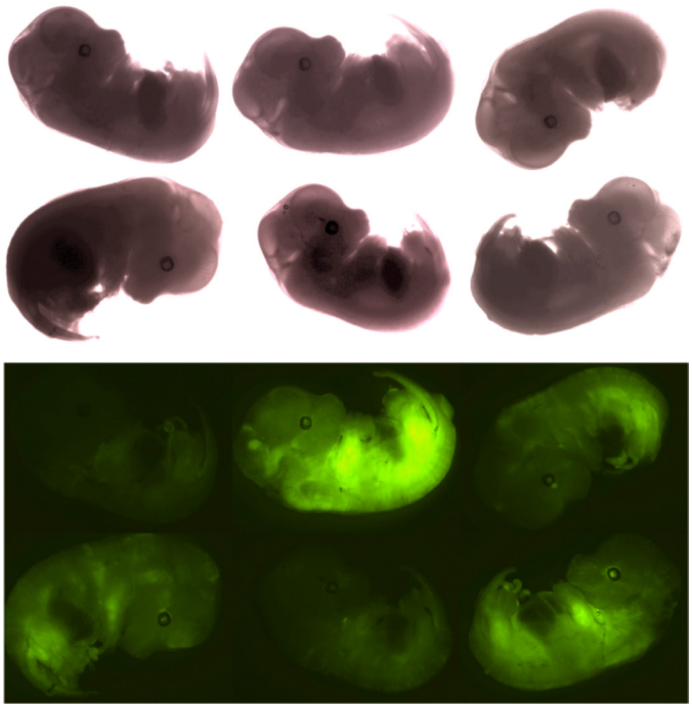
Jiho Choi,
Kyle Flattery, undergraduate researcher
Konrad Hochedlinger, PhD
Aaron Huebner, PhD
Inna Lipchina, PhD
Abby Sarkar*
Benjamin Schwarz, MD, PhD
Ryan Walsh*
Caitlin Murphy, administrative assistant

* PhD Candidate

The Hochedlinger lab is studying the mechanisms of cellular reprogramming using transcription-factor-mediated conversion of somatic cells into induced pluripotent stem (iPSCs). iPSCs are typically derived by retroviral transduction of the embryonic transcription factors Oct4, Sox2, c-Myc and Klf4, which reset the differentiation state of an adult cell into that of a pluripotent cell. The underlying transcriptional and epigenetic changes remain largely elusive. Importantly, iPSCs have been derived from different species—including human patients—and therefore provide a unique platform to model degenerative disorders such as Alzheimer's disease, Parkinson's disease and diabetes. Moreover, iPSCs could be ultimately used in regenerative medicine to replace damaged cells and tissues with genetically matched cells.

We have identified biomarkers to track and prospectively isolate intermediate cell populations during the reprogramming process, and we are currently using these populations to understand the transcriptional, epigenetic and proteomic changes in cells undergoing reprogramming. In addition, we have shown that terminally differentiated beta cells and lymphocytes can be reprogrammed into iPSCs, thus demonstrating that induced pluripotency is not limited to rare adult stem cells as has been suggested. Interestingly, however, we discovered that immature hematopoietic cells give rise to iPSCs more efficiently than any tested mature cell types, suggesting that the differentiation stage of the starting cell can influence the efficiency of reprogramming. At the molecular level, we have identified the p53 and p16/p19 tumor suppressor pathways as well as the Tgf-beta signaling cascade as roadblocks during the reprogramming process, pointing out striking similarities between pluripotent cells and cancer cells.

One major roadblock for the therapeutic use of iPSCs is the fact that integrating viruses are used to deliver the reprogramming genes to cells, resulting in genetically altered iPSCs. By using adenoviruses expressing the



Fluorescent images of E13.5 chimeras generated from injecting Nanog−/− iPSCs that constitutively express GFP into wild-type GFP-negative blastocysts (top, phase; bottom, GFP).

Images: B. Schwarz.

reprogramming factors transiently in cells, we were able to produce iPSCs devoid of any viral elements and thus any genetic manipulation. More recently, we have developed a reprogrammable mouse carrying a single doxycycline-inducible cassette with the four reprogramming genes in all tissues. We are employing this system to perform genetic and chemical screens to identify molecules important during the reprogramming process as well as for comparative studies between iPSCs and embryonic stem cells. For example, we discovered that the Dlk1-Dio3 imprinted gene cluster is aberrantly silenced by hypermethylation in many iPSC lines, which correlates with their impaired developmental potential. We recently showed that ascorbic acid treatment prevents aberrant silencing, thus providing the first small compound that improves the quality of iPSCs. Furthermore, our lab is investigating the role of three-dimensional chromatin structure in pluripotency and reprogramming by using circular chromosome conformation capture (4C)-sequencing. This work revealed an unexpectedly complex interaction network of the *Nanog* locus with rest of the genome, which is critical for the maintenance and establishment of pluripotency (see also caption image).

In addition, we are interested in studying the role of Sox2 in adult tissues. While Sox2 has been mostly interrogated in the context of pluripotent stem cells and cellular reprogramming, recent data suggest that it may play important functions in adult tissues as well. For example, Sox2 is essential for neural stem cell maintenance, and its coding region is amplified in lung and esophageal cancer, thus implicating Sox2 in adult tissue regeneration and tumorigenesis. Intriguingly, we have identified Sox2-expressing cells in several adult tissues where it has not previously been characterized, including squamous epithelia lining the stomach, anus and cervix as well as in testes, lens and glandular stomach. Future work in the lab is aimed at understanding the role of Sox2 and Sox2+ cells in tissue homeostasis and cancer by utilizing conditional knockout, lineage tracing and cell ablation mouse models.

Selected Publications:

Bar-Nur O, Brumbaugh J, Verheul C, Apostolou E, Pruteanu-Malinici I, Walsh RM, Ramaswamy S, **Hochedlinger K**. Small molecules facilitate rapid and synchronous iPSC generation. *Nature Methods* (in press)

Schwarz BA, Bar-Nur O, Silva JC, **Hochedlinger K**. Nanog is dispensable for the generation of induced pluripotent stem cells. *Curr Biol.* 2014 Feb 3;24(3):347-50.

Apostolou E, Ferrari F, Walsh RM, Bar-Nur O, Stadtfeld M, Cheloufi S, Stuart HT, Polo JM, Ohsumi TK, Borowsky ML, Kharchenko PV, Park PJ, **Hochedlinger K**. Genome-wide Chromatin Interactions of the Nanog Locus in Pluripotency, Differentiation, and Reprogramming. *Cell Stem Cell*. 2013 Jun 6;12(6):699-712.

Polo JM, Anderssen E, Walsh RM, Schwarz BA, Nefzger CM, Lim SM, Borkent M, Apostolou E, Alaei S, Cloutier J, Bar-Nur O, Cheloufi S, Stadtfeld M, Figueroa ME, Robinton D, Natesan S, Melnick A, Zhu J, Ramaswamy S, **Hochedlinger K**. A molecular roadmap of reprogramming somatic cells into iPS cells. *Cell*. 2012 Dec 21;151(7):1617-32.

Stadtfeld M, Apostolou E, Ferrari F, Choi J, Walsh RM, Chen T, Ooi SS, Kim SY, Bestor TH, Shioda T, Park PJ, **Hochedlinger K**. Ascorbic acid prevents loss of Dlk1-Dio3 imprinting and facilitates generation of all-iPS cell mice from terminally differentiated B cells. *Nat Genet*. 2012 Mar 4;44(4):398-405, S1-2.

Arnold K, Sarkar A, Yram MA, Polo JM, Bronson R, Sengupta S, Seandel M, Geijsen N, **Hochedlinger K**. Sox2(+) adult stem and progenitor cells are important for tissue regeneration and survival of mice. *Cell Stem Cell*. 2011 Oct 4;9(4):317-29.

Hanno Hock, MD, PhD

The Hock laboratory explores the molecular basis of blood cell formation and the pathogenesis of leukemia and lymphoma. Specifically, we study the transcription factors that regulate gene activity during normal blood cell development and how the transcriptional apparatus goes awry in cancer. For example, we have developed important insights into a network of transcription factors that help maintain blood stem cells in the bone marrow; this work could lead to new strategies for increasing the yield of stem cells for bone marrow transplantation. Another project in our laboratory focuses on deciphering the multistep process that leads to lymphoblastic leukemia of childhood, with the goal of identifying new drug targets for this devastating disease. Finally, we are interested in how DNA packaging affects the interaction between genes and transcription factors, especially with regard to oncogenes and tumor suppressor genes important in human cancer.

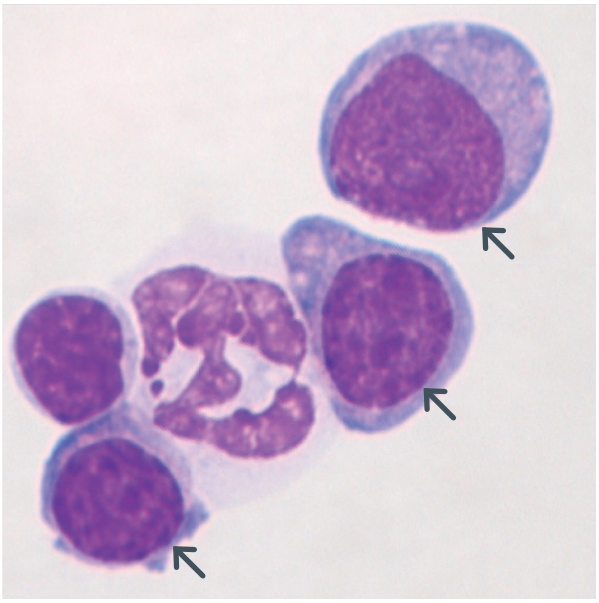
Hock Laboratory

Hock Laboratory
Adlen Foudi, PhD
Hanno Hock, MD, PhD
Daniel Kramer
Ondrej Krejci, PhD
Ryan LeGraw

Our laboratory is interested in the molecular control of normal and malignant stem cells with an emphasis on the hematopoietic system. Blood cells need to be continuously replenished by a small population of hematopoietic stem cells (HSCs) that have the capacity to both self-renew and mature stepwise into all known blood lineages. HSCs are also the ancestors of leukemia and lymphoma cells. As HSCs mature, they undergo successive changes in gene expression. The transcriptional apparatus must ensure that genes specific to immature cells are repressed as differentiation proceeds while genes that are necessary for mature cells become activated. This activating and inactivating of genes is achieved by cooperative action of a variety of lineage-specific and general transcription factors and the complex molecular machinery that regulates the accessibility of different regions of the genome in chromatin. We investigate how transcription factors establish differentiation-specific transcriptional programs and how such programs can become derailed in cancer, leukemia and lymphoma.

Transcriptional control of normal and malignant hematopoietic stem cells in the adult bone marrow

Hematopoiesis in the bone marrow emanates HSCs. We are studying the basic biology of HSCs. Specifically we explore how a network of transcription factors that includes Tel-Etv6, Gfi1, Gfi1b and Gata2 maintains HSCs in the bone marrow (Hock et al. 2004, Genes & Development; Hock et al. 2004, Nature). The goal is to exploit the biology of transcriptional regulation of HSCs to maintain, expand, and possibly even generate HSCs ex vivo so that more patients will have the option of bone marrow transplantation. In a closely related effort, we are exploring the molecular programs of stem cells in leukemia and lymphoma to identify differences in



Dr. Hock's laboratory works on molecular mechanisms of normal differentiation and malignant transformation. The image shows normal blood cells and leukemic cells (arrows) from an novel experimental model generated in the lab.

their molecular regulation compared with normal HSCs. Such differences may allow us to specifically target tumor stem cells while sparing normal blood formation.

Deciphering the molecular events leading to acute lymphoblastic leukemia of childhood

About one in 2000 children develop this catastrophic illness, most often with a t(12;21) translocation. Despite very aggressive treatments, not all children can be cured, and some suffer from long-term side effects of their therapy. Rational development of more specific, less toxic treatments requires a precise understanding of the molecular mechanisms that cause the disease. We have discovered that TEL-AML1, the first hit in childhood leukemia, generates a preleukemic, latent lesion in HSCs. We are now exploring how additional genetic hits cooperate to derail normal blood development and generate leukemia. Deciphering the multistep pathogenesis of this entity is likely to serve as a paradigm for the development of other malignant diseases.

Exploration of novel epigenetic regulators in stem cells

Our understanding of how specialized cells of the body establish their identity by regulating access to genes continues to increase. For example, a large fraction of the genes active in brain cells are inactive in blood cells and, therefore, are stored in a very dense, inaccessible state. As most molecules involved in the regulation of gene accessibility have only recently been identified, studying their biology is likely to provide unique opportunities for the development of entirely novel therapies. Our laboratory is investigating the utility of a group of proteins termed MBT-proteins, which is very important for condensing DNA and modifying histones. Evidence suggests that this protein family may play important roles in normal and malignant blood formation, but its precise functions remain poorly understood. Our laboratory has recently discovered an entirely novel, essential function of the family member L3mbtl2 in pluripotent stem cells.

Selected Publications:

Foudi A, Kramer DJ, Qin J, Ye D, Behlich AS, Mordecai S, Preffer FI, Amzallag A, Ramaswamy S, Hochedlinger K, Orkin SH and **Hock H**. Distinct, strict requirements for Gfi-1b in adult bone marrow red cell and platelet generation. *J Exp Med* 211, 909 – 927. 2014.

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Eminli S, Foudi A, Stadtfeld M, Maherali N, Ahfeldt T, Mostoslavsky G, **Hock H**, Hochedlinger K. Differentiation stage determines potential of hematopoietic cells for reprogramming into induced pluripotent stem cells. *Nat Genet*. 41(9):968-76, 2009 Sep.

A. John Iafrate, MD, PhD

Research in **the Iafrate laboratory** focuses on bringing new genetic technologies to cancer diagnostics and their application to the practice of pathology. In collaboration with the Massachusetts General Hospital Translational Research Laboratory, we have overcome numerous hurdles to develop high-throughput technologies for rapid and efficient genetic analysis of tumor samples from cancer patients. These tools have revolutionized cancer diagnostics at the Massachusetts General Hospital and have been adopted by other cancer centers, both nationally and internationally. We are also exploring the development of predictive biomarkers for lung and brain cancer and developing new tools to better understand the role of copy number variants (CNVs) in human disease.

Iafrate Laboratory

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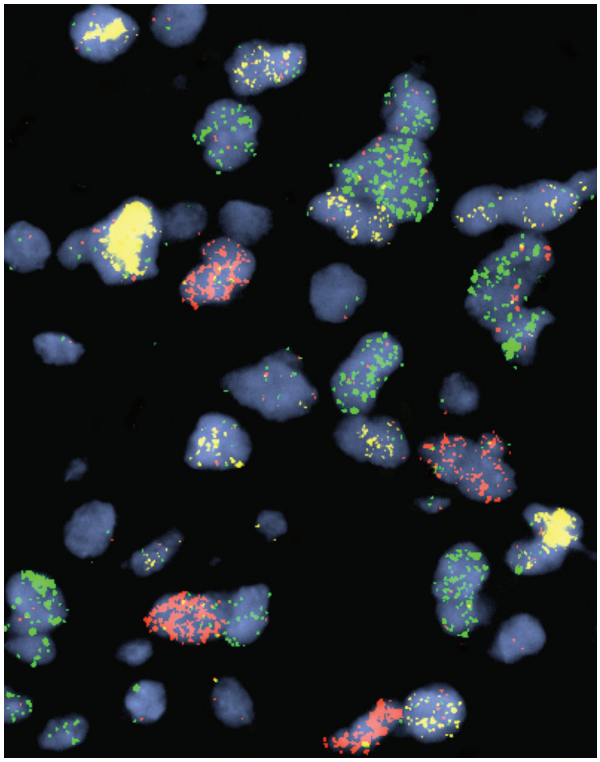
Hyo Sup Shim, MD
Zongli Zheng, PhD

Our lab has focused efforts on developing highly complex molecular analyses of tumor genetics using novel technologies. We developed the SNaPshot genotyping assay, an assay capable of detecting over 100 recurrent cancer mutations, which has enabled Mass General to make personalized cancer medicine a priority. We have a strong interest in the clinical implementation of genetic screening technologies that can help direct targeted therapies, focusing on lung, pancreatic and brain tumors. Our recent contributions in the treatment of a subset of non-small cell lung carcinoma (NSCLC) with rearrangements of the ALK tyrosine kinase and with rearrangements of the ROS1 tyrosine kinase with a small molecule kinase inhibitor (crizotinib) underscore the promise of personalized cancer care. For the phase 1 trial of crizotinib in advanced stage NSCLC, we used a fluorescence in situ hybridization (FISH) assay to screen for ALK rearrangements in archived pathology lung cancer specimens. We screened greater than 1,500 patients to identify the >100 patients who eventually were enrolled into the "expanded cohort" of ALK-positive patients in the phase 1 trial. The observed 65% response rate and 10 month progression-free survival resulted in rapid FDA approval of both crizotinib and the companion FISH diagnostic. We have been actively investigating next generation sequencing to detect chromosomal rearrangements in tumor tissue, with on-going studies are assessing the relative sensitivity in much larger clinical cohorts. The method we have developed, termed "anchored multiplex PCR" is an efficient target enrichment technology, allowing for 100s of targets to be simultaneously analyzed from small tissue samples. Our long-term goal is to develop high-throughput genetic screening approaches for all cancer patients.

We have also initiated studies of tumor heterogeneity; these efforts focus on gene amplification of receptor tyrosine kinases in glioblastoma. This work has revealed a new subclass of brain tumors with mosaic gene amplification of up to three kinases in distinct but intermingled cell populations within the same tumor, forming a mosaic pattern. We found that each subpopulation was actively proliferating and contributing to tumor growth. Detailed genetic analysis found that



Genetic heterogeneity in glioblastoma tumors

different subpopulations within a particular tumor shared other gene mutations, indicating they had originated from the same precursor cells. Mapping the location of different subpopulations in the brain of a glioblastoma patient suggested that each subpopulation may serve a different function in the growth and spread of the tumor. We are exploring the therapeutic implications of such driver gene heterogeneity in model systems of glioblastoma.

Our laboratory has also focused on human genetics, namely on copy number variation (CNVs). These polymorphisms involve copy number gains or losses of large genomic regions (kilobases up to several megabases) that were identified using high-resolution genomic microarrays to compare the genomes of phenotypically normal individuals. Our continuing work is focused on the detailed structural analysis of CNVs using high-resolution, fluorescence microscopy imaging techniques, quantitative polymerase chain reaction (PCR) and bacterial artificial chromosome (BAC) sequencing. We have developed novel fluorescence in situ hybridization (FISH) probes based on deletion CNVs that can be used to determine genetic identity in situ. These probes are applied to chimerism analysis in transplantation and will aid in the study of engraftment, rejection and graft-versus-host disease. Importantly, these probes are located on autosomes, so for the first time chimerism analysis can be performed in same-sex transplants.

Selected Publications:

Zheng Z, Liebers M, Zhelyazkova B, Cao Y, Panditi D, Chen J, Robinson HE, Chmielecki J, Pao W, Engelman JA, **Iafrate AJ**, Le LP. Anchored multiplex PCR for targeted next-generation sequencing. *Nat Medicine*. In press, 2014.

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Kwak EL, Bang Y, Camidge DR, Shaw AT, Solomon B, Maki RG, Ou SI, Dezube BJ, Jänne PA, Costa DB, Varella-Garcia M, Kim W, Lynch TJ, Fidias P, Stubbs H, Engelman JA, Sequist LV, Tan W, Gandhi L, Mino-Kenudson M, Wei GC, Shreeve SM, Ratain MJ, Settleman J, Christensen JG, Haber DA, Wilner K, Salgia R, Shapiro GI, Clark JW, **Iafrate AJ**. Response of non-small cell lung cancers with anaplastic lymphoma kinase (ALK) gene rearrangements to a targeted ALK inhibitor. *N Engl J Med*. 363(18):1693-703, 2010.

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Othon Iliopoulos, MD

The Iliopoulos laboratory works on understanding the biochemical mechanisms of cancer angiogenesis and cancer metabolism in order to identify and validate new targets for anticancer drug development. Cancer cells need oxygen and nutrients to survive, grow and metastasize. To meet these needs, tumor cells activate a sophisticated program known as cancer angiogenesis to stimulate the growth of surrounding blood vessels. In addition, they reshape their metabolic requirement so that they outcompete the normal cells in growth. Our research program focuses on understanding the differences between cancer-induced blood vessels and those that feed normal tissues. We are also exploring the molecular mechanisms through which cancer cells reconfigure their metabolism to outcompete normal cells for life-sustaining nutrients; this work has the potential to lead to new targets for drug discovery.

Iliopoulos Laboratory

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Biology of Tumor Angiogenesis and Tumor Metabolism

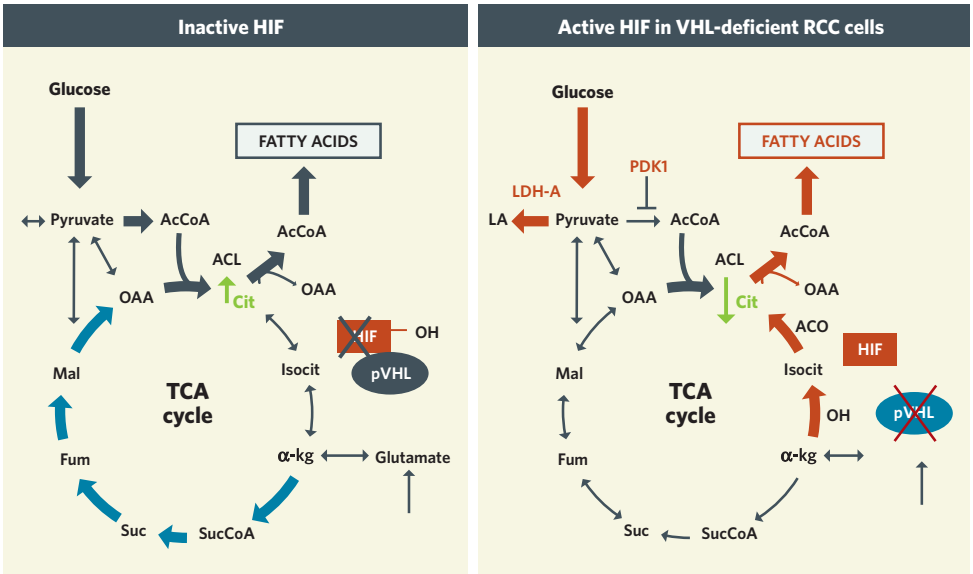
Our laboratory is interested in tumor angiogenesis and tumor metabolism. Our current studies utilize biochemical, cellular and genetic approaches to dissect the mechanisms by which eukaryotic cells—both normal and neoplastic—sense and respond to hypoxia.

Hypoxia activates several intracellular signaling pathways, leading to secretion of growth and angiogenic factors and to dramatic metabolic changes. Cellular hypoxia activates a family of transcription factors termed hypoxia inducible factors (HIFs). HIF signaling is also directly activated and co-opted by any tumor-initiating mutation. HIF is a powerful regulator of cancer angiogenesis, carbon and lipid metabolism, stem cell proliferation, and tissue differentiation. Inhibition of HIF in preclinical animal models leads to cancer suppression. Levels of HIF expression in human solid and hematologic malignancies have strong prognostic value.

Detailed understanding of the molecular events that regulate cancer angiogenesis and metabolism will lead to rational selection of molecular targets for anticancer drug development.

Discovery and Development of Hypoxia Inducible Factor 2a (HIF2a) inhibitors

We have identified small molecules that repress HIF translation in a highly specific way by targeting a bifunctional protein that serves either as an mRNA binding protein (i.e., iron regulatory protein 1, or IRP1) or as a cytosolic enzyme of intermediary metabolism (i.e., aconitase 1, ACO1). These molecules are active in vivo and synergistically inhibit all downstream targets of HIF, thereby negatively impacting angiogenesis and tumor metabolism. We are currently using these HIF inhibitors as chemical biology tools to analyze HIF signaling and its effect on metabolism. In parallel studies, we are testing their anticancer function in preclinical experiments.



Expression of Hypoxia Inducible Factor HIF2a rewires the central carbon metabolism in renal cell cancer.

Genetic Studies on VHL-HIF-Hypoxia Pathway

The Hypoxia-HIF-VHL pathway is remarkably conserved between *Drosophila* and mammalian cells. We use *Drosophila* genetics to identify genes modifying the hypoxia-VHL-HIF pathway. We developed a *Drosophila* model for VHL disease and HIF activation and are using this model to identify second-site modifiers of Sima and dVHL functions.

Biomarkers for Early Identification of Renal Cell Carcinoma (RCC)

Loss of VHL function and upregulation of HIF activity is a hallmark of human RCC. We combined analysis of HIF-activated genes and signaling pathways specifically deregulated in RCC with proteomic analysis of patient-derived plasma and tumor samples, and we identified a set of candidate RCC biomarkers. In addition, we have developed assays to measure the expression of these biomarkers in patient blood. These biomarkers can serve for early identification of RCC and as surrogate markers for disease activity in patients enrolled in clinical trials.

Selected Publications:

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Zimmer M, Ebert BL, Neil C, Brenner K, Papaioannou I, Melas A, Tolliday N, Lamb J, Pantopoulos K, Golub T, **Iliopoulos O**. Small-molecule inhibitors of HIF-2a translation link its 5'UTR iron-responsive element to oxygen sensing. *Molecular Cell*. 32(6):838-48. 2008 Dec 26.

*Co-corresponding authors

J. Keith Joung, MD, PhD

The Joung laboratory is developing strategies to reprogram the genome and epigenome of living cells to better understand biology and treat disease. We have developed and optimized molecular tools for customized genome editing that enable scientists to alter the DNA sequence of a living cell—from fruit flies to humans—with great precision. These technologies are based on designer DNA-binding and RNA-guided proteins engineered to recognize and cleave specific genomic sequences. We also use these targeting methodologies to direct various other regulatory elements to enable activation, repression, or alteration of histone modifications of specific genes. These tools have many potential uses in cancer research and may one day lead to more efficient gene therapy capable of correcting disease-related mutations in human cells.

Joung Laboratory

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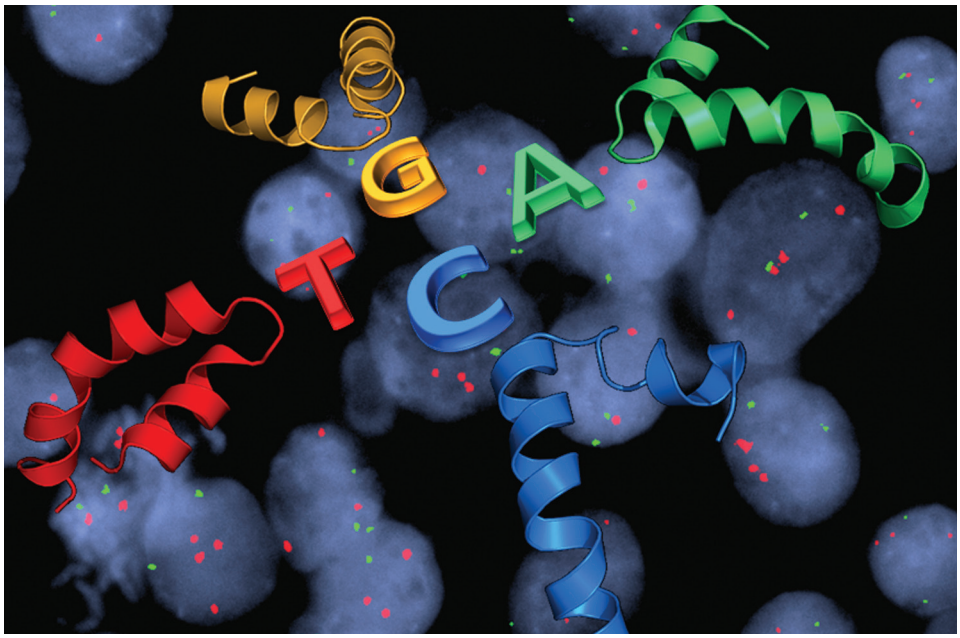
The Joung Laboratory develops technologies for genome and epigenome engineering of living cells and organisms using engineered zinc finger, transcription activator-like effector (TALE), and RNA-guided CRISPR/Cas-based systems and explores their applications for biological research and gene therapy.

Genome Editing Using Targeted Nucleases

Genome editing technology was recently named runner-up for "Breakthrough of the Year" for 2012 and 2013 by *Science* magazine and "Method of the Year" for 2011 by *Nature Methods*. We have previously invented two rapid, robust, and publicly available methods for engineering ZFNs known as OPEN (Oligomerized Pool Engineering; Maeder et al., *Mol Cell* 2008) and CoDA (Context-Dependent Assembly; Sander et al., *Nat Methods* 2011). In addition, we have also developed and optimized methods for engineering TALENs including an automated, high-throughput method known as FLASH (Fast Ligation-based Automated Solid-phase High-throughput) assembly (Reyon et al., *Nat Biotechnol*. 2012). We have also recently described reagents that enable the rapid construction of CRISPR RNA-guided nucleases (RGNs) (Hwang et al., *Nat Biotechnol*. 2013).

Much of our recent work with genome-editing nucleases has focused on CRISPR RGNs. We and our collaborators were the first to demonstrate that these nucleases can function *in vivo* (Hwang & Fu et al., *Nat Biotechnol*. 2013), modifying endogenous genes in zebrafish and the first to show that they can induce significant off-target mutations in human cells (Fu et al., *Nat Biotechnol*. 2013). To improve the specificities of these nucleases, we have developed two platforms that show greatly reduced off-target effects: one based on the use of truncated guide RNAs (Fu & Sander et al., *Nat Biotechnol*. 2014) and the other in which we engineered dimerization-dependent CRISPR RNA-guided nucleases (Tsai et al., *Nat Biotechnol*. 2014).



Structures of TAL effector repeat domains for binding each of the four DNA bases overlaid on FISH images of cells modified by gene-editing nucleases.

Epigenome Editing Using Targeted Transcription Factors

We have recently demonstrated that the TALE and CRISPR RGN platforms can also be utilized to create artificial customizable transcription factors that can robustly alter expression of endogenous human genes (Maeder et al., *Nat Methods* 2013a; Maeder et al., *Nat Methods* 2013b). In addition, we have collaborated with the group of Brad Bernstein to develop fusions of the histone demethylase LSD1 with TALE domains that can induce targeted histone alterations at endogenous human enhancers (Mendenhall et al., *Nat Biotechnol.* 2013). Finally, we have also developed fusions of engineered TALE domains with the catalytic domain of the TET1 enzyme, enabling the targeted demethylation of CpGs in human cells (Maeder et al., *Nat Biotechnol.* 2013). We are exploring the use of these and other proteins in both a directed fashion as well as with combinatorial libraries to induce specific phenotypes and cellular states in human cells.

Selected Publications:

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Sander JD, **Joung JK**. CRISPR-Cas systems for editing, regulating and targeting genomes. *Nat Biotechnol.*, 2014 Apr;32(4):347-55. Review.

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Fu Y, Foden JA, Khayter C, Maeder ML, Reyon D, **Joung JK***, Sander JD*. High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells. *Nat Biotechnol.*, 2013 Jun 23.

Hwang WY, Fu Y, Reyon D, Maeder ML, Tsai SQ, Sander JD, Peterson RT, Yeh JR, **Joung JK**. Efficient genome editing in zebrafish using a CRISPR-Cas system. *Nat Biotechnol.*, 2013 Mar;31(3):227-9.

*Co-corresponding authors

David M. Langenau, PhD

Most pediatric patients whose sarcoma or leukemia recurs will succumb to their disease. The focus of **the Langenau lab** is to uncover the mechanisms that drive progression and relapse in pediatric tumors with the long-term goal of identifying new therapeutic drug targets to treat relapse and refractory disease. One approach we have used is to add drugs to the water of novel zebrafish models of pediatric sarcoma and leukemia that mimic human malignancy. We then imaged tumor growth in the zebrafish and utilize detailed imaging studies to visualize tumor cells in live animals to assess how cellular heterogeneity drives continued tumor growth. Capitalizing on insights gained from our zebrafish models of cancer, we are now extending our findings to human T-cell acute lymphoblastic leukemia and rhabdomyosarcoma.

Langenau Laboratory

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Karin McCarthy
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* PhD Candidates

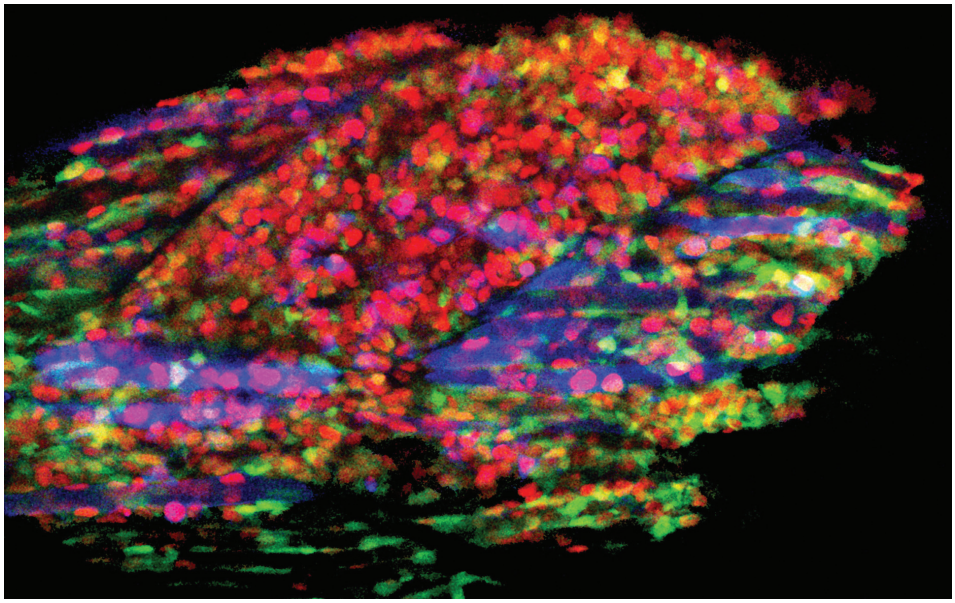
The Langenau laboratory research focus is to uncover relapse mechanisms that alter growth, therapy resistance, and tumor propagating cell frequency in pediatric cancer. Utilizing zebrafish models of T-cell acute lymphoblastic leukemia (T-ALL) and embryonal rhabdomysoarcoma (ERMS), we have undertaken chemical and genetic approaches to identify novel modulators of growth and relapse.

Uncovering progression-associated driver mutations in T-cell acute lymphoblastic leukemia

T-ALL is an aggressive malignancy of thymocytes that affects thousands of children and adults in the United States each year. Recent advancements in conventional chemotherapies have improved the five-year survival rate of patients with T-ALL. However, patients with relapsed disease are largely unresponsive to additional therapy and have a very poor prognosis. Ultimately, 70% of children and 92% of adults with relapsed T-ALL will die of the disease, underscoring the clinical imperative for identifying the molecular mechanisms that cause leukemia cells to re-emerge at relapse. Utilizing a novel zebrafish model of relapsed T-ALL, large-scale trangenesis platforms, and unbiased bioinformatic approaches, we have uncovered new oncogenic drivers associated with aggression, therapy resistance and relapse. A large subset of these genes exert important roles in regulating human T-ALL proliferation, apoptosis and response to therapy. Discovering novel relapse-driving oncogenic pathways will likely identify new drug targets for the treatment of T-ALL.

Visualizing and killing cancer stem cells in embryonal rhabdomyosarcoma

ERMS is a common soft-tissue sarcoma of childhood and phenotypically recapitulates fetal muscle development arrested at early stages of differentiation. Microarray and cross-species comparisons of zebrafish, mouse and human ERMS uncovered the finding that the RAS pathway is activated in a majority of ERMS. Building on this discovery, our laboratory has developed a transgenic zebrafish model of kRASG12D-induced ERMS



Visualizing cancer stem cells in live zebrafish affected with embryonal rhabdomyosarcoma. GFP expression is confined to the myf5+ ERMS-propagating cells while differentiated nontumor propagating cells are labeled with a nuclear histone-RFP fusion and membrane associated Cyan

that mimics the molecular underpinnings of human ERMS. We used fluorescent transgenic zebrafish that label ERMS cell subpopulations based on myogenic factor expression, to identify functionally distinct classes of tumor cells contained within the ERMS mass. Specifically, the myf5-GFP+ self-renewing cancer stem cell drives continued tumor growth at relapse and is molecularly similar to a nontransformed, activated muscle satellite cell. Building on the dynamic live cell imaging approaches available in the zebrafish ERMS model, our laboratory has undertaken chemical genetic approaches to identify drugs that kill relapse-associated, self-renewing myf5-GFP+ ERMS cells. We are currently assessing a subset of drugs for their ability to regulate growth of human ERMS cells.

Selected Publications:

Blackburn JS, Liu S, Wilder JL, Dobrinski KP, Lobbardi R, Moore FE, Martinez SA, Chen EY, Lee C, **Langenau DM**. Clonal evolution enhances leukemia propagating cell frequency in T-cell acute lymphoblastic leukemia through AKT/mTORC1 pathway activation. *Cancer Cell*. 2014; 25(3):366-78.

Chen EY, DeRan M, Ignatius MS, Grandinetti KB, Clagg R, McCarthy K, Lobbardi RM, Brockmann J, Keller C, Wu X, **Langenau DM**. GSK3 inhibitors induce the canonical WNT/b-catenin pathway to suppress growth and self-renewal in embryonal rhabdomyosarcoma. *PNAS*, 2014; 111(14):5349-54.

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David N. Louis, MD

The Louis laboratory studies the biological basis of human brain tumors in order to understand which molecules go awry in the formation of brain tumors. Understanding the molecular basis of human brain tumors enables improved diagnosis, better assessment of patient prognosis, more precise prediction of response to existing therapies, greater understanding of resistance to current chemotherapies, and design of novel treatments. For example, our studies showing that molecular genetic alterations are powerful predictors of therapeutic response and survival in patients with anaplastic oligodendrogliomas and other oligodendroglial tumors have led to changes in diagnostic protocols worldwide for these tumors.

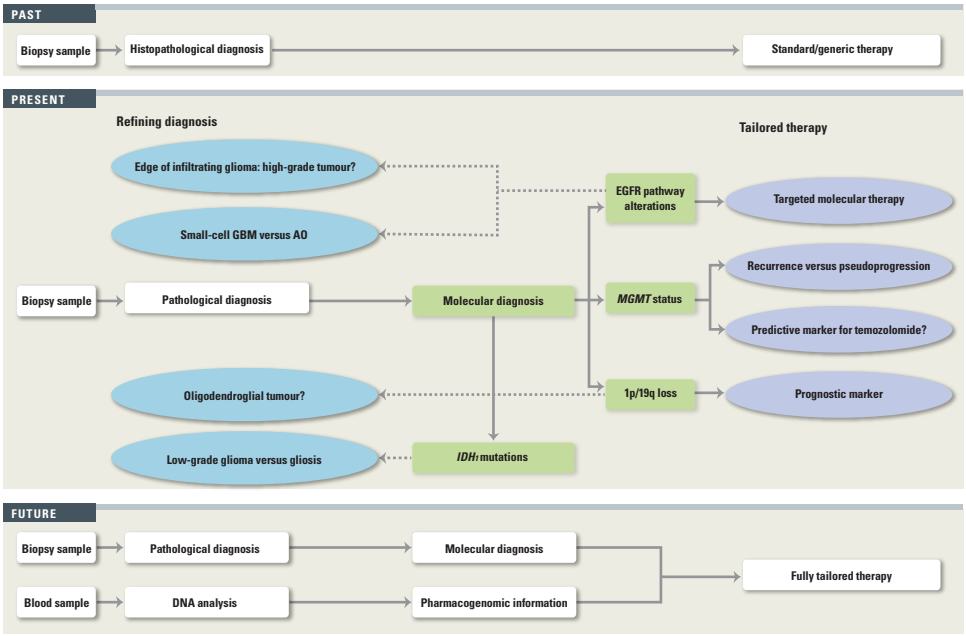
Louis Laboratory

James Kim
David N. Louis, MD
Catherine Nutt, PhD

Our laboratory investigates the molecular genetic basis of human brain tumors. Brain tumors are the second most frequent malignancy of childhood, and their incidence in adults increases with advancing age. These tumors are also among the most devastating of human malignancies, affecting the organ that defines the "self," often severely compromising quality of life. Malignant gliomas of the cerebral hemispheres in adults are the most common brain tumors and are the focus of our laboratory efforts.

Elucidating the molecular basis of glioma formation may impact both diagnostic and therapeutic aspects of clinical neuro-oncology. We have demonstrated alterations characteristic of specific glioma subtypes and grades. We originally demonstrated that molecular genetic analysis could be used to define clinicopathologically relevant subsets of glioblastomas (e.g., those with TP53 mutations and those with EGFR amplification). Glioblastomas with IDH1/2 and TP53 mutations are often so-called secondary glioblastomas, meaning the glioblastoma has arisen in a prior lower-grade astrocytoma; this scenario typically occurs in younger adult patients. TP53 mutations are also common in glioblastomas having many giant cells. EGFR gene amplification, on the other hand, typically occurs in so-called primary (or *de novo*) glioblastomas, which appear to arise more rapidly in older adult patients, as well as in small-cell glioblastomas. Notably, some of these genetic alterations can be used to improve pathological diagnosis of these entities, and the laboratory has demonstrated that markers such as mutant R132H IDH1 expression can be used to distinguish reactive astrocytosis from low-grade astrocytoma.

We have also shown that molecular genetic alterations are powerful predictors of therapeutic response and survival in patients with anaplastic oligodendrogliomas and in patients with other oligodendroglial tumors. Those patients, whose tumors have 1p loss, essentially always respond to therapy, and those with combined 1p and 19q loss that lack other detectable alterations have durable responses and long survival times (i.e., more than 10 years). In contrast, those patients whose tumors lack these genetic alterations but harbor others, such as EGFR amplification, rarely respond to chemotherapy in a durable manner and have short survivals (i.e., fewer than two years). These findings have already



Molecular diagnostic applications in glioma management. From Jansen et al., Lancet Neurology 2010.

led to incorporation of molecular diagnostic testing around the world for these parameters.

Dr. Louis is currently leading the efforts to incorporate molecular testing into the next updates of the World Health Organization Classification of Central Nervous System Tumors.

The lab has also demonstrated that glioblastomas treated with the alkylating agent temozolomide (which is now the standard of care for such cases) frequently inactivate mismatch repair genes, leading to more rapid growth during therapy and to therapeutic resistance, and has worked collaboratively with the Bernstein and Suva labs on epigenetic and single-cell studies of high-grade gliomas. The laboratory has also been involved in molecular genetic studies of other forms of human brain tumors, such as meningiomas.

Selected Publications:

Louis DN. Perry A, Burger P, et al. International Society of Neuropathology-Haarlem consensus guidelines for nervous system tumor classification and grading. *Brain Pathol* 2014 Jul 2. doi: 10.1111/bpa.12171.

Suvà ML, Rheinbay E, Gillespie SM, Patel AP, Riggi N. Wakimoto H, Rabkin SD, Martuza RL, Chi AS, Rivera MN, Wortman I, Shalek A, Rozenblatt-Rosen O, Regev A, **Louis DN**, Bernstein BE. Reconstructing and reprogramming the tumor-propagating potential of glioblastoma stem-like cells. *Cell* 157:580-594, 2014.

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Camelo-Piragua S, Jansen M, Ganguly A, Kim JCM, Cosper AK, Dias-Santagata D, Nutt CL, Iafrate AJ, **Louis DN**. A sensitive and specific diagnostic panel to distinguish diffuse astrocytoma from astrocytosis: chromosome 7 gain with mutant IDH1 and p53. *J Neuropathol Exp Neurol*. 70:110-115, 2011.

Mohapatra G, Engler DA, Starbuck KD, Kim JC, Bernay DC, Scangas GA, Rousseau A, Batchelor TT, Betensky RA, **Louis DN**. Genome-wide comparison of paired fresh frozen and formalin-fixed paraffin-embedded gliomas by custom BAC and oligonucleotide array comparative genomic hybridization: facilitating analysis of archival gliomas. *Acta Neuropathol*. 121:529-43, 2011.

Jansen M, Yip S, **Louis DN**. Molecular pathology in adult gliomas: diagnostic, prognostic, and predictive markers. *Lancet Neurol*. 9:717-26, 2010.

Shyamala Maheswaran, PhD

Metastasis, the leading cause of cancer related mortality, is a highly orchestrated process involving angiogenesis, invasion, intravasation and survival in the vasculature and extravasation and growth at distal sites. **The Maheswaran laboratory** is focused on understanding the mechanism of this process using in vitro and in vivo model systems and circulating tumor cells, which are putative metastatic precursors. Epithelial to mesenchytmal transition (EMT), an embryonic process reinstated in tumor cells, is a critical modulator of cancer metastasis. EMT is induced by several transcription factors and signaling pathways, and it enhances tumor cell motility and resistance to apoptosis. Using breast cells which are induced to undergo EMT, we intend to gain greater insight into the mechanisms involved in EMT induced tumor progression and identify signaling nodes that render tumor cells susceptible to targeted therapeutic intervention.

Maheswaran Laboratory*

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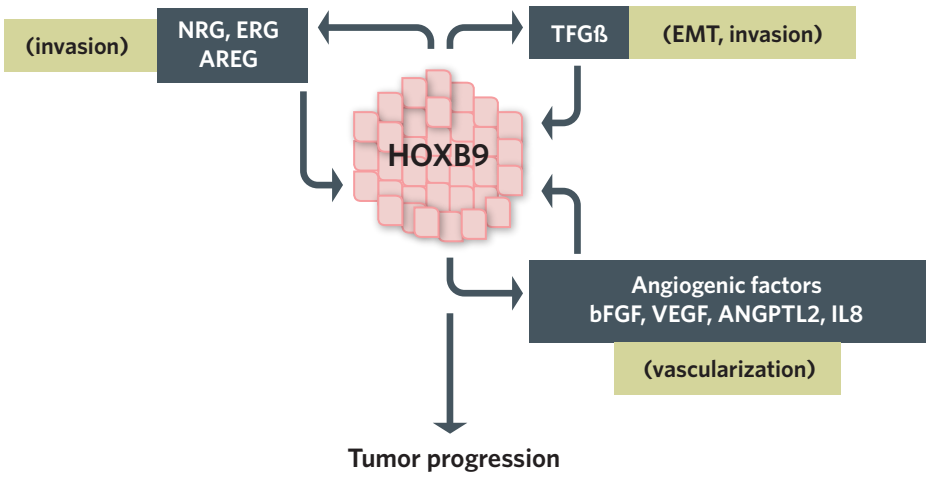
*co-directed with Daniel Haber, MD, PhD

Elucidating the mechanism of tumor metastasis using HOXB9 and BTG2 expressing breast cancers as a model system

My research goal is to understand the biology of breast cancer metastasis using cell culture and animal models and to validate these findings in clinical specimens. Aberrant expression of transcription factors, which has been implicated in the tumorigenesis of several types of cancers, can constitute a mechanism that induces the expression of growth and angiogenic factors in tumors leading to their local increase in the tumor microenvironment to favor tumor progression. The transcription factor HOXB9 is overexpressed in a subset of aggressive breast cancers. Suppression of its partner, BTG2—a p53 inducible gene—in breast cancer is also associated with increased metastasis, recurrence and early death. We have modeled breast cancer metastasis using experimental systems that mimic these molecular aberrations. These model systems demonstrate that molecular dysfunction involving gain of HOXB9 expression and loss of BTG2 expression induce tumoral secretion of cytokines such as TGFß and ErbB ligands and angiogenic factors into the microenvironment. Secretion of these growth factors induces signaling pathways that promote tumor cell proliferation, migration and invasion, angiogenesis, and distal metastasis. Moreover, they also alter tumor cell fates, leading to the acquisition of mesenchymal and stem-like phenotypes which influence tumor cell responses to radiation and other therapeutic interventions. In mouse models, breast tumor xenografts in which HOXB9 is overexpressed or BTG2 is suppressed are more sensitive to drugs that target the HER pathway and tumor angiogenesis. The goals of the lab are 1) to identify the mechanisms by which these molecular aberrations alter the tumor microenvironment and to delineate the autocrine and paracrine mechanisms that



HOXB9 overexpressing breast tumors produce growth factors to promote tumor progression.

influence tumor progression, 2) to identify the pathways that can be targeted either alone or in combination to suppress tumor progression and metastasis in this setting, and 3) to determine whether gain of HOXB9 and/or loss of BTG2 can be used as markers to identify patients who will be responsive/resistant to breast cancer therapies. By modulating HOXB9 and BTG2 expression in breast cancer cells, we intend to identify the molecular mechanisms that will render this subset of aggressive breast cancers susceptible to therapies that target these pathways.

Molecular characterization of circulating tumor cells

In collaboration with Drs. Daniel Haber and Mehmet Toner, I am also interested in the cellular and molecular characterization of circulating tumor cells (CTCs). This interest ties in well with the overall goal of the lab, which is to study cancer metastasis. In cancer patients, a rare population of tumor-derived cells is found in the circulation and is likely the source for distant metastatic disease. Detecting CTCs has far-reaching implications for both clinical care and cancer biology. CTCs are rare, comprising 1 in 10^9 cells in the blood of patients with metastatic breast cancer. This isolation presents a tremendous technical challenge for existing cell separation technologies. The microfluidic technology developed in Dr. Mehmet Toner's laboratory enables gentle, efficient and specific isolation of live CTCs in a single step. CTCs isolated from breast, prostate, pancreatic and lung cancer patients using this cutting edge technology will be characterized and standardized to provide a noninvasive tool for early disease detection and for monitoring response/resistance to therapy; viable cells will be cultured to gain insight into the growth, drug resistance and metastatic properties of these epithelial cancers.

Selected Publications:

Aceto N, Bardia A, Miyamoto DT, Donaldson MC, Wittner BS, Spencer JA, Yu M, Pely A, Engstrom A, Zhu H, Brannigan BW, Kapur R, Stott SL, Shioda T, Ramaswamy S, Ting DT, Lin CP, Toner M, Haber DA*, **Maheswaran S***. Circulating tumor cell clusters are oligoclonal precursors of breast cancer metastasis. *Cell*. 158(5):1110-22, 2014.

Yu M, Bardia A, Aceto N, Bersani F, Madden MW, Donaldson MC, Desai R, Zhu H, Comaills V, Zheng Z, Wittner BS, Stojanov P, Brachtel E, Sgroi D, Kapur R, Shioda T, Ting DT, Ramaswamy S, Getz G, Iafrate AJ, Benes C, Toner M, **Maheswaran S**, Haber DA. Cancer therapy. Ex vivo culture of circulating breast tumor cells for individualized testing of drug susceptibility. *Science*. 345(6193):216-20, 2014

Yu M, Bardia A, Wittner BS, Stott SL, Smas ME, Ting DT, Isakoff SJ, Ciciliano JC, Wells MN, Shah AM, Concannon KF, Donaldson MC, Sequist MV, Brachtel E, Sgroi D, Baselga J, Ramaswamy S, Toner M, Haber DA, **Maheswaran S**. Circulating Breast Tumor Cells Exhibit Dynamic Changes in Epithelial and Mesenchymal Composition. *Science*. 339(6119): 580-584, 2013.

Chiba N, Comaills V, Shiotani B, Takahashi F, Shimada T, Tajima K, Winokur D, Hayashida T, Willers H, Brachtel E, Vivanco MD, Haber DA, Zou L, **Maheswaran S**. Homeobox B9 induces epithelial-to-mesenchymal transition-associated radioresistance by accelerating DNA damage responses. *Proc Natl Acad Sci U S A*. 109(8):2760-5, 2012.

Takahashi F, Chiba N, Tajima K, Hayashida T, Shimada T, Takahashi M, Moriyama H, Brachtel E, Edelman EJ, Ramaswamy S, **Maheswaran S**. Breast tumor progression induced by loss of BTG2 expression is inhibited by targeted therapy with the ErbB/HER inhibitor lapatinib. *Oncogene*. 30(27):3084-95, 2011.

*Co-corresponding authors

Andrea I. McClatchey, PhD

The McClatchey laboratory focuses on understanding how cells organize their outer membrane or cortex, which, in turn, determines their identity, behavior, and interface with the extracellular environment. Cancer cells exhibit defective membrane organization and therefore interact inappropriately with other cells and with their environment. Our research stems from a longstanding interest in understanding the molecular basis of neurofibromatosis type 2 (NF2), a familial cancer syndrome that is caused by mutation of the *NF2* tumor suppressor gene. The *NF2*-encoded protein, Merlin, and closely related ERM proteins (Ezrin, Radixin, and Moesin) are key architects of the cell cortex.

McClatchey Laboratory

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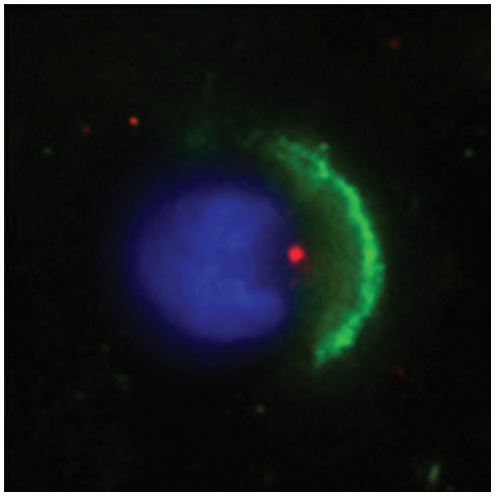
Understanding morphogenesis and tumorigenesis

The vast array of forms and functions exhibited by different cell types is made possible by the organization of specialized domains within the cell cortex such as cell:cell and cell:matrix adhesions, the intestinal brush border, neuronal growth cone and immunological synapse. The assembly of such cortical domains involves the coordination of processes occurring at the plasma membrane with those in the underlying cytoskeleton. Central to this coordination is the formation of protein complexes at the plasma membrane that position membrane receptors, control their abundance and activity, and link them to the cortical cytoskeleton, thereby serving both regulatory and architectural functions. The overarching goal of my laboratory is to understand how the organization of protein complexes at the cell cortex contributes to morphogenesis and tumorigenesis. This interest stems from a longstanding dedication to elucidating the molecular basis of neurofibromatosis type 2 (NF2), a familial cancer syndrome that is caused by mutation of the *NF2* tumor suppressor gene.

The *NF2*-encoded protein Merlin is closely related to the ERM proteins (Ezrin, Radixin and Moesin) that link membrane proteins to the cortical cytoskeleton, thereby both stabilizing membrane complexes and stiffening the cell cortex. The proximal goal of our work is to delineate the molecular function of Merlin and identify therapeutic targets for NF2; our work also directly addresses fundamental aspects of basic and cancer cell biology.

Through the generation and analysis of mouse models, we identified critical roles for Merlin and the ERM proteins in morphogenesis, homeostasis and tumorigenesis in many tissues including the liver, kidney, intestine, skin and mammary gland. Complementary molecular and cell-based studies suggest that these phenotypes are caused by defective cortical distribution of membrane receptors such as EGFR/ErbBs, cell junction components, and/or protein complexes that guide the orientation and function of the mitotic spindle. For example, our recent studies revealed that a key activity of Merlin is to restrict the distribution of Ezrin at the cell cortex. In the absence of Merlin, as in *NF2*-mutant cancers, unrestricted



The membrane-cytoskeleton linking protein Ezrin forms a cortical 'cap' (green) that instructively positions the centrosome (red) and eventually guides mitotic spindle orientation in dividing cells (the nucleus is stained blue).

cortical Ezrin drives both the aberrant distribution of membrane receptors such as EGFR/ErbBs and aberrant centrosome-to-cortex communication, yielding defective spindle orientation and integrity. These studies provided novel insight into how the organization of the cell cortex defines the identity and behavior of individual cell types and into how aberrant cortical organization contributes to unscheduled cell proliferation and tumor development.

Ongoing studies extend both basic and translational implications of this work. We are currently focused on defining the molecular mechanism by which Merlin/ERMs organize the biochemical and physical properties of the cell cortex and how this, in turn, controls receptor distribution and spindle orientation/integrity. We are also working in collaboration with other CCR faculty to understand how Merlin/ERM activities and, conversely, *NF2*-mutant phenotypes are influenced by mechanical forces such as those experienced in tissues. Importantly, we are also pursuing two novel translational avenues that stem directly from our basic studies: 1) The role of unregulated ErbB signaling in *NF2*-mutant tumors, particularly schwannomas, which are the hallmark of human NF2; and 2) Targeting aberrant centrosome/spindle function in *NF2*-mutant tumors; indeed, we have found that cells derived from all four major human *NF2*-mutant tumor types (schwannoma, meningioma, renal carcinoma and mesothelioma) exhibit centrosome/spindle defects and therefore may be sensitive to centrosome/spindle-targeting drugs. We believe that the continued partnering of these basic and translational studies will not only lead to novel therapeutic options for *NF2*-mutant tumors but also advance our understanding of these basic cellular activities that are known to contribute to other human cancers.

Selected Publications:

Hebert AM, Duboff B, Casaletto JB, Gladden, AB, **McClatchey AI**. Merlin/ERM proteins establish cortical asymmetry and centrosome position. *Genes Dev*. 26(24): 2709-23, 2012 Dec 15.

Casaletto JB, Saotome I, Curto M, **McClatchey AI**. Ezrin-mediated apical integrity is required for intestinal homeostasis. *Proc Natl Acad Sci U S A*. 108(29):11924-9, 2011 Jul 19.

Gladden AB, Hebert AM, Schneeberger EE, **McClatchey AI**. The NF2 tumor suppressor, Merlin, regulates epidermal development through the establishment of a junctional polarity complex. *Dev Cell*. 19(5):727-39, 2010 Nov 16.

Benhamouche S, Curto M, Saotome I, Gladden AB, Liu CH, Giovannini M, **McClatchey AI**. Nf2/Merlin controls progenitor homeostasis and tumorigenesis in the liver. *Genes Dev.* 24(16):1718-30, 2010 Aug 15.

Morris ZS, **McClatchey AI**. Aberrant epithelial morphology and persistent epidermal growth factor receptor signaling in a mouse model of renal carcinoma. *Proc Natl Acad Sci U S A*. 106(24):9767-72, 2009 Jun 16.

Cole BK, Curto M, Chan AW, **McClatchey AI.** Localization to the cortical cytoskeleton is necessary for Nf2/merlin-dependent epidermal growth factor receptor silencing. *Mol Cell Biol*. 28(4):1274-84, 2008 Feb.

Raul Mostoslavsky, MD, PhD

Research in **the Mostoslavsky laboratory** focuses on a family of proteins first discovered in yeast that plays a critical role in many human diseases, including cancer. The yeast protein Sir2 enables yeast cells to survive under conditions of nutrient stress and functions as a modulator of lifespan. While recent studies indicate that some of the mammalian sirtuin (SIRT) homologues also play a role in stress resistance and metabolic homeostasis, their precise molecular functions remain unclear. Most of our work involves the Sir2 mammalian homolog known as SIRT6. Our research suggests that SIRT6 modulates glucose metabolism and DNA repair and may function as a tumor suppressor gene. Using transgenic mouse models and other experimental systems, we are exploring the role of SIRT6 in tumorigenesis and other disease processes.

Mostoslavsky Laboratory

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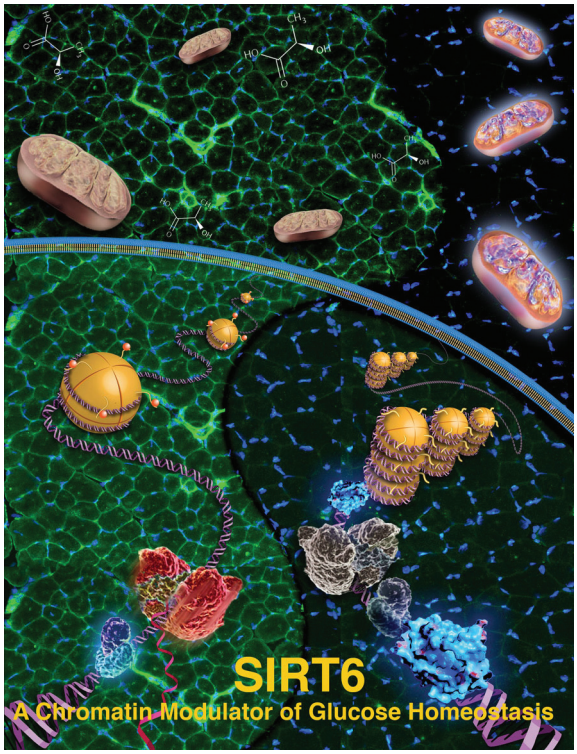
Cells need to maintain their nuclear DNA accurately in order to function properly. Indeed, defects in DNA integrity are associated with cancer, aging and immunodeficiency. Therefore, numerous DNA repair systems in mammalian cells function to endow us with long and relatively tumor-free lives. The DNA and the histones are arranged in the nucleus in a highly condensed structure known as chromatin. Cellular processes that unwind the double helix— such as transcription, replication and DNA repair—have to overcome this natural barrier to DNA accessibility.

Multicellular organisms also need to control their use of cellular energy stores. Glucose metabolism plays a crucial role in organismal homeostasis, influencing energy consumption, cell proliferation, stress resistance and lifespan. Defective glucose utilization causes numerous diseases ranging from diabetes to an increased tendency to develop tumors. For cells to respond appropriately to changes in energy status or to DNA damage, a close coupling of DNA repair, chromatin remodelling and metabolic pathways is likely to be involved.

Our lab is interested in understanding the influence of chromatin on DNA repair and the relationship between the DNA damage response and the metabolic adaptation of cells. We focus on the study of a group of proteins called SIRTs, the mammalian homologues of the yeast Sir2. Sir2 is a chromatin silencer that functions as an NAD-dependent histone deacetylase to inhibit DNA transcription and recombination. Although we have several collaborations involving the mammalian SIRT1 protein, most of our work has focused on another mammalian Sir2 homologue, SIRT6. We have recently found that SIRT6 binds to chromatin and regulates DNA repair functioning as an anchor of the chromatin remodeler SNF2H. In addition, we have shown that SIRT6 regulates metabolic responses in cells and that mice lacking SIRT6 exhibit severe metabolic defects, including hypoglycemia and hypoinsulinemia. SIRT6 appears to modulate glucose flux inside the cells, functioning as a histone H3K9 deacetylase to silence glycolytic genes acting as a coexpressor of Hif1alpha, in this way directing glucose away from glycolysis



SIRT6: A Chromatin Modulator of Glucose Homeostasis

to reduce intracellular ROS levels. This function appears critical for glucose homeostasis, as SIRT6 deficient animals die early in life from hypoglycemia. Remarkably, our recent studies implicate SIRT6 as a tumor suppressor that regulates cancer metabolism through mechanisms that by-pass known oncogenic pathways. Cancer cells prefer fermentation (i.e., lactate production) to respiration. Despite being described by biochemist and Nobel laureate Otto Warburg decades ago (i.e., the Warburg effect), the molecular mechanisms behind this metabolic switch remain a mystery. We believe SIRT6 may function as a critical modulator of the Warburg effect, providing a long-sought molecular explanation to this phenomenon.

Our current studies are directed at determining how the DNA repair and metabolic functions of SIRT6 may be related to each other. We use a number of experimental systems, including biochemical and biological approaches, as well as genetically engineered mouse models.

Projects:

1. Defining which enzymatic activity is critical for SIRT6 function and determining the proteins targeted by this activity
2. Deciphering how SIRT6 regulates chromatin structure
3. Determining the role of SIRT6 in DNA repair and tumorigenesis using mouse models
4. Elucidating the role of histone modifications and chromatin dynamics in DNA repair
5. Determining molecular crosstalks between epigenetics and metabolism.

Selected Publications:

Toiber D, Erdel F, Bouazoune K, Silberman DM, Zhong L., Mulligan P, Sebastian C, Cosentino C, Martinez-Pastor B, Giacosa S, D'Urso A, Naar AM, Kingston R, Rippe K, and **Mostoslavsky R**. SIRT6 recruits SNF2H to DNA break sites, preventing genomic instability through chromatin remodeling. *Molecular Cell*. In Press, 2013.

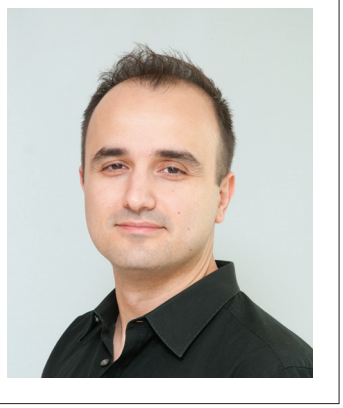
Sebastian C, Zwaans BM, Silberman DM, Gymrek MA, Goren A, Zhong L, Ran O, Truelove J, Guimaraes AR, Toiber D, Cosentino C, Greenson JK, MacDonald AI, McGlynn L, Maxwell F, Edwards J, Giacosa S, Guccione E, Weisledder R, Bernstein BE, Regev A, Shiels PG, Lombard DB and **Mostoslavsky R**. The Histone Deacetylase SIRT6 is a tumor suppressor that controls cancer metabolism. *Cell*. 151, 1185-1199. 2012.

Zhong L, D'Urso A, Toiber D, Sebastian C, Henry RE, Vadysirisack DD, Guimaraes A, Marinelli B, Wikstrom JD, Nir T, Clish CB, Vaitheesvaran B, Iliopoulos O, Kurland I, Dor Y, Weissleder R, Shirihai OS, Ellisen L, Espinosa JM, **Mostoslavsky R**. The histone deacetylase SIRT6 regulates glucose homeostasis via Hif1α. *Cell*. 140, 280-293, 2010.

Finkel T, Deng C-H, **Mostoslavsky R**. Recent progress in the biology and physiology of sirtuins. *Nature*. 460, 587-591, 2009.

Mostoslavsky R, Chua KF, Lombard DL, Pang WW, Fischer MR, Gellon L, Liu P, Mostoslavsky G, Franco S, Murphy MM, Mills KD, Patel P, Hsu J, Hong AL, Ford E, Cheng H-L, Kennedy C, Nunez N, Bronson R, Frendewey D, Auerbach W, Valenzuela D, Karow M, Hottiger MO, Hursting S, Barrett JC, Guarente L, Mulligan R, Demple B, Yancopolous GD, and Alt FW. Genomic instability and aging-like phenotype in the absence of mammalian SIRT6. *Cell*. 124:315-329, 2006.

Mostoslavsky R, Singh N, Tenzen T, Goldmit M, Gabay C, Elizur S, Qi P, Reubinoff BE, Chess A, Cedar H, Bergman Y. Asynchronous replication and allelic exclusion in the immune system. *Nature*. 414, 221-225, 2001.



Mo Motamedi, PhD

Humans have a variety of different cell types, which perform a multitude of functions necessary for life. Interestingly, all cells within an individual share the identical set of genes. So how do cells acquire different identities and functions? Recent work has revealed that during development cellular identity is established and maintained by a process called epigenetics, a molecular memory system by which cells turn some of their genes on and off, establishing distinct gene expression patterns and cellular identities. Epigenetic mechanisms ensure that correct gene expression patterns are inherited during cell division so that stable cell identities can be maintained throughout development. In cancers, cells lose their ability to retain their correct identity and display aberrant gene expression patterns. Epigenetic aberrations occur at all stages of malignancies, from tumor formation to metastasis. **The Motamedi laboratory** uses the powerful model system of the fission yeast to explore this problem. Our goal is to understand the precise molecular mechanisms involved in regulating epigenetics in an effort to uncover novel targets for fighting cancers.

Motamedi Laboratory

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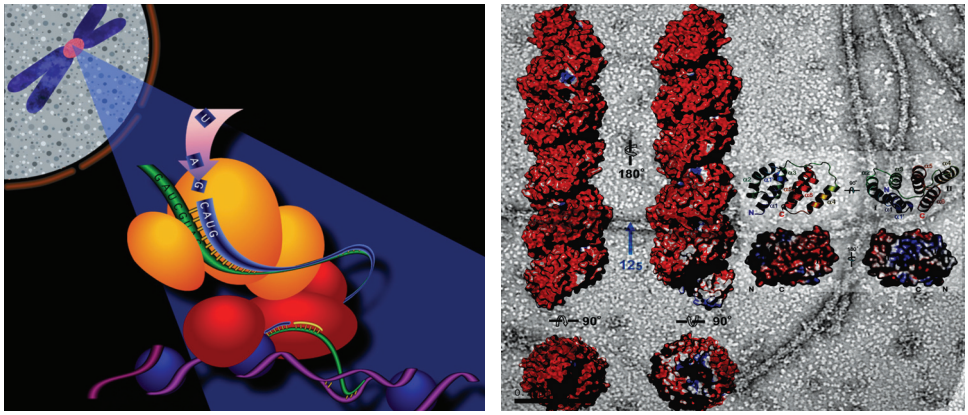
* Graduate student

Epigenetic changes are stable and heritable alterations to gene expression patterns without concomitant mutations in the responsible genes. Disruption to epigenetic regulation leads to aberrant gene expression patterns, which underlie a variety of human maladies, including all cancers. Epigenetic aberrations have been shown to contribute to all stages of oncogenesis from initiation to metastasis. Understanding how epigenetic circuits are established, maintained and inherited at the molecular level is critical for the development of novel targets and therapeutic tools in the battle against cancer. Most of what is known about the molecular mechanism of epigenetic inheritance comes from decades of research in model organisms such as *Saccharomyces cerevisiae, Schizosaccharomyces pombe*, and *Drosophila melanogaster*. This research has led to the discovery of highly conserved protein families and chromatin marks which are now being targeted for therapeutic or diagnostic purposes.

Noncoding RNAs and chromatin

The first model about how long and small noncoding RNAs mediate epigenetic inheritance of chromatin states was proposed in the fission yeast. Our model posits that noncoding RNAs, tethered to chromatin, provide a platform for the assembly of RNA-processing and chromatin-modifying proteins, leading to transcriptional regulation of the neighboring genes. In addition to acting as platforms, RNA molecules target chromatin regulatory proteins to specific chromosomal regions. These principles now have emerged as a conserved mechanism by which noncoding RNAs partake in epigenetic inheritance of chromatin states and regulate gene expression globally. Recent work in cancer has revealed that regulation of epigenetic states by noncoding RNAs is intimately associated with all stages of oncogenesis. Thus uncovering the molecular details of this mechanism is one of the most



The image on the left depicts RNA-mediated epigenetic gene silencing at the fission yeast centromeres, during which nascent long non-coding(lnc) RNAs, tethered to chromatin, act as platforms for the recruitment of silencing proteins. New synthesis of lncRNAs (shown as incorporation of new ribo-nucleotides) followed by lncRNA processing into short siRNAs (yellow RNA in the Red complex) lead to amplifications of the RNA silencing signal. The image on the right depicts the polymerization domain of one of the key silencing proteins, Tas3. This self-polymerization property is required for the 'spreading' of silencing factors from initiation centers to the surrounding chromosomal regions. This mechanism is required for proper chromosome segregation and maintenance of genomic stability.

promising fields of research in molecular biology.

In the Motamedi lab, we study how noncoding RNAs and chromatin complexes cooperate to mediate epigenetic gene silencing. We use a combination of genetic, biochemical, cell biological, genomic and proteomic approaches to ask mechanistic questions about how epigenetic states are established, maintained and reprogrammed in cells. Because many of the proteins involved in this process are highly conserved among eukaryotes, we will apply this knowledge to investigate the contribution of the homologous proteins to epigenetic inheritance and, in the long run, to cancers in human cells.

DNA repair and genomic stability

Another interest of the Motamedi lab is DNA repair and genome stability. In eukaryotic cells, the abundance of repetitive DNA sequences (centromeres, telomeres, rDNA, etc.) and the presence of an efficient recombination system pose a serious challenge to genomic stability. Aberrant recombination among repetitive DNA elements results in loss or duplication of genetic information often contributing to an increase in mutation rates and genome instability. To maintain genomic stability, cells compact their repetitive DNA into a special structure called heterochromatin, which prevents spurious recombination among repeats, thus stabilizing the genome. Cells defective in heterochromatin formation exhibit high rates of chromosome loss in mitosis, genomic instability, and increased mutation rates. In cancers, heterochromatin is lost in nearly all cancers contributing to their increase in mutation rates and cancers. In the Motamedi lab, we study how chromatin and noncoding RNAs cooperate to maintain heterochromatin and genomic stability. Our goal is to gain novel insight into the conserved mechanisms by which cells make their repetitive DNA elements refractory to recombination and regulate the access of these factors to these regions.

Overall our goal is to harness the powerful genetic, biochemical, and cell biological tools available in the fission yeast to drive novel discoveries in pathways affected in cancers.

Selected Publications:

Joh RI, Palmieri CM, Hill IT, **Motamedi MR**. Regulation of histone methylation by noncoding RNAs. BBA *Gene Regulatory Mechanisms* 2014.

Buker S, **Motamedi MR**. Purification of native Argonaute complexes from the fission yeast Schizosaccharomyces pombe. *Methods Mol Biol*. 725: 1-13. Argonaute proteins: Methods and Protocols, 2011.

Li H*, **Motamedi MR***, Yip C, Wang Z, Walz T, D. J. Patel, D. Moazed. An alpha motif at Tas3 C terminus mediates RITS cis-spreading and promotes heterochromatic gene silencing.†*Mol Cell*. 34: 155-167, 2009. †*This article was previewed in Dev Cell. 16: 630-632, 2009*.

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Smith KM, Kothe GO, Matsen CB, Khlafallah TK, Adhvaryu KK, Hemphill M, Freitag M, **Motamedi MR**, Selker E. The fungus Neurospora-racrassa displays telomeric silencing mediated by multiple sirtuins and by methylation of histone H3 lysine 9. *Epigenetics Chromatin*. 1: 5, 2008.

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*co-authors

Anders M. Näär, PhD

The Näär laboratory investigates the mechanisms by which genes are switched on or off and how these processes go awry in diseases such as cancers and cardio-metabolic disorders. For example, we have discovered previously unknown molecular mechanisms involved in controlling the output of genes important in cholesterol and fat metabolism. Studies of these mechanisms, involving complex circuits of gene regulators and tiny snippets of RNA called microRNAs, are yielding new therapeutic strategies to target metabolic defects contributing to the etiology of many types of cancers as well as cardiometabolic diseases such as obesity, type 2 diabetes, non-alcoholic fatty liver diseases, and coronary artery disease.

Näär Laboratory

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Joy Nishikawa, PhD
Yoojin Sohn, BS
Alexandre Wagschal, PhD
Lifeng Wang, PhD
Su Wu, PhD

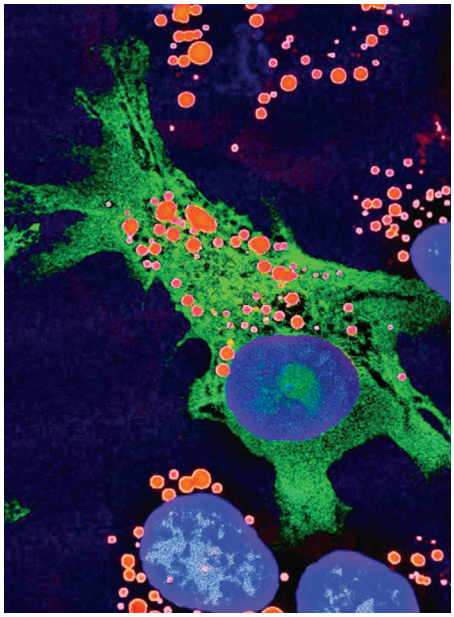
Our research is focused on elucidating molecular mechanisms of gene regulation, with emphasis on disease-associated pathways contributing to cholesterol/lipid disorders, certain types of cancers, and multidrug resistance in fungal infections.

Cholesterol/lipid regulation by the SREBP transcription factors

Part of our effort is centered on understanding how transcriptional regulators activate or repress target gene expression. One area of interest concerns the regulatory circuits governing cholesterol/lipid homeostasis. Aberrant regulation of cholesterol and other lipids contributes to major human diseases such as atherosclerosis, type 2 diabetes, metabolic syndrome, Alzheimer's disease, and several types of cancers, thus highlighting the importance of understanding how cholesterol/lipid homeostasis is controlled. Our work on the sterol regulatory element-binding protein (SREBP) transcription factor family, master regulators of cholesterol/lipid biosynthesis and metabolism, has provided key mechanistic insights into gene regulatory pathways guiding metabolic homeostasis. For example, we have found that a specific subunit (ARC105/MED15) of the Mediator co-activator, a large multiprotein assembly, plays a critical role in mediating SREBP-dependent activation of genes controlling cholesterol/lipid homeostasis (Yang et al. *Nature* 2006). Our studies have also revealed a critical role for orthologs of the NAD^+-dependent deacetylase SIRT1 in negative regulation of SREBPs during fasting from *C. elegans* to mammals, with important implications for human cholesterol/lipid disorders (Walker et al. *Genes Dev* 2010). We have also uncovered a novel SREBP-regulatory feedback circuit linking production of the key membrane phospholipid phosphatidylcholine to SREBP-dependent control of hepatic lipogenesis (Walker et al. *Cell* 2011). These insights together may yield novel treatments for cardiometabolic diseases and cancers.

MicroRNA regulation of cholesterol/lipid homeostasis

Cholesterol and lipids are trafficked in the blood as lipoprotein particles, such as low-density lipoprotein (LDL) and high-density lipoprotein (HDL), which ferry their fatty cargo to different cells and tissues. Intriguingly, we have found conserved microRNAs (miR-33a/b)



The image shows a cell (expressing Green Fluorescent Protein) with large lipid droplets (red) that accumulate in cells where the cholesterol/lipid regulator SREBP is activated. The nucleus is stained blue with DAPI. Aberrant activity of SREBP has been linked to cardiometabolic disorders and cancers.

embedded within intronic sequences in the human SREBP genes. Our studies revealed that miR-33a/b target the cholesterol efflux pump ABCA1 for translational repression. ABCA1 is important for HDL synthesis and reverse cholesterol transport (RCT) from peripheral tissues, including macrophages/foam cells, and mutations in the ABCA1 gene have been implicated in atherosclerosis. Moreover, our work has shown that miR-33a/b also control the expression of genes involved in fatty acid oxidation, as well as the regulation of energy homeostasis. These findings suggest that miR-33a/b may represent novel targets of antisense-based therapeutics to increase ABCA1 levels, promote macrophage/foam cell cholesterol efflux, stimulate de novo HDL production and RCT, and ameliorate cardiovascular disease (Najafi-Shoushtari et al. *Science* 2010; Rottiers et al. *CSH Symp Quant Biol* 2012; Rottiers & Näär, *Nature Rev. Mol. Cell Biol*. 2012; Rottiers et al. *Science Transl Med* 2013).

Multidrug resistance in pathogenic fungi

Immunocompromised individuals such as cancer patients undergoing chemotherapy are highly susceptible to fungal infections (e.g., *Candida* species), which frequently become drug-resistant upon antifungal treatment. We have elucidated the molecular mechanism by which the important human pathogenic fungus *Candida glabrata* becomes resistant to standard azole antifungal treatment (Thakur et al. *Nature* 2008). Orthologs of the Pdr1p transcription factor control the multidrug resistance pathway in *C. glabrata*, and mutations in Pdr1p causes constitutive activation and MDR in clinical isolates of *C. glabrata*. We have found that Pdr1p interacts with the ARC105/MED15 homolog Gal11p in *C. glabrata*, and we have used this detailed molecular understanding to devise high-throughput screens to find small molecules targeting the interaction of Pdr1p with Gal11p. This work led to the identification of a potent inhibitor of Pdr1p-dependent gene activation and MDR in drug resistant *C. glabrata* harboring mutated Pdr1p. This compound exhibits efficacy in mouse models as a novel anti-MDR co-therapeutic to re-sensitize drug-resistant *C. glabrata* to standard azole treatment.

Selected Publications:

Rottiers V, Obad S, Petri A, McGarrah R, Lindholm MW, Black JC, Sinha S, Goody RJ, Lawrence MS, Delemos AS, Hansen HF, Whittaker S, Henry S, Brookes R, Najafi-Shoushtari SH, Chung RT, Whetstine JW, Gerszten RE, Kauppinen S, and **Näär AM**. Pharmacological inhibition of a microRNA family in non-human primates by a seed-targeting 8-mer antimiR oligonucleotide. *Science Translational Medicine*. 2013.

Toiber D, Erdel F, Silberman D, Zhong L, Mulligan P, Bouazoune K, Sebastian C, Cosentino C, Martinez-Pastor B, Giacosa S, D'Urso A, **Näär AM**, Kingston R, Rippe K, and Mostoslavsky R. SIRT6 recruits SNF2H to sites of DNA breaks, preventing genomic instability through chromatin remodeling. *Molecular Cell*. 2013.

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Randall T. Peterson, PhD

The Peterson laboratory focuses on discovering bioactive small molecules by high-throughput in vivo screening. Whereas chemical screening has traditionally focused on simple, in vitro assays, many biological phenomena are difficult to reduce to an in vitro assay. The Peterson lab is using the tools of chemical biology to investigate these complex in vivo phenomena. By conducting high-throughput screens with intact, living zebrafish, we can discover small molecules that alter virtually any biological process. The lab is applying this approach in three areas: 1) developmental biology, including cardiovascular development and germ cell development; 2) disease physiology, including heart failure, anemia and leukemia; and 3) animal behaviors. In each of these areas, the novel small molecules discovered are providing new biological insights and/or novel therapeutic opportunities.

Peterson Laboratory

Aarti Asnani, MD
Colleen Brady, PhD
Andrew Gonzales
Devin Harrison
Youngnam Jin, PhD
Xiang Li, PhD
Yan Liu, PhD
Anjali Nath, PhD
Randall T. Peterson, MD
Andrew Rennekamp, PhD
You Wang, PhD

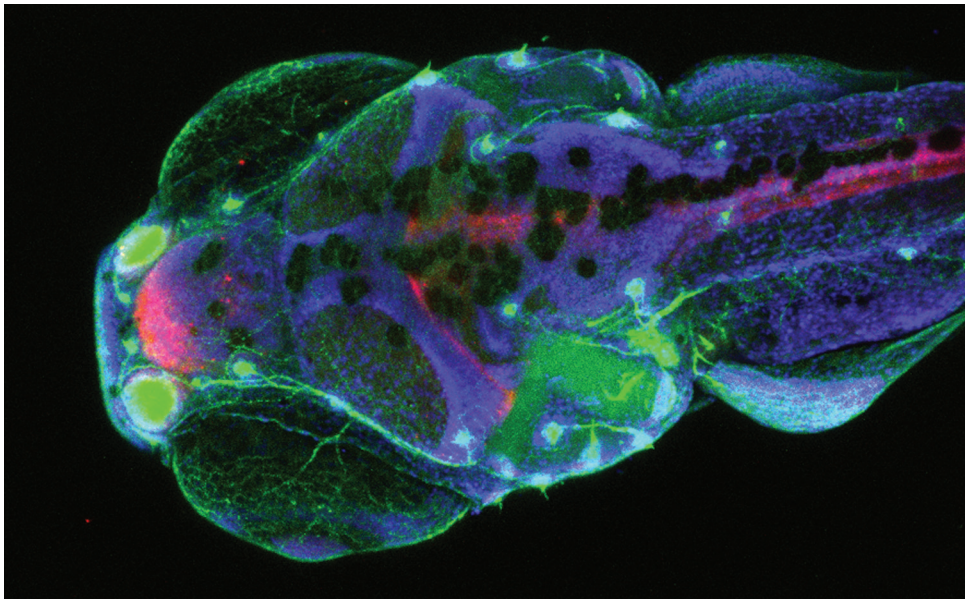
Developmental Biology

Small molecules are powerful tools for studying developmental biology because they provide timing and dosage control over developmental pathways that is difficult to achieve with genetic mutations. Unfortunately, only a handful of developmental pathways can currently be targeted with small molecules. We are discovering novel chemical modifiers of developmental pathways by exposing zebrafish embryos to libraries of structurally diverse small molecules and identifying those that induce specific developmental defects. Using screens of this type, we have discovered dozens of compounds that cause specific defects in hematopoiesis, embryonic patterning, pigmentation, and morphogenesis of the heart, brain, ear and eye and germ cell lineage.

One notable lab success in recent years has been the discovery of dorsomorphin and related BMP receptor antagonists. These small molecules were discovered during a zebrafish screen for compounds that alter development of the embryonic dorsal-ventral axis. As the first compounds to antagonize BMP signaling, the molecules have become powerful tools for studying BMP functions, and the molecules have already been used in hundreds of other studies around the world. In addition, the compounds have proven to be effective in treating animal models of BMP-related disorders, including heterotopic ossification and anemia. The compounds are currently in late stages of preclinical development.

Disease Physiology

One focus of our group is modeling human diseases in zebrafish. We use these models to screen large chemical libraries for small-molecule modulators of the disease-related phenotypes. The compounds we discover help us elucidate disease mechanisms and serve as starting points for developing new drug candidates. Disease physiology is often complex and involves interactions between multiple organs and tissue types. Consequently, many diseases cannot be studied effectively using in vitro assays. The zebrafish is an excellent vertebrate model system to study many complex,



Nerve bundles stained in the head of a transparent zebrafish.

non-cell autonomous diseases because the diseases can be studied in a native, whole-organism setting. In addition, compounds discovered in zebrafish screens have the advantage of having been selected for their ability to be active, efficacious and well tolerated in animals.

One notable example from the lab was discovery of compounds that suppress the effects of the AML1-ETO oncogene in acute myeloid leukemia (AML). We generated a model of AML by expressing the human AML1-ETO oncogene in zebrafish. These zebrafish accumulate granulocytic blast cells that resemble those found in humans with AML. In a robotic expression screen of thousands of small molecules, we discovered that nimesulide can reverse the oncogenic effects of AML1-ETO, an effect that is conserved in mammalian models of AML.

Animal Behaviors

Behaviors are accessible readouts of the molecular pathways that control neuronal signaling. Our group develops tools and techniques for high-throughput behavioral phenotyping in the zebrafish. These tools have potential to improve our understanding of neuronal signaling and may accelerate the pace of neuroactive drug discovery.

Selected Publications:

Kokel D, Cheung CY, Mills R, Coutinho-Budd J, Huang L, Setola V, Sprague J, Jin S, Jin YN, Huang XP, Bruni G, Woolf CJ, Roth BL, Hamblin MR, Zylka MJ, Milan DJ, **Peterson RT**. Photochemical activation of TRPA1 channels in neurons and animals. *Nat Chem Biol*. 9(4):257-63, 2013.

Hwang WY, Fu Y, Reyon D, Maeder ML, Tsai SQ, Sander JD, **Peterson RT**, Yeh JR, Joung JK. Efficient genome editing in zebrafish using a CRISPR-Cas system. *Nat Biotechnol*. 31(3):227-9, 2013.

van Ham TJ, Kokel D, **Peterson RT**. Apoptotic cells are cleared by directional migration and elmo1-dependent macrophage engulfment. *Curr Biol*. 22(9):830-6, 2012.

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Rihel J, Prober DA, Arvanites A, Lam K, Zimmerman S, Jang S, Haggarty SJ, Kokel D, Rubin LL, **Peterson RT**, Schier AF. Zebrafish behavioral profiling links drugs to biological targets and rest/wake regulation. *Science*. 327(5963):348-51, 2010.

Yeh JR, Munson KM, Elagib KE, Goldfarb AN, Sweetser DA, **Peterson RT**. Discovering chemical modifiers of oncogene-regulated hematopoietic differentiation. *Nat Chem Biol*. 5(4):236-43, 2009.

Shiv Pillai, MD, PhD

The Pillai laboratory asks questions about the biology of the immune system and human genetics. Some of these questions are: 1) Can we manipulate the immune system to treat cancer and to increase immunological memory? 2) Can we understand how genetics and the environment affect lymphoid clones to drive common diseases? and 3) Can this latter information be used to better understand and develop new therapies for chronic inflammatory human diseases such as arthritis, lupus and Crohn's disease? Our discovery of the role of an enzyme called Btk in the activation of B cells has contributed to the generation of Btk inhibitors that are effective in B cell malignancies and in trials of autoimmunity. One of the pathways we are currently studying suggests new approaches for the treatment of autoimmune disorders. We have also found a novel way to strengthen immune responses and enhance helper T cell memory that provides hope for developing more effective personalized immune-system based treatments for cancer.

Pillai Laboratory

Cariappa Annaiah, MD
Ezana Demissie*
Yasuyuki Iuchi MD
Maria Kulikova
Vinay Mahajan, MD, PhD
Hamid Mattoo PhD
Shiv Pillai, MD, PhD
Ian Rosenberg, PhD
Kendra Taylor, PhD
Kai Xin, PhD

* PhD Candidates

A novel human T cell subset that drives fibrosis (NIAID Autoimmune Center of Excellence at MGH)

In studies on the immunology of IgG4 related disease and scleroderma, performed in collaboration with John Stone in Rheumatology, we have identified an unusual, clonally expanded and potentially "fibrogenic" human CD4+ effector T cell subset. The role of this T cell subset is currently being investigated.

Using small molecules to enhance immunity in cancer and chronic viral infections

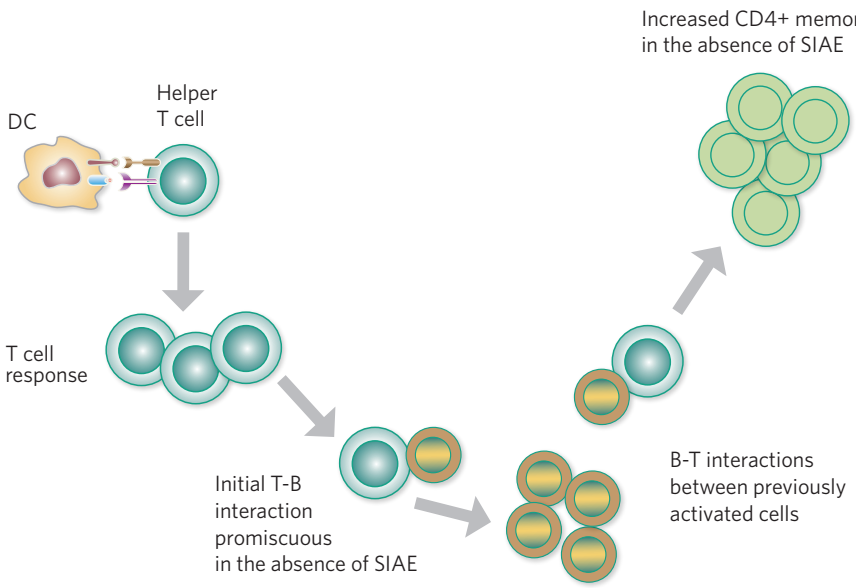
We have shown that the absence of sialic acid acetyl esterase (SIAE) contributes to enhanced somatic hypermutation in B cells and an increase in CD4+ and CD8+ T cell memory. The mechanisms by which these alterations occur are being investigated. We have, in collaborative studies with Ben Cravatt at Scripps, identified small molecule inhibitors of SIAE and are attempting to develop approaches to enhance immunity in cancer and chronic viral infections.

Studies on the genesis of plasmacytoid dendritic cells

The origins of plasmacytoid dendritic cells have long been controversial. We have identified unique bone marrow progenitors that exclusively give rise to plasmacytoid dendritic cells without differentiating into conventional dendritic cells. This work is generating new insights as to the nature of the myeloid versus lymphoid split during hematopoietic cell development.

Epigenetic alterations in the SIAE pathway and human autoimmunity

A major area of interest for our lab is the genetic basis of human autoimmunity, the role of rare variants in genes that regulate B cell tolerance, and the epigenetic regulation of the SIAE pathway. Alterations of an epigenetic



Promiscuous T-B collaboration and enhanced T cell memory in the absence of SIAE.

nature in this pathway have been observed in over half of all lupus and rheumatoid arthritis subjects, and are currently being investigated.

DNA methylation, B cell self-renewal and chronic lymphocytic leukemia

We have long been interested in cell fate decisions in B cell development and in the development of self-renewing B cell subsets. The roles of DNMT3a in B-1a B cell self-renewal and of specific methylation events in chronic lymphocytic leukemia are being investigated.

Selected Publications:

Mahajan V, Mattoo H, Deshpande V, **Pillai S**, Stone JH. IgG4-related disease. *Annual Review of Pathology.* 9, 315-47, 2014.

Mattoo H, Mahajan VS, Della-Torre E, Sekigami Y, Carruthers M, Wallace ZS, Deshpande V, Stone JH, **Pillai S**, De novo oligoclonal expansions of circulating plasmablasts in active and relapsing IgG4-related disease. *J Allergy Clin Immunol*, 2014.

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Sridhar Ramaswamy, MD

The Ramaswamy laboratory is working to understand how solid tumor metastasis, dormancy, and drug resistance interrelate. Our major goal is to use insight from our studies to devise new strategies for the combination targeting of advanced cancers. Our multidisciplinary approach integrates clinical studies in solid tumor patients with experimental approaches in cancer, computational, & systems biology.

Ramaswamy Laboratory

Cleidson Alves, PhD
Arnaud Amzallag, PhD
Ipsita Dey-Guha, PhD
Sheheryar Kabraji, MD
Robert Morris, PhD
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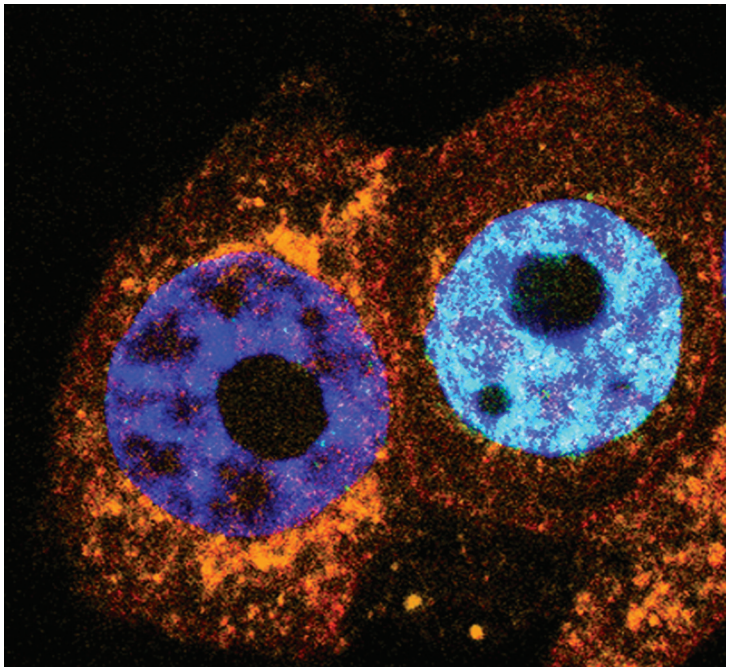
Sridhar Ramaswamy, MD
Laila Ritsma, PhD
Ken Ross, PhD
Salony, PhD
Xavier Sole, PhD
Ben Wittner, PhD

Asymmetric Cancer Cell Division

We have a special interest in the molecular basis of asymmetric cancer cell division. We have found that rapidly proliferating cancer cells occasionally divide asymmetrically to produce slowly proliferating "G0-like" progeny that are highly treatment resistant both in vitro and in cancer patients. We have developed reliable methods for the identification, isolation, tracking and experimental study of these G0-like cells. Our molecular and cellular studies have revealed that partial suppression of the AKT/PKB signaling pathway during mitosis induces a signal transduction and epigenomic network that regulates asymmetric cancer cell division and the production of G0-like cells. Since virtually all tumors depend on AKT signaling for their growth and survival, we believe that understanding the mechanisms underlying this quantitative regulation of AKT signaling and asymmetric cancer cell division in precise detail might enable us to develop entirely new strategies to diagnose and therapeutically target a wide variety of different cancer types where slowly proliferating and dormant cancer cells are difficult to eradicate. Current projects include 1) identifying upstream pathways that asymmetrically suppress AKT signaling in dividing cancer cells; 2) defining the signaling and epigenomic postures of G0-like progeny using next-generation sequencing, proteomic, and metabolomic approaches; 3) dynamically visualizing asymmetrically dividing cancer cells using live-cell imaging approaches in vitro and in vivo; and 4) determining how asymmetric cancer cell division may contribute to human tumor metastasis, dormancy and treatment resistance in vivo.

Cancer Cell Metastasis

We are working to understand how human cancer genomes regulate solid tumor progression. We are particularly interested in defining transcriptional networks that regulate metastasis, dormancy and drug response. Several years ago, we found that multigene transcriptional signatures are expressed by a majority of malignant cells within tumors that are destined to metastasize. These studies spurred the development and deployment of widely used gene-signature-based clinical diagnostics for the diagnosis and risk stratification of cancer patients with different tumor types. We subsequently found that virtually all of these poor prognosis signatures indirectly reflect the activity within tumors of the MYC transcription factor. Moreover, we found that in certain contexts MYC may specifically regulate cancer cell invasion and metastasis apart from its well-studied roles in proliferation and survival. Since MYC is arguably the most commonly altered human oncogene, understanding how quantitative increases in MYC activity contributes to



Asymmetric cancer cell division. (Published in Proc Natl Acad Sci USA. 108:12845-12850, 2011. Reprinted courtesy of Ipsita Dey-Guha, PhD, Massachusetts General Hospital Cancer Center).

metastasis might suggest new strategies for therapeutically targeting advanced cancers. Current projects include 1) DNA-seq, RNA-seq and ChIP-seq profiling to comprehensively define the metastasis-related MYC transcriptional state; and 2) functional studies probing the MYC transcriptional network in vivo.

Center for Computational Discovery

A major challenge in modern cancer research is the generation, storage, analysis and interpretation of complex experimental data. Individual experiments using cutting-edge technologies can generate terabytes of data that must be quantitatively mined to identify important cancer genes, pathways and drug associations to drive the discovery of new biomarkers and drug targets. Scientists in Massachusetts General Hospital's Center for Computational Discovery (CCD) have significant expertise in the analysis of high-throughput biological data from across the current technological spectrum including next-generation sequencing (i.e., DNA, RNA, ChIP-seq), microarrays (e.g., SNP, CHG, Expression, Tiling, ChIP-Chip), proteomics (array-based), genome-scale RNAi and chemical screens, and high-throughput microscopy. CCD scientists are developing new methods for the analysis, display and storage of large data sets generated with these cutting-edge technologies. CCD scientists also work closely with a wide spectrum of investigators throughout the Mass General Cancer Center on a variety of translational and fundamental research projects at any given time, both as collaborators and as consultants. In approaching new projects, we apply established analytic tools and also develop, implement and deploy customized tools depending on specific requirements. Current projects involve 1) cancer mechanisms; 2) stem cell epigenomics and biology; 3) cancer genome discovery in tumors and circulating tumor cells; 4) cancer cell line pharmacogenomics; 5) biomarker discovery and validation using data integration, meta-analysis, and predictive modeling.

Selected Publications:

Dey-Guha I, Alves CP, Salony, Sole X, Yeh AC, Darp RA, **Ramaswamy S**. A mechanism that produces slowly proliferating cancer cells. *Mol Cancer Res*. Under review, 2014.

Polo JM, Anderssen E, Walsh RM, Schwarz B, Borkent M, Apostolou E, Stadtfeld M, Figueroa ME, Robinton D, Natesan S, Melnick A, **Ramaswamy S***, Hochedlinger K*. A molecular roadmap of reprogramming somatic cells into iPS cells. *Cell*. 151:1617-1632, 2012.

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Ramaswamy S*, Ross KN, Lander ES, Golub TR*. A molecular signature of metastasis in primary solid tumors. *Nat Genet*. 33:49-54, 2003.

Ramaswamy S, Tamayo P, Rifkin R, Mukherjee S, Yeang CH, Angelo M, Ladd C, Reich M, Latulippe E, Mesirov JP, Poggio T, Gerald W, Loda M, Lander ES, Golub TR. Multi-class cancer diagnosis using tumor gene expression signatures. *Proc Natl Acad Sci USA*. 98:15149-15154, 2001.

*co-authors

Miguel N. Rivera, MD

Research in **the Rivera laboratory** focuses on the identification and characterization of developmental pathways involved in pediatric tumors and sarcomas. These tumors are strongly associated with developmental processes and, in particular, with abnormalities in the mechanisms that regulate stem cell populations during the formation of specific organs. Our work combines genomic technologies for direct analysis of tumors with functional analysis of new pathways that are common to embryonic development and cancer. Given that these developmental processes are poorly understood at present, we anticipate that our work will point to new approaches for therapeutic intervention.

Rivera Laboratory

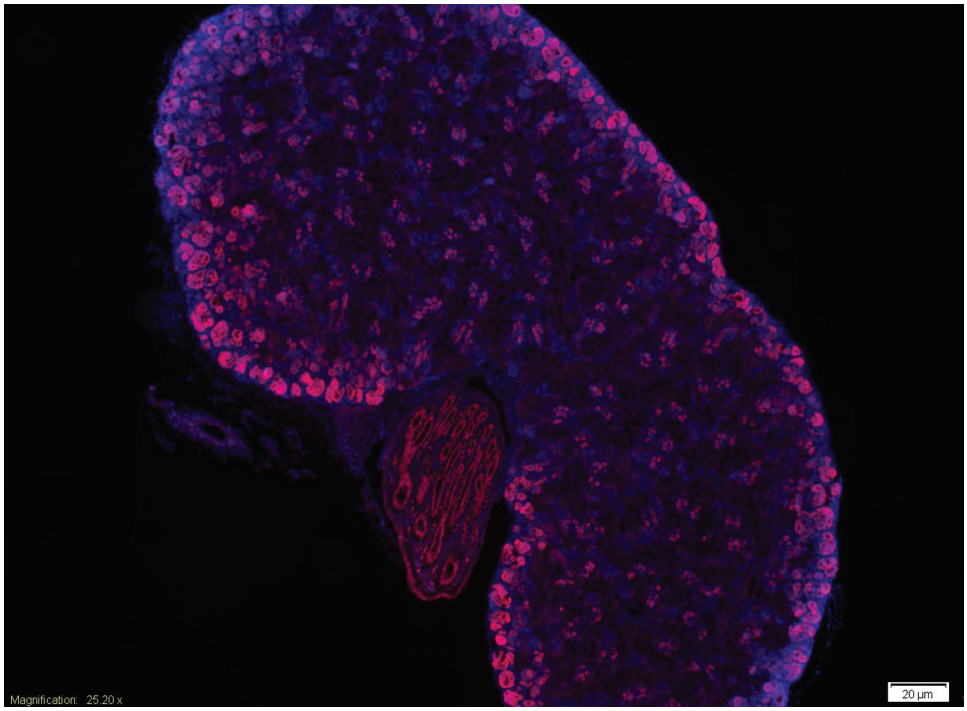
Kshitij Arora, MD
Mary Awad
Gaylor Boulay, PhD
Nicolo Riggi, MD, PhD
Miguel N. Rivera, MD
Nikki Rossetti

Role of the WTX gene family in cancer and development

Wilms tumor, the most common pediatric kidney cancer, is a prime example of the connection between cancer and development because it arises from kidney-specific stem cells and is composed of several cell types that resemble the earliest stages of kidney development. We identified WTX, a new X-linked tumor suppressor gene which is inactivated in up to 30% of cases of Wilms tumor, by comparing the DNA of primary tumor samples with that of normal tissues using array comparative genomic hybridization (CGH). WTX is the founding member of a new protein family (FAM123) and is expressed in the stem cell compartment of the developing kidney as well as in a variety of other tissues during embryogenesis. In collaboration with the Haber and Bardeesy laboratories, we have demonstrated that inactivation of WTX in mice leads to severe alterations in the development of several organs that arise from mesenchymal precursors, including kidneys, bones and fat. We are now studying the function of WTX and related proteins using several in vitro and in vivo model systems.

Epigenomic approaches to the identification of novel pathways in cancer

Genome-wide chromatin profiling, which combines chromatin immunoprecipitation and high-throughput sequencing, is a new technology that has been used to study the epigenetic code of embryonic stem cells. As opposed to expression arrays, chromatin profiling provides a unique view of cellular differentiation programs by allowing the identification of both active and repressed domains in the genome through the analysis of histone modifications. Prominent active marks tend to be associated with transcription factors that play key roles in a given lineage. A mixture of repressive and active signals is often indicative of genes that are poised for transcription but are not yet active due to an incomplete process of differentiation. We have applied this technology to Wilms tumor and have uncovered a new set of genes with potentially critical functions in blocking



Immunofluorescence image of a developing mouse kidney. The transcription factor Pax2 (red) is present in the stem cells that can give rise to Wilms tumor (adjacent to the surface of the organ) and in precursors to collecting ducts.

differentiation programs in tumor cells. Given that the mechanisms that regulate this tumor and its precursor stem cell population are not well defined, epigenomic profiling provides a powerful tool for directly identifying new pathways of potential clinical importance. We are now testing the function of the genes identified in Wilms tumor as well as extending our epigenomic analysis to other tumor types where developmental pathways are expected to play a key role.

Selected Publications:

Kim WJ, **Rivera MN**, Coffman EJ, Haber DA. The WTX tumor suppressor enhances p53 acetylation by CBP/p300. *Mol Cell*. 45(5):587-97, 2012 Mar 9.

Moisan A*, **Rivera MN***, Lotinun S, Akhavanfard S, Coffman EJ, Cook EB, Stoykova S, Mukherjee S, Schoonmaker JA, Burger A, Kim WJ, Kronenberg HM, Baron R, Haber DA, Bardeesy N. The WTX tumor suppressor regulates mesenchymal progenitor cell fate specification. *Dev Cell*. 20(5):583-96, 2011 May 17.

Aiden AP*, **Rivera MN***, Rheinbay E, Ku M, Coffman EJ, Truong TT, Vargas SO, Lander ES, Haber DA, Bernstein BE. Wilms tumor chromatin profiles highlight stem cell properties and a renal developmental network. *Cell Stem Cell*. 6(6):591-602, 2010 Jun 4.

Rothenberg SM*, Mohapatra G*, **Rivera MN***, Winokur D, Greninger P, Nitta M, Sadow PM, Sooriyakumar G, Brannigan BW, Ulman MJ, Perera RM, Wang R, Tam A, Ma XJ, Erlander M, Sgroi DC, Rocco JW, Lingen MW, Cohen EE, Louis DN, Settleman J, Haber DA. A genome-wide screen for microdeletions reveals disruption of polarity complex genes in diverse human cancers. *Cancer Res*. 70(6):2158-64, 2010 Mar 15.

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*co-authors

James William Rocco, MD, PhD

The Rocco laboratory works to improve treatment of head and neck cancers. Tumors of the tonsil and the back of the throat—a region called the oropharynx—are occurring more frequently due to human papillomavirus (HPV), the virus that also causes most cervical cancer. Although oropharyngeal tumors often respond to combined chemotherapy and radiation, about 2,000 people in the United States die of them every year, and those who are cured can suffer severe long-term consequences of treatment. Being able to predict outcome before treatment would help personalize therapy and identify patients who need more aggressive treatment. The focus of our laboratory is to identify characteristics of tumors that are related to patient outcomes and examine the ways that these characteristics might make cancer cells more or less responsive to therapy. We aim to bring our results back to the clinic, both by improving our ability to predict outcomes and by devising therapies that are targeted to the characteristics of an individual patient's tumor.

Rocco Laboratory

Rebecca Hammon, MD
Stephanie Hsiao, PhD
Armida Lefranc-Torres, MD
William Michaud, PhD
Edmund Mroz, PhD
Miguel Pereira, MD
Eric Wu, MA
James Rocco, MD, PhD

Our studies of how cytotoxic therapy affects tumor cells have led to a promising translational application in oropharyngeal squamous cell carcinoma (OPSCC), an increasingly prevalent form of head and neck cancer associated with human papillomavirus (HPV) infection.

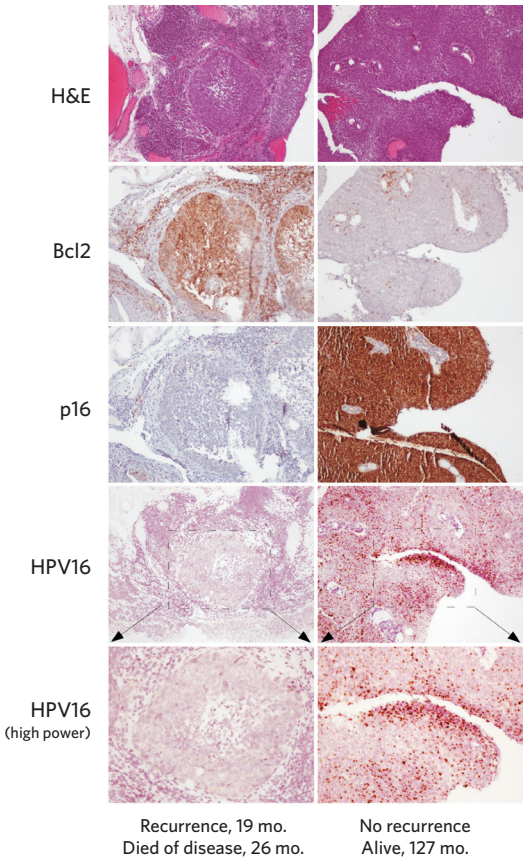
Laboratory studies of therapeutic targets in head and neck cancer

The platinum-based concurrent chemoradiation therapy frequently used to treat head and neck tumors acts in part through the p63/p73 network of proteins. In this network, a form of p63 called deltaNp63 prevents one form of p73 from starting a cellular program that leads to cell death. We found that cisplatin leads to loss of deltaNp63, allowing p73-driven cell death. However, some tumor cells can stay alive after cisplatin treatment if they have high levels of the anti-apoptotic protein Bcl2. We are now studying whether drugs that inhibit Bcl2 function might provide complementary or alternative treatment for tumors whose high Bcl2 levels tend to oppose standard therapy. Our work on Bcl2 and the intrinsic and extrinsic apoptotic pathways has also led us to study the role of caspase 8 in responses of head and neck cancer cells to cisplatin.

Mechanism-based biomarkers in oropharyngeal tumors

The incidence of HPV-associated OPSCC, now over 75% of OPSCC cases and accounting for nearly half of OPSCC deaths, has increased 7.5% per year for the past two decades. The high morbidity and frequent failure of chemoradiation therapy for this increasingly prevalent disease indicate the need for improved methods to predict treatment outcome.

Extending our basic-science results to the clinic, we found that chemoradiation tends to fail in patients whose tumors have high Bcl2 expression. Notably, we found that Bcl2 and HPV status are not related to each other and provide independent estimates of treatment



Bcl2 expression and HPV status are both related to outcome in oropharyngeal cancer. Tumor sections from a patient who was cured by concurrent chemoradiation treatment (right) and from a patient who died of disease despite treatment (left), stained with hematoxylin/eosin (H&E) or for human papilloma virus (HPV), the p16 protein (typically high in HPV-positive cases), or the anti-apoptotic protein Bcl2. Overall, Bcl2-high/HPV-negative tumors have poor outcomes, while >90% of patients with Bcl2-low/HPV-positive tumors are cured. Patients with Bcl2-high/HPV-positive or Bcl2-low/HPV-negative tumors have intermediate risk of recurrence. From Nichols et al, Clinical Cancer Research 16: 2138-46, 2010.

failure. We are currently validating these findings in a large, prospective clinical study, while applying the novel technology of BH3 profiling to examine the functional status of Bcl2 and related proteins that regulate cell death.

Regulation of p16 expression in tumor suppression and senescence

The $p16^{INK4A}$ protein helps prevent division of tumor-prone cells by slowing the cell cycle and inducing cellular senescence. This process costs the organism, however, by reducing the regenerative potential of stem cells required for tissue maintenance and increasing the risk of tissue damage from senescent cells. We discovered that the C-terminal binding protein (CtBP), a target of several stress-signaling pathways, regulates p16 expression through loss of Polycomb-based repression. This epigenetic memory is likely a major tumor-suppressive mechanism that is inherited by a cell's progeny and may explain how gradual minor insults to tissues can lead to eventual growth arrest and senescence. Finding ways to reset this memory could help prevent or reverse some forms of tissue damage and aging.

Intra-tumor genetic heterogeneity and outcome in head and neck cancer

We developed a way to use next-generation DNA sequencing to measure the intra-tumor heterogeneity that arises from multiple subclones of cancer cells within a tumor. We found that the overall survival of head and neck cancer patients whose tumors had high heterogeneity was substantially shorter than the survival of patients with low-heterogeneity tumors. This relation of outcome to tumor heterogeneity was particularly striking for patients who received chemotherapy as part of their treatment. High heterogeneity may limit the effectiveness of therapies that target individual cellular proteins or pathways, as genetically distinct cancer cells within a tumor may not have the same targets.

Selected Publications:

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Dennis Sgroi, MD

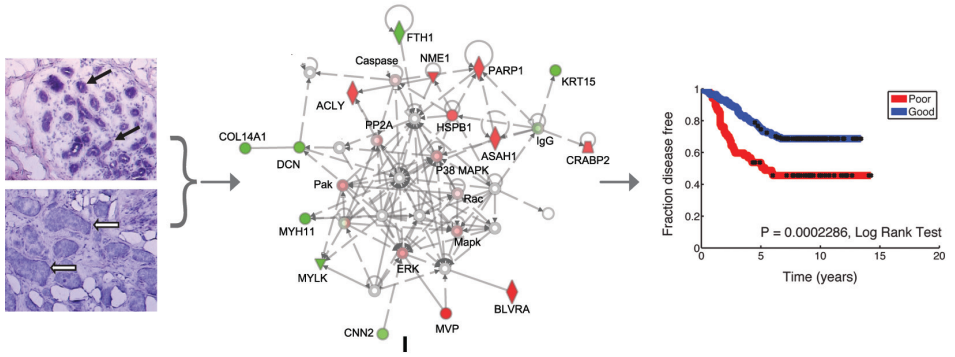
The overarching goals of research in **the Sgroi laboratory** are to develop better ways to identify patients who are at risk for the development of breast cancer and to identify those breast cancer patients who are likely to benefit from targeted drug therapies. We are taking several different approaches to achieving these goals. First, we are deciphering specific molecular events that occur during the earliest stages of tumor development and using this knowledge to develop biomarkers that will predict for increased risk of progression to cancer. Second, using DNA microarray technologies, we are searching for novel breast cancer biomarkers to identify patients with hormone-receptor-positive breast cancer who are most likely to benefit from extended hormonal therapy. Finally, we are taking a combined approach—based on analysis of tissue from breast cancer patients and various laboratory studies—to identifying biomarkers that will predict how individual breast cancer patients will respond to novel targeted therapeutics.

Sgroi Laboratory

Stacy Francis BS
Piiha-Lotta Jerevall-Jannok, PhD
Dennis Sgroi, MD
Angela Volorio, MD

Our research focuses on understanding the molecular genetic events associated with the pathogenesis of human breast cancer. My laboratory has developed technological approaches to study gene expression in the earliest microscopic precursor lesions as well as in the latest stages of human breast cancer. Specifically, we have been successful in combining laser capture microdissection, high-density cDNA array, and real-time quantitative PCR (RTQ-PCR) technologies to identify novel gene expression patterns in human breast cancer. Using this approach, we have demonstrated for the first time that atypical intraductal hyperplasia and ductal carcinoma in situ are direct precursors to invasive ductal carcinoma. More specifically, we have shown that the various pathological stages of breast cancer progression are highly similar at the transcriptional level, and that atypical intraductal hyperplasia—the earliest identifiable stage of breast cancer—is a genetically advanced lesion with an expression profile that resembles that of invasive breast cancer. More recently, we have studied the gene expression changes of the stromal microenvironment during breast cancer progression, and we have demonstrated that the transition from preinvasive to invasive breast cancer is associated with distinct stromal gene expression changes.

In collaboration with Barry Karger, PhD, of the Barnett Institute, we have used advanced tandem mass spectrometry to perform comparative proteomic profiling of normal breast epithelium with neoplastic epithelium of the preinvasive and invasive stages of breast cancer. Through a novel bioinformatic approach, we recently integrated our transcriptomic and proteomic data sets to identify a novel, robust biomarker of clinical outcome in estrogen-receptor-positive breast cancer patients. We are currently applying tandem mass spectometry technologies to standard formalin-fixed, paraffin-embedded breast tumor samples as a means to identify biomarkers of therapeutic response to HER2- and estrogen-receptor pathway-driven tumors.



The comparative analysis of the transcriptome and proteome of normal breast epithelium and malignant breast epithelium (top panel) combined with a proteome network analysis has led to the discovery of a novel robust network-based biomarker (center) with clinical relevance (right).

Presently, my laboratory is focused on applying high-throughput DNA microarray and proteomic technologies as a means to predict the clinical behavior of human breast cancer in the setting of specific hormonal and chemotherapeutic regimens. We have independently developed two complementary biomarkers—the Molecular Grade Index (MGI) and the HOXB13/IL17BR (H/I). MGI is a molecular surrogate for histological grade and a highly precise biomarker for risk of breast cancer recurrence. The HOXB13:IL17BR index, on the other hand, is a biomarker of endocrine responsiveness in ER+ breast cancer, as it has been shown to predict for benefit from adjuvant tamoxifen and extended adjuvant aromatase inhibitor therapy. Most recently, we demonstrated that the combination MGI and H/I, called the Breast Cancer Index (BCI), outperforms the Oncotype Dx Recurrence Score for predicting risk of recurrence. As a result of our collective data, we anticipate assessing BCI in clinical trials of extended adjuvant hormonal therapy. Given that HOXB13 expression in clinical breast cancers is associated with endocrine therapy responsiveness, we are currently investigating the functional activity of HOXB13 and assessing its possible role as a surrogate marker for a nonclassical estrogen receptor signaling pathway.

Lastly, using an artificial zinc-finger transcription factors combinatorial library technology, we developed an in vitro breast cancer model of drug resistance to a clinically important antiendocrine therapeutic agent. Our results demonstrate that this approach can be used successfully to induce stable drug resistance in human cancer cell lines and to identify a gene expression signature that is associated with a clinically relevant drug-resistance phenotype. These experiments provide an important proof of principle for the use of combinatorial zinc-finger transcription factor libraries to induce and to study important cellular phenotypes, including human cancer drug resistance. We are currently using this approach to identify potential biomarkers for HER2-directed and PARP1-directed therapies.

Selected Publications:

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Lee J, Hirsh AS, Wittner BS, Maeder ML, Singavarapu R, Lang M, Janarthanan S, McDermott U, Yajnik V, Ramaswamy S, Joung JK, **Sgroi DC**. Induction of stable drug resistance in human breast cancer cells using a combinatorial zinc finger transcription factor library. *PLoS One*. 6:e21112, 2011.

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Toshihiro Shioda, MD, PhD

The Shioda laboratory is interested in how exposure of pregnant women to environmental pollutants affects health of their children throughout their lifespan. *The Developmental Origins of Health and Diseases* (DOHaD) hypothesis claims that exposure of fetuses in the uterus to pollutants may permanently damage the epigenetic mechanisms regulating gene expression in various types of stem cells, causing adult-onset diseases including obesity and cancer. If epigenetic damages are introduced into the genome of germ cells, transgenerationally transmittable disorders may emerge without involving mutations in the nucleotide base sequences of the genomic DNA. One of the major hurdles in this field is the lack of convenient models for mechanistic studies of the DOHaD phenomena. Taking advantage of the latest progress in reproductive and stem cell biology and the cutting-edge deep sequencing technology, we are presently focusing on generation and characterization of cell culture models of germline stem cells such as primordial germ cells derived from human and mouse iPS cells.

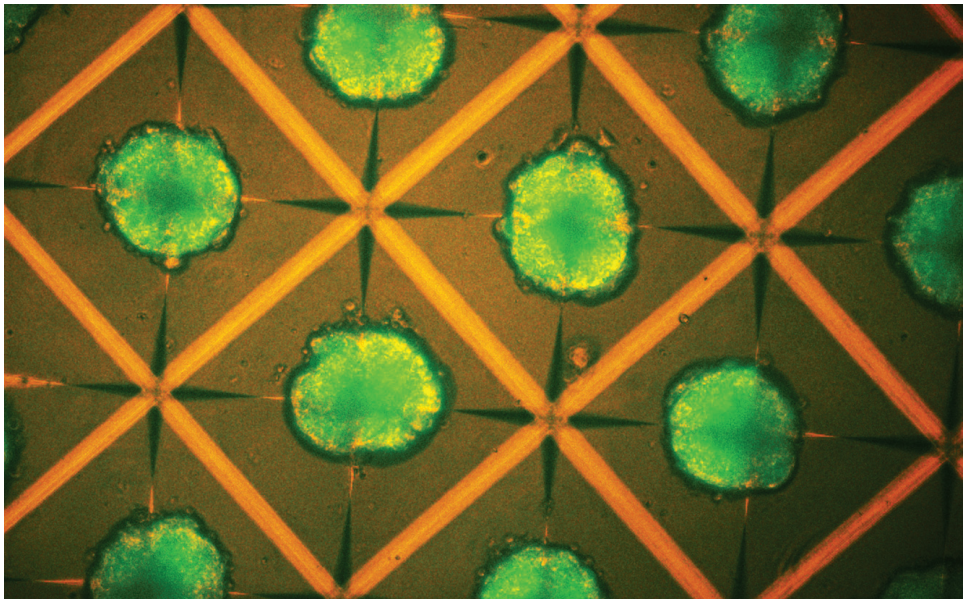
Shioda Laboratory

Molecular Profiling Laboratory

Mary Biotidara
Mayinuer Maitituoheti, MD, PhD
Yuko Miki
Na Qu, PhD
Keiko Shioda
Toshihiro Shioda, MD, PhD
Shiomi Yawata

In vitro production of primordial germ cells from human and mouse pluripotent stem cells

Experimental investigations of the exposure of pregnant women and their fetuses to potentially disease-causing conditions is impossible. In an effort to establish credible and convenient surrogate models, we have been trying to generate human PGCs in cell culture from the pluripotent iPS cells. To validate the human PGC-like cells (PGC-LCs), we are also generating mouse PGC-LC models. Genome-wide deep sequencing analyses of mouse PGC-LCs and natural mouse PGCs for mRNA expression, DNA methylation, DNA hydroxymethylation, and histone modifications have established significant similarities between PGC-LCs and PGCs, demonstrating the PGC-hallmark global DNA demethylation in PGC-LCs. By genome-wide comparison of these (epi)genomic marks between human and mouse PGC-LCs and mouse natural PGCs, we attempt to determine the advantages and limitation of the use of the human PGC-LC models for mechanistic toxicogenomics research in the absence of access to human natural PGCs. The differentiation status of the presently available human PGC-LCs still remains incomplete compared to the mouse PGC-LCs. Because the standard human iPS and embryonic stem (ES) cells seem to be already differentiated into the epiblast-like status (primed pluripotency) whereas mouse iPS and ES cells are completely pluripotent (ground-status pluripotency), we hypothesized that the primed status is a significant barrier to differentiation into PGC-LCs. Mouse PGC precursors emerge from the proximal epiblast at E6.25 as a small number of cells expressing Blimp1/Prdm1, and this occurs only once and within a very narrow time of window. Probably reflecting this restriction, mouse epiblast-like cells differentiated from iPS cells are capable of



Fluorescence image of mouse embryoid bodies developed from iPS cells in microwells. The red squares are boundaries of each microwell, and the embryoid bodies are GFP-positive reflecting activation of the Oct-4 promoter. Our lab is generating in vitro models of germline cells from mouse embryoid bodies and analyzing epigenomic events associating the genomic reprogramming events that occur during the germline differentiation from somatic precursor cells.

generating PGC-LCs only within 48~72 hours after initiation of the iPS cell conversion into epiblast-like cells. To address this hypothesis, we have generated the ground-status human iPS cells, and attempts are presently ongoing in my laboratory to generate PGC-LCs from the ground-status and primed human iPS cells.

Environmental epigenomics

Exposure of pregnant mice to the environmental endocrine disruptors such as Bisphenol A or tributyltin causes transgenerationally transmittable disorders such as mammary gland hyperplasia or obesity. Collaborating with multiple extramural laboratories that perform animal exposure studies, our laboratory has been searching for possible epigenetic changes in germline cells as well as tissues showing the adult-onset morphological phenotypes, such as mammary glands or adipocyte-producing mesenchymal stem cells. The goal of these projects is to identify the chemical-specific "epigenetic lesions" that are responsible for the late-onset and/or transgenerational disease phenotypes in the genomes of the exposed fetuses and their progenies. For example, exposure of pregnant mice to tributyltin, a commonly used anti-fouling agent, causes transgenerationally transmittable obesity of the offspring. Tributyltin is a strong PPAR-γ agonist, and the transgenerational obesity is also caused by exposure of pregnant mice to rosiglitazone, a clinically used anti-diabetic drug and a PPAR agonist. The mesenchymal stem cells isolated from these obese animals tend to differentiate into the adipocytic lineage at the expense of osteogenic lineage. Our preliminary studies which we are conducting in collaboration with Dr. Bruce Blumberg, who discovered these phenomena, have identified possible epigenetic aberrations caused by the in utero exposure to the PPAR agonists.

Selected Publications:

Yu M, Bardia A, Aceto N, Bersani F, Madden MW, Donaldson MC, Desai R, Zhu H, Comaills V, Zheng Z, Wittner BS, Stojanov P, Brachtel E, Sgroi D, Kapur R, **Shioda T**, Ting DT, Ramaswamy S, Getz G, Iafrate AJ, Benes C, Toner M, Maheswaran S, and Haber DA. Ex vivo culture of circulating breast tumor cells for individualized testing of drug susceptibility. *Science* 345:216-220 (2014).

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Shannon Stott, PhD

The Stott laboratory is comprised of bioengineers and chemists focused on translating technological advances to relevant applications in clinical medicine. Specifically, we are interested in using microfluidics and imaging technologies to create tools that increase understanding of cancer biology and of the metastatic process. In collaboration with the Toner, Haber and Maheswaran laboratories, we have developed a microfluidic device that can isolate extraordinarily rare circulating tumor cells (CTCs) from the blood of cancer patients. We are striving to employ new imaging modalities to extract as much information as possible from these rare cells while pushing the technology further for early cancer detection. Ultimately, we hope that by working in close partnership with the molecular and cell biologists at the Mass General Cancer Center, we can create new tools that directly impact patient care.

Stott Laboratory*

Sam Au, PhD
Thomas Carey
Fernanda Carvalho, MS
Anh Hoang, PhD
Xiaocheng Jiang, PhD
Aimal Khankhel
Wooseok Kim
Kathleen Miller
Eduardo Reátegui, PhD
Rebecca Sandlin, PhD
Aaron Shank
Shannon Stott, PhD
Keith Wong, PhD
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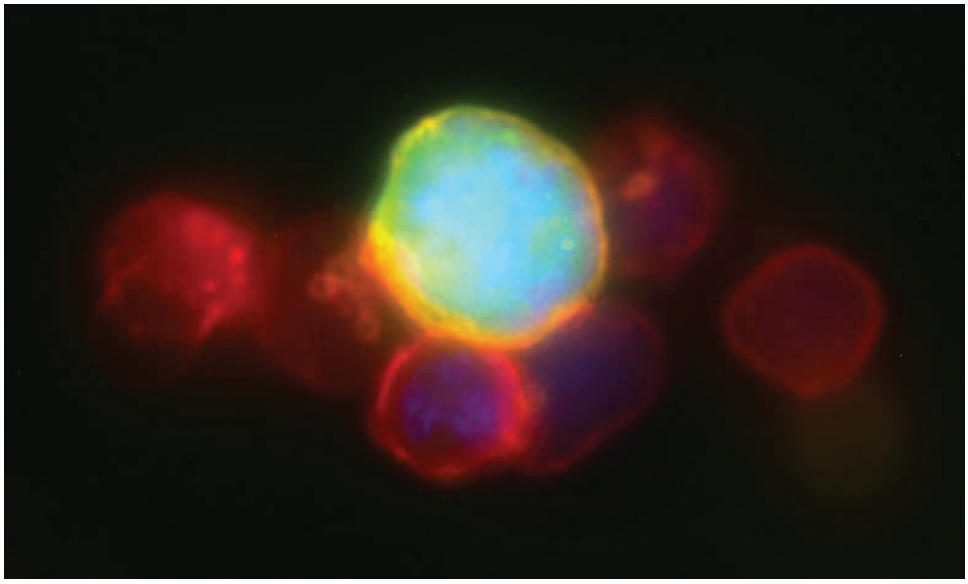
Rapid technological advances in microfluidics, imaging and digital gene-expression profiling are converging to present new capabilities for blood, tissue and single-cell analysis. Our laboratory is interested in taking these advances and creating new technologies to help build understanding of the metastatic process. Our research focus is on 1) the development and application of microfluidic devices for the isolation and characterization of CTCs, 2) novel imaging strategies to characterize cancer cells and the dynamics of metastasis, and 3) the enrichment and analysis of exosomes and microvesicles using microfluidics.

Microfluidics for Circulating Tumor Cell Analysis

One of the proposed mechanisms of cancer metastasis is the dissemination of tumor cells from the primary organ into the blood stream. A cellular link between the primary malignant tumor and the peripheral metastases has been established in the form of CTCs in peripheral blood. While extremely rare (1 in 10 billion cells), these cells provide a potentially accessible source for early detection, characterization and monitoring of cancers that would otherwise require invasive serial biopsies. The emerging fields of medical technology and microfluidics offer a radically different approach to rare cell detection, which is particularly relevant to the isolation of CTCs. Working in collaboration with Drs. Mehmet Toner, Shyamala Maheswaran and Daniel Haber, we have designed a high throughput microfluidic device, the CTC-Chip, that allows the isolation and characterization of CTCs from the peripheral blood of cancer patients. Using blood from patients with metastatic and localized cancer, we have demonstrated the ability to isolate, enumerate and molecularly characterize putative CTCs with high sensitivity and specificity. Additionally, microclusters of CTCs have been captured in a rare number of patient samples. These clusters of CTCs present an intriguing phenomenon; however their significance has yet to be determined. Ongoing projects include translating the technology for early cancer detection, exploring these clusters of CTCs, increasing capture sensitivity through targeting platelets that can mask the surface



Micrograph of a circulating tumor cell (CTC, green, center) surrounded by white blood cells (red). The CTC is staining positive for platelet markers (orange), suggesting that the cancer cell surface is cloaked by platelets. Image courtesy of Xiaocheng Jiang.

antigens of cancer cells, and the design of biomaterials for the release of the rare cells from the device surface.

High-content and high-throughput imaging of cancer cells

Cancer cells can be highly heterogeneous, with rare metastasis precursors capable of giving rise to a metastatic lesion mixed in with other tumor cells undergoing apoptosis. Thus, due to this heterogeneity, quantitative, robust analysis for individual cells may be critical for determining a particular cancer cells' their clinical relevance in different disease contexts. Due to limitations in the number of distinct spectra that can be used in wide-field fluorescence imaging, high throughput characterization of cells and tissue is traditionally done with three to four colors. Our lab is exploring alternative imaging modalities, such as multi-spectral imaging (MSI), to enable quantitative analysis of multiple (8+) markers on a single cell. Our interest in MSI is driven by the technology's capability to image as many colors as distinct antibodies available and by dramatic reductions in sample autofluorescence.

We are interested in using this technology to interrogate signaling activity in CTCs isolated from the blood of cancer patients. These data will be used to gain an increased understanding in the relationship between pharmacologic measurements and clinical outcomes, ultimately leading to the optimization of patient therapy.

Microvesicle and Exosome Characterization

Microvesicles have been implicated in promoting tumor progression by manipulating the surrounding microenvironment. Researchers have hypothesized that microvesicles shed from the membranes of tumors transport RNA and proteins that promote tumor growth, and studies have shown that microvesicles are present in the serum of patients with glioblastoma. Ongoing work in my lab, performed in collaboration with Dr. Xandra Breakefield of the Mass General Neurogenetics Unit, is exploring the use of microfluidics to purify microvesicles from serum and use of their RNA content as a biomarker, specifically the expression of epithelial growth factor receptor (EGFRvIII).

Selected Publications:

Yu M*, Bardia A*,Wittner BS, **Stott SL**, Smas ME, Ting DT, Isakoff SJ, Ciciliano JC, Wells MN, Shah AM, Concannon KF, Donaldson MC, Sequist LV, Brachtel E, Sgroi D, Baselga J, Ramaswamy S, Toner M, Haber DAˆ, Maheswaran S.ˆ "Circulating Breast Tumor Cells in Humans Exhibit Dynamic Changes in Epithelial and Mesenchymal Cell Composition" *Science*, 339 (6119), 2013.

Miyamoto DT*, Lee RJ*, **Stott SL***, Ting DT, Wittner BS, Ulman M, Smas ME, Lord JB, Brannigan BW, Tratuwein J, Bander NH, Wu CL, Sequist LV, Smite MR, Ramaswamy S, Toner M, Maheswaran S, Haber DA, "Androgen receptor signaling in circulating tumor cells as a marker of hormonally responsive prostate cancer" *Cancer Discovery*, Oct23 epub, 2012.

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*co-authors, ˆJoint corresponding

Mario Luca Suvà, MD, PhD

The Suvà laboratory is focused on the biology of brain tumors, in particular glioblastoma and oligodendroglioma. We dissect how cellular heterogeneity and plasticity contribute to tumor cells properties. We study primary human samples up to the single-cell level and establish genetically and epigenetically relevant cellular models from patient tumors. We model how brain cancer cells exploit their plasticity to establish phenotypically distinct populations of cells, with a focus on programs governing glioma stem cells. Additionally, the laboratory investigates how mutations affecting genes involved in chromatin regulation contribute to cellular transformation. Given the tremendous heterogeneity of genetic aberrations in brain tumors, we seek to identify common programs integrated at the chromatin level that would offer novel therapeutic options in these dismal diseases.

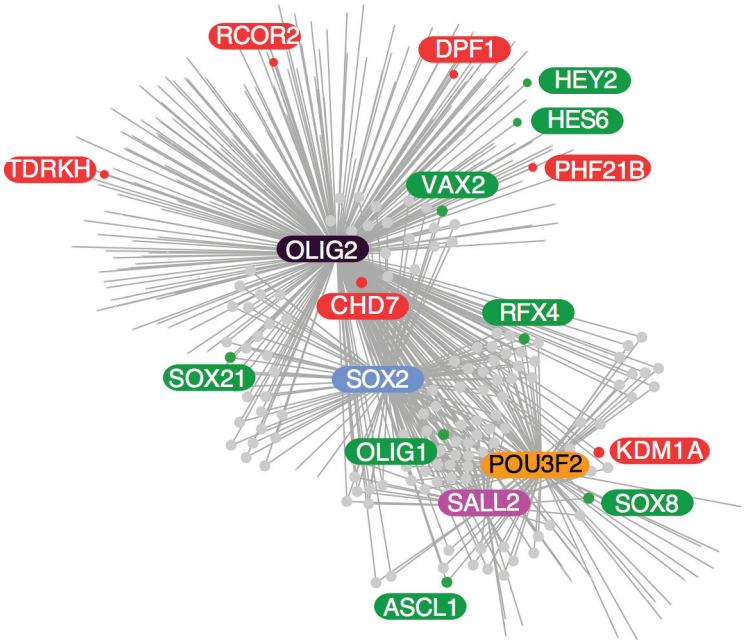
Suvà Laboratory

Leah Escalante, BS
Mariella Filbin, MD, PhD
Christine Hebert, BS
Shawn Gillespie, BS
Mario Luca Suvà, MD, PhD
Andrew Venteicher, MD, PhD

Large scale sequencing studies have implicated increasing numbers of transcription factors (TFs), chromatin regulators (CRs) and histones as direct targets of mutations and rearrangements in cancer. These genetic alterations are now recognized to play critical roles in cellular transformation by altering key transcriptional programs involved in cellular differentiation, plasticity and proliferation. Aberrant epigenetic programs and hierarchies of cellular differentiation are concepts particularly relevant to the biology of gliomas, complex infiltrating brain tumor affecting both adults and children that remain incurable. Glioma cells display unique dependencies on programs of neural development, maintaining distinctive transcriptional circuits that reflect their differentiation status. There are strong evidences that these epigenetic programs have a major influence on glioma cell properties, with stem-like cells driving tumor-propagation and recurrence, while more differentiated cells lack these capabilities. These programs are dictated and sustained by master TFs, CRs and associated cellular networks that direct activation or repression of cis-regulatory elements. Our laboratory establishes genetically and epigenetically relevant cellular models from patient tumors, and utilizes epigenomic profiling, genome-editing technologies, cellular reprogramming and single-cell RNA sequencing to reconstruct cellular circuits and uncover novel dependencies in gliomas.

Targeting neurodevelopmental programs in primary human glioblastoma stem cells.

We have integrated large scale epigenomics with functional experiments and cellular reprogramming in primary glioblastoma, the most common genetic variant of the disease. With this approach, we have demonstrated that a core set of four neurodevelopmental TFs (SOX2, POU3F2, SALL2 and OLIG2) code the unique properties of glioblastoma stem-like cells, including their *in vivo* tumor-propagating potential. We have shown that this core combination of TFs is expressed by subsets of stem cells in patient tumors and have begun to dissect their transcriptional program. We suggest that these programs are either pre-existing epigenetic states hijacked by genetic mutations or aberrant states



Regulatory network of glioblastoma stem cells. Chromatin regulators (red), transcription factors (green) and candidate master regulators (SOX2, POU3F2, SALL2, OLIG2) are highlighted.

generated during cellular transformation. Our working hypothesis is that aberrant neurodevelopmental programs could represent key targets that can be therapeutically exploited not only to eliminate existing stem-like populations, but potentially to prevent their generation through bi-directional plasticity. Our lab is currently utilizing novel genome-editing technologies to generate functional knock-out of critical nodes in the network to identify novel dependencies in glioblastoma and assess novel therapeutic options.

Annotation of functional genomic elements in secondary glioblastoma, pediatric glioblastoma and oligodendroglioma.

At least two additional genetic routes lead to glioblastoma development, namely secondary glioblastoma bearing signature *IDH* mutations and pediatric glioblastoma with *H3F3A* mutations. These mutually exclusive mutations are of particular interest, as they are both thought to impact on the epigenome of cells, possibly through shared mechanisms. In collaboration with groups in the MGH Brain Tumor Center, we are applying deep chromatin profiling to genetically defined cultures of secondary glioblastoma and *H3F3A* mutant pediatric glioblastoma. As additional model, our group is mapping the epigenome of oligodendrogliomas, another type of glioma. Our goal is to identify the regulatory elements and their associated networks that control cellular state across the spectrum of human gliomas.

Gliomas heterogeneity assessed at single-cell level.

Tumor heterogeneity poses a major challenge to cancer diagnosis and treatment. It can manifest as variability between tumors, or within cells from the same tumor, that may harbor different mutations or exhibit distinct phenotypic or epigenetic states. Such intratumoral heterogeneity is increasingly appreciated as a determinant of treatment failure and disease recurrence. In a collaboration between the neurosurgery department at MGH, the Bernstein Lab at MGH and the Regev Lab at the Broad Institute, we are pursuing our previous efforts in single-cell transcriptional profiling. We are currently investigating single cells from many different types of gliomas to assess tumor heterogeneity at an unprecedented depth.

Selected Publications:

Patel AP, Tirosh I, Trombetta JJ, Shalek AK, Gillespie SM, Wakimoto H, Cahill DP, Nahed BV, Curry WT, Martuza RL, Louis DN, Rozenblatt-Rosen O, **Suvà ML***, Regev A*, Bernstein BE*. Single-cell RNA-seq highlights intra-tumoral heterogeneity in primary glioblastoma. *Science* 2014 Jun 20;344(6190):1396-401.

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Rheinbay E†, **Suvà ML**†, Gillespie SM, Wakimoto H, Patel AP, Shahid M, Oksuz O, Rabkin SD, Martuza RL, Rivera MN, Louis DN, Kasif S, Chi AS, Bernstein BE. Chromatin profiles reveal an aberrant transcription factor network connected to Wnt signaling and essential for glioblastoma stem cell maintenance. *Cell Reports*. 2013 May 30;3(5):1567-79.

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Janiszewska M†, **Suvà ML**†, Riggi N, Houtkooper RH, Auwerx J, Clément-Schatlo V, Radovanovic I, Rheinbay E, Provero P, Stamenkovic I. Imp2 controls oxidative phosphorylation and is crucial for preserving glioblastoma cancer stem cells. *Genes & Development*. 2012 Sep 1;26(17):1926-44.

*co-senior authorship
†co-first authorship

David A. Sweetser, MD, PhD

The Sweetser laboratory investigates how leukemia and other cancers develop with the goal of developing novel, more effective therapies with fewer side effects. We are investigating how the Groucho/TLE family of co-repressors function as potent tumor suppressors of acute myeloid leukemia and their roles in normal development and cell function. Knock-out mice for Tle1 and Tle4 have identified critical roles for these proteins in hematopoiesis, bone, lung, and brain development, as well as a critical role in limiting inflammation. In collaboration with Jing-Ruey Yeh, PhD, and David Langenau, PhD, at Massachusetts General Hospital we are using the zebrafish as a model for cancer development and drug discovery. The laboratory is also using whole exome sequencing to characterize underlying cancer predisposition genes in patients with a variety of pediatric malignancies. As the MGH site director for the newly established HMS Undiagnosed Diseases Center, Dr. Sweetser is also leading a group of clinicians and researchers actively engaged in elucidating the underlying basis of a wide variety of human diseases.

Sweetser Laboratory

Selvi Ramasamy, PhD
Thomas Shin
David A. Sweetser, MD, PhD
Rae'e Yamin

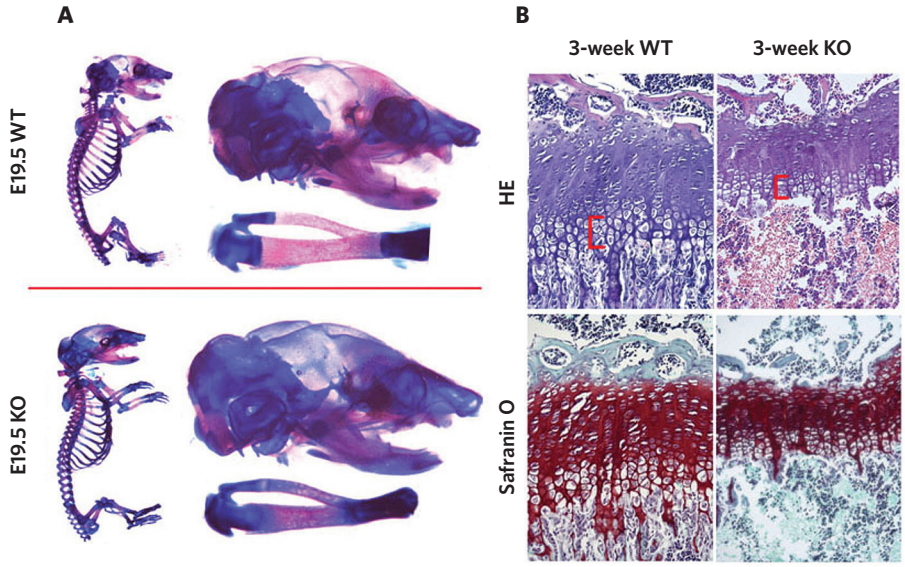
Genetics of Acute Myeloid Leukemia

Our laboratory is working to elucidate cooperating networks underlying leukemogenesis and to help develop novel targeted therapies for cancer. Current projects are detailed below.

Evaluation of the role of the Groucho/TLE family of corepressors in development and leukemogenesis.

Our laboratory has defined TLE1 and TLE4 as members of a novel family of tumor suppressor genes, the TLE/Groucho proteins, the inactivation of which appears to be a key cooperating event with other oncogenes in the development of a subset of acute myeloid leukemias.

The Groucho/TLE family of corepressor proteins is known to modulate many of the major pathways involved in development and oncogenesis, including Wnt/β-catenin, Notch, Myc, NFκB, and TGFβ. However, researchers are only beginning to understand their potential role in oncogenesis. These genes appear to behave as tumor suppressor genes in the pathogenesis of other myeloid malignancies and lymphomas. However, the role of this gene family in malignancies is complex, as in synovial cell sarcoma where TLE1 is overexpressed and behaves as an oncogene by pairing with the SS18-SSX fusion oncogene and ATF2 to silence other tumor suppressor genes. Current work in the lab seeks to clarify the role these proteins play in malignancy as well as in normal development. We have shown the ability of these proteins to potently regulate Myc leukemogenesis can be demonstrated in both murine and zebrafish models of leukemia. In addition, TLE1 and TLE4 are potent inhibitors of the AML1-ETO oncogene in the most common subtype of AML. We are currently elucidating the mechanisms of this inhibition that appears to



Tle4 is not only a tumor suppressor gene in AML, but is critical for normal bone mineralization and bone marrow support.

involve both regulation of gene transcription and chromatin structure.

Our laboratory is also working to understand the role these proteins play in normal development. To assist in this evaluation, we have generated conditional Tle1 and Tle4 knockout mice and are currently characterizing role these proteins play in the development of a variety of tissues. Our studies to date indicate TLE1 is a potent repressor of inflammation via its ability to repress NFKB, while TLE4 is a critical modulator of neuronal and B-cell differentiation and is required for hematopoietic stem cell maintenance, as well as bone development.

Identification of novel inhibitors of AML1-ETO

We have collaborated with the Yeh laboratory to identify several novel small molecule inhibitors of AML1-ETO using a zebrafish high-throughput biological screen. Our results, published in early 2012, identified several classes of agents capable of inhibiting AML1-ETO, and we have demonstrated the efficacy of these agents in treating mouse models of leukemia.

Identifying Genetic Predispositions to Cancer

It is being increasingly recognized that genetic predispositions play a role in the development of many cancers, especially those in children. We are using whole exome sequencing of several cancer types in children to help identify germline mutations that can influence cancer development. Individuals with these mutations may be at higher risk for relapse or the development of additional cancers, and warrant more intensive and extensive surveillance.

The Undiagnosed Diseases Network

The Harvard Medical School hospital consortium of MGH, Brigham and Women's Hospital and Children's Hospital has been recently selected as one of six new sites comprising a nationwide Undiagnosed Diseases Network. As Chief of Medical Genetics at MGH, and the MGH site director for the UDN, Dr. Sweetser is coordinating a team of expert clinicians and researchers, and is using whole exome and whole genome sequencing, and a variety of investigative resources to identify the underlying basis of a variety of challenging human diseases.

Selected Publications:

Giampietro, PF, Armstrong L, Stoddard A, Blank RD, Livingston J, Raggio CL, Rasmussen K, Pickart M, Lorier R, Turner A, Sund S, Sobrera N, Neptune E, **Sweetser DA**, Santiago-Cornier A, Broeckel, U. Whole Exome Sequencing Identifies a POLRID Mutation Segregating in a Father and Two Daughters with Findings of Klippel-Feil and Treacher Collins Syndromes, *Am J Med Genet*, 2014, in press.

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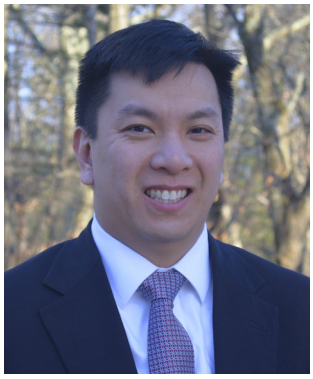
Sweetser DA, Peniket AJ, Haaland C, Blomberg AA, Zhang Y, Zaidi ST, Dayyani F, Zhao Z, Heerema NA, Boultwood J, Dewald GW, Paietta E, Slovak ML, Willman CL, Wainscoat JS, Bernstein ID, and Daly SB. Delineation of the minimal commonly deleted segment and identification of candidate tumor suppressor genes in del(9q) acute myeloid leukemia. *Genes, Chromosomes and Cancer*. 44:279-91, 2005.



David Ting, MD

Pancreatic cancer remains one of the most deadly cancers where the vast majority of patients are diagnosed too late and conventional therapies have largely been ineffective, making early detection and novel drug targets greatly needed. RNA sequencing technologies have recently provided unprecedented resolution of how cancer cells behave. Recent analysis of pancreatic tumors has found a significant amount of "non-coding" RNAs being produced in cancer cells, but not in normal tissues that have provided new insight into this disease and has implications as novel early detection biomarkers. In addition, **the Ting Laboratory** has been utilizing innovative microfluidic chip technologies to capture circulating tumor cells (CTCs) in the blood of pancreatic cancer patients as a means to understand why pancreatic cancers spread so quickly and as a potential non-invasive tool to diagnose our patients earlier.

Ting Laboratory

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Pancreatic ductal adenocarcinoma (PDAC) is an aggressive cancer with a 5% overall survival at 5 years, and therefore, new strategies for early detection and therapeutics are greatly needed. The Ting Laboratory has utilized RNA-sequencing technology to understand the complex transcriptional landscape of PDAC. We have used this technology to identify non-coding sequences (ncRNA) that are differentially expressed in cancer versus normal tissues. This has provided novel insight into the pathogenesis of PDAC and offers a method to identify novel biomarkers and therapeutic targets. In addition, we have been able to capture pancreatic circulating tumor cells (CTCs) with an innovative microfluidic chip technology and successfully applied RNA-sequencing to these cells to understand their role in the metastatic cascade.

Satellite Non-coding RNAs

RNA sequencing of primary PDAC tumors and a variety of normal tissues demonstrated that approximately half of all PDAC transcripts sequenced were unannotated, while nearly all reads in normal pancreas could be aligned, offering a unique opportunity for novel biomarker discovery in PDAC. Initial analysis of this data identified significant transcription emanating from pericentromeric heterochromatic regions of the genome previously thought to be inactive due to heavy epigenetic silencing. Pericentromeric heterochromatin is comprised of large tandem arrays of repetitive elements called satellites and these regions are known to be differentially methylated in a variety of malignancies. Cell line models have demonstrated that the accumulation of satellite transcripts can be induced by DNA demethylation, heat shock, or the induction of apoptosis, and their overexpression disrupts kinetichore formation causing genomic instability. Analysis of all human satellites identified the HSATII satellite as being exquisitely specific for pancreatic cancer compared to normal pancreatic tissue. HSATII expression was confirmed by RNA in *situ* hybridization (RNA-ISH) and was present in preneoplastic pancreatic intraepithelial

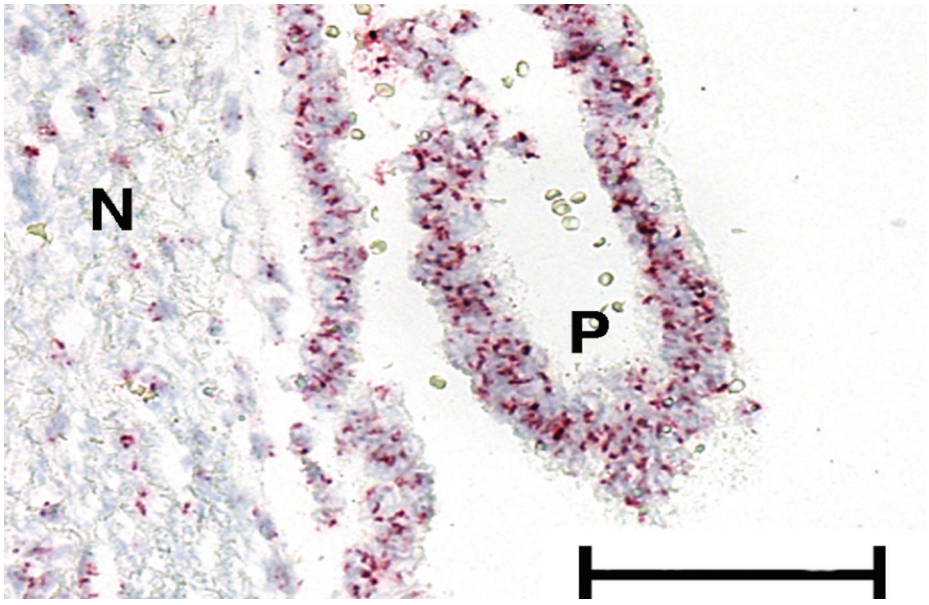

Image of a preneoplastic pancreatic intraepithelial neoplasm (P) positive for the HSATII ncRNA (Red dots).Normal adjacent reactive stroma (N) with minimal expression. Counterstain hematoxylin (blue). Scale bar = 100 μm.

neoplasia (PanIN) suggesting satellite expression occurs early in tumorigenesis, which provides for a potential biomarker for early detection. Furthermore, satellite expression correlated with the expression of a set of genes enriched in stem cells suggests a link between satellites and altered cancer cell fate. We are now trying to understand the biological role of satellites in pancreatic cancer as well as develop RNA-sequencing pipelines to discover other novel ncRNAs.

Pancreatic Circulating Tumor Cells

CTCs are cells that have entered the vasculature and are thought to harbor the precursors of metastasis. Using a novel microfluidic device developed by Daniel Haber's and Mehmet Toner's group at MGH, we have been able to isolate pancreatic CTCs and perform RNA sequencing on these rare cells. RNA-sequencing of these pancreatic CTCs has identified aberrant WNT signaling as an important pathway in the metastatic process. In particular, the TAK1 kinase was found to be a key part of WNT signaling in CTCs and confers the ability to resist apoptosis in the setting of non-adherent conditions (i.e. anoikis resistance).

The temporal development of CTCs in tumorigenesis is not well understood, but evidence for CTC shedding in early localized cancers suggests that these cells are heterogeneous and that only a small subset of CTCs have the biological potential to metastasize. To better understand CTC heterogeneity we have now developed methods for RNA-sequencing at single cell resolution. This has provided a complete transcriptome evaluation of these rare cells and we are developing tools to best characterize single cell RNA-sequencing data. The early emergence of CTCs and the opportunity to understand the biology of metastasis in transit offers the potential for developing non-invasive, early detection tools and new strategies to target metastasis.

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Yu M, Bardia A, Aceto N, Bersani F, Madden MW, Donaldson MC, Desai R, Zhu H, Comaills V, Zheng Z, Wittner BS, Stojanov P, Brachtel E, Sgroi D, Kapur R, Shioda T, **Ting DT**, Ramaswamy S, Getz G, Iafrate AJ, Benes C, Toner M, Maheswaran S, and Haber DA, Cancer therapy. Ex vivo culture of circulating breast tumor cells for individualized testing of drug susceptibility. *Science*, (2014); 345(6193): 216-20.

Javaid S, Zhang J, Anderssen E, Black JC, Wittner BS, Tajima K, **Ting DT**, Smolen GA, Zubrowski M, Desai R, Maheswaran S, Ramaswamy S, Whetstine JR, and Haber DA, Dynamic chromatin modification sustains epithelial-mesenchymal transition following inducible expression of Snail-1. *Cell Rep*, (2013); 5(6): 1679-89.

Yu M, Bardia A, Wittner BS, Stott SL, Smas ME, **Ting DT**, Isakoff SJ, Ciciliano JC, Wells MN, Shah AM, Concannon KF, Donaldson MC, Sequist LV, Brachtel E, Sgroi D, Baselga J, Ramaswamy S, Toner M, Haber DA, Maheswaran S. Circulating breast tumor cells exhibit dynamic changes in epithelial and mesenchymal composition. *Science* (2013); 339(6119): 580-4.

Yu M*, **Ting DT***, Stott SL, Wittner BS, Ozsolak F, Paul S, Ciciliano JC, Smas ME, Winokur D, Gilman AJ, Ulman MJ, Xega K, Contino G, Alagesan B, Brannigan BW, Milos PM, Ryan DP, Sequist LV, Bardeesy N, Ramaswamy S, Toner M, Maheswaran S, Haber DA. RNA sequencing of pancreatic circulating tumour cells implicates WNT signalling in metastasis. *Nature* (2012); 487(7408): 510-3.

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*Equal contribution

Shobha Vasudevan, PhD

The Vasudevan laboratory focuses on the role of noncoding RNAs in cancer cells. Tumors demonstrate heterogeneity, harboring a small proportion of assorted quiescent cells that switch from rapid proliferation—characteristic of other cancer cells—to a specialized, reversibly arrested state that decreases their susceptibility to chemotherapy. Quiescent cancer cells can resist conventional therapeutics and contribute to cancer recurrence, resuming proliferation and cancerous growth upon chemotherapy removal. Previous data revealed that microRNAs, noncoding RNAs that control vital genes in cancer and growth, are important for the persistence of quiescent cancer cells. The primary goal of our research program is to characterize the expression and roles of regulatory noncoding RNAs and AU-rich elements (AREs) in quiescence and tumor progression. A complementary focus is to investigate the regulation of noncoding RNAs and AREs in response to quiescent conditions in tumors, stem cells and germ cells. Our goal is to develop a greater understanding of the versatile roles of regulatory RNAs in cancer as a basis for designing new drug therapies.

Vasudevan Laboratory

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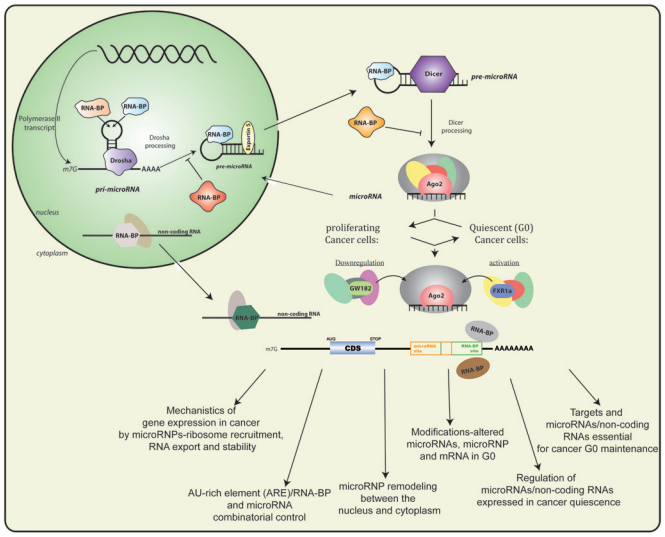
Samuel Truesdell, M.S.
Shobha Vasudevan, PhD

AU-rich elements (AREs) are highly conserved mRNA 3′-untranslated region (UTR) regulatory elements while microRNAs are small noncoding RNAs that target distinct 3′UTR sites and control post-transcriptional gene expression of clinically relevant messages, including those of cytokines and potent growth factors. MicroRNAs, like AREs, are known post-transcriptional regulators in cell proliferation, development and death. Their deregulation leads to rapid and dramatic changes in expression levels promoting a broad range of critical effects, including tumor growth, chemoresistance, metastasis, recurrences, leukemias, lymphomas and developmental disorders.

The primary goal of our research program is to investigate the underlying mechanisms of gene expression control of critical, cancer-associated cytokine and growth factor genes by noncoding RNAs, microRNAs and AREs, as well as their interactions and synergisms with RNA binding protein complexes (RNPs) in response to quiescent conditions in tumors that lead to tumor progression and recurrence. An associated direction is to investigate the regulation of expression and function of regulatory noncoding RNAs and RNPs by distinct tumor-associated conditions, using cancer cell lines, stem cells and *Xenopus laevis* germ cells. An important focus of our research is to functionally characterize the selective interactions between regulatory noncoding RNAs, RNPs, and their mRNA targets that encode for critical growth and cell state regulators, and develop specific therapeutic approaches against tumor resistance and recurrence.

Several studies indicate that cells that survive clinical therapy include dormant, quiescent G0-like cells, observed as a small—but clinically relevant —population in leukemias and in several solid tumors associated with



Regulation of gene expression in cancer by noncoding RNAs and RNPs.

poor survival rates. Quiescence or G0 is a unique, adaptive, nonproliferating state that provides an advantageous escape from harsh situations and chemotherapy, allowing cells to evade permanent outcomes of tumor-negative environments such as senescence, differentiation or apoptosis. Instead, the cell is suspended reversibly in an assortment of transition phases that retain the ability to return to proliferation and contribute to tumor heterogeneity, resistance and recurrence. Quiescence involves gene expression reprogramming, upregulating those mRNAs and regulatory RNAs—including specific microRNAs—required for survival and persistence in the G0 state. The key finding of our studies on cytokine and growth factor gene expression, which forms the basis of our research program, is that AREs, microRNAs and RNPs are transformed by such cellular conditions to alter expression patterns of specific, clinically important genes. We further identified post-transcriptional effectors associated with these RNAs under distinct conditions by developing an in vivo crosslinking-coupled affinity purification method to purify endogenous RNP complexes. These findings opened a novel, unexplored area of research into gene expression control in response to tumor-associated conditions by highly potent RNA regulators. These investigations have major implications for understanding gene expression that contributes to tumor progression, resistance and recurrence.

The lab has four core directions:

1. To functionally characterize microRNAs and specific noncoding regulatory RNAs and identify their associated cofactors and target mRNAs that control expression of clinically important cytokines, cancer and cell state regulators, using previously developed in vivo crosslinking coupled affinity purification methods and confirmatory assays.
2. To investigate the mechanism of gene expression control and interconnections of the identified RNA regulators, AREs, microRNA target sites and RNPs.
3. To elucidate the regulation of expression and function of noncoding RNAs, AREs and RNPs by specific tumor-associated conditions.
4. To characterize the selective interactions between small regulatory RNAs and their mRNA targets in order to develop antisense manipulations of these interactions as specific therapeutic approaches. These studies should lead to a greater understanding of the versatile role of regulatory small noncoding RNAs in the pathogenesis of cancers and to novel approaches in RNA-based therapeutic applications.

Selected Publications:

Lee S, Truesdell SS, Bukhari SIA, Lee JH, LeTonqueze O and **Vasudevan S**. Upregulation of eIF5B controls cell cycle arrest and specific developmental stages. *PNAS*, 2014 in press.

Liu M, Roth A, Yu M, Morris R, Bersani F, Rivera MN, Lu J, Shioda T, **Vasudevan S**, Ramaswamy S, Maheswaran S, Diederichs S, Haber DA. The IGF2 intronic miR-483 selectively enhances transcription from IGF2 fetal promoters and enhances tumorigenesis. *Genes & Dev*: 27(23):2543-8, 2013.

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Johnathan R. Whetstine, PhD

The Whetstine laboratory is interested in understanding how the chromatin microenvironment regulates gene expression while maintaining a stable genome. We interrogate this relationship by studying the role of histone-modifying enzymes in both human culture and *C. elegans* models. We have initiated these types of studies by focusing on a specific class of chromatin regulators, the JmjC-containing histone demethylases. Since the discovery of these chromatin regulators, my laboratory has started screening tumors for genomic anomalies (copy changes and mutations) in this class of enzyme. Additionally, we have begun examining their molecular roles at a biochemical, molecular and *in vivo* level. Based on our observations, we are determining whether these genomic alterations will allow us to modify conventional chemotherapy to treat tumors with alterations in JmjC enzymes and establishing whether these changes will serve as novel molecular diagnostics. In addition, we have uncovered an important role for these enzymes in regulating both cell cycle and copy number control and have initiated a number of studies to expand upon this recent discovery. The laboratory is currently expanding these same types of studies into other novel chromatin regulators such as histone methyltransferases and deacetylases.

Whetstine Laboratory

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Kelly Biette*
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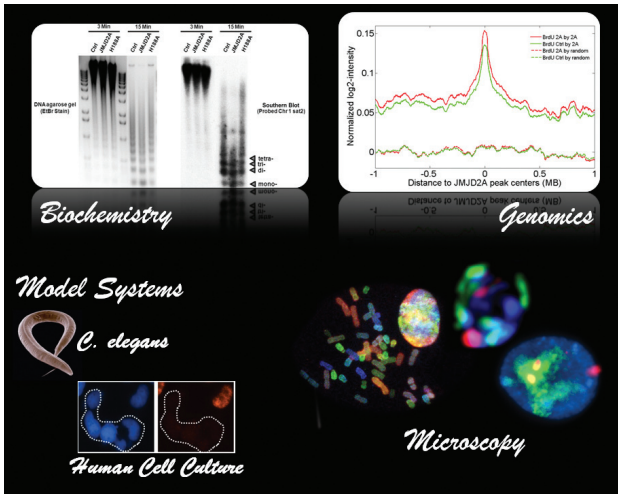
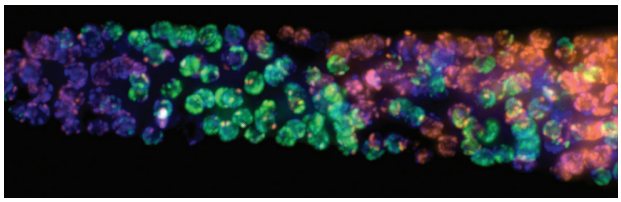
* Graduate student

Histone methylation and acetylation dynamics: impact on development and cancer pathology

Events within the nucleus are governed by a number of processes, but increasing information emphasizes the relationship between post-translational modifications (PTMs) on the histones within the chromatin and proper developmental patterning and pathologies like cancer. The N-terminal tails of histones are subject to a plethora of PTMs including phosphorylation, ubiquitination, acetylation and methylation. Each modification can affect chromatin architecture, but the sum of these modifications may be the ultimate determinant of the chromatin state and biological outcome. Research has shown that multiple lysine (K) residues on the tails of histone H3 and H4 are sites for methylation. The site and degree of methylation (mono-, di-, or tri-) are linked to transcriptional activation and repression, cell cycle progression, and DNA damage response. Many biological processes like heterochromatin formation and X-inactivation are regulated by histone methylation; therefore, aberrant methylation can result in human diseases such as cancer. For this reason, organisms have developed enzymes that are responsible for both adding and removing the methyl mark. Our group studies the impact that histone-modifying proteins have on development, behavior and cancer pathology.

My laboratory is focused on understanding the impact that both methylation and acetylation dynamics have in both human cell culture and





The top image represents the types of approaches that the Whetstine Laboratory is using to understand the impact that chromatin and the associated modulatory factors have on development and cancer.

The lower image visualizes DNA replication timing through the use of a pulse chase-labeled C. elegans adult germline. Altered histone modifications delayed the replication process in this histone demethylase JMJD-2 mutant. These data are presented in Black JC, Allen A, Van Rechem C, Forbes E, Longworth M, Tschöp K, Rinehart C, Quiton J, Walsh R, Smallwood A, Dyson NJ, Whetstine JR. Conserved antagonism between JMJD2A/KDM4A and HP1γ during cell cycle progression. Mol Cell. 40(5):736-48, 2010 Dec 10.

C. elegans. In particular, we are investigating the impact that the histone 3 lysine 9/36 tri-demethylases have on differentiation, neural behavior and tumorigenesis by understanding their roles in transcriptional regulation of the coding and noncoding regions of the genome, in cell cycle progression through regulating chromatin structure, and in the stability of the genome. We are also interrogating the mechanisms associated with regulating histone demethylase function. For example, we have demonstrated that KDM4A is modulated throughout the cell cycle by the SCF E3 ubiquitin ligase complex, which is an important regulator of demethylase levels and function during the cell cycle. Most recently, we have demonstrated that JMJD2A/KDM4A is amplified in a number of tumors, correlates with poor outcome in ovarian cancer patients and regulates the site-specific copy gain of regions implicated in chemotherapy resistance. Through the use of proteomics and genomics, we have been able to identify important associated proteins regulating these KDM4A driven events at regions being directly modulated.

The laboratory will interrogate the functional role of histone demethylases by using genomic (ChIP-chip, ChIP-seq, microarrays, and RNA-seq), proteomic (MS-MS complexes and PTMs), cytological (live imaging and deconvolution confocal microscopy) and genetic (C. elegans, human cell lines, and zebrafish) approaches (Figure 1). Using these strategies, we have uncovered roles for the *C. elegans* JMJD-2 enzyme in genomic stability and DNA replication (Figure 2). We have extended these studies to demonstrate a conserved role for human JMJD2A in DNA replication and demonstrated that ubiquitin plays a key role in this regulation. Using similar approaches, we have also uncovered an important link between histone deacetylase 1 (HDAC-1) and the regulation of extracellular matrix biology in both humans and *C. elegans*, a finding that has direct implications in cancer chemotherapy. Overall, the laboratory will integrate a number of approaches and systems to determine the important biological pathways regulated by histone demethylases and histone deacetylases.

Selected Publications:

Van Rechem C, and **Whetstine JR**.Examining the Impact of Gene Variants on Lysine Methylation. *Biochim Biophys Acta* 2014: ahead of print.

Black JC, Manning AL, Van Rechem C, Kim J, Ladd B, Cho J, Pineda CM, Murphy N, Daniels DL, Montagna C, Lewis PW, Glass K, Allis CD, Dyson NJ, Getz G, **Whetstine JR**. KDM4A Lysine Demethylase Induces Site-Specific Copy Gain and Rereplication of Regions Amplified in Tumors. *Cell.* 154, 541-555, 2013.

Black JC, **Whetstine JR**. Tipping the lysine methylation balance in disease. *Biopolymers*. 99(2):127-35, 2013 February.

Black JC, Van Rechem C, **Whetstine JR**. Histone lysine methylation dynamics: establishment, regulation, and biological impact. *Mol Cell*. 48(4):491-507, 2012 November.

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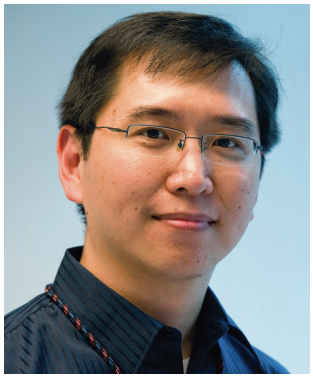
Black JC, Allen A, Van Rechem C, Forbes E, Longworth M, Tschöp K, Rinehart C, Quiton J, Walsh R, Smallwood A, Dyson NJ, **Whetstine JR**. Conserved antagonism between JMJD2A/KDM4A and HP1γ during cell cycle progression. *Mol Cell*. 40(5):736-48, 2010 Dec 10.



Lee Zou, PhD

Cancer is a complex disease associated with genetic and epigenetic alterations in the genome. To prevent these detrimental alterations, cells have evolved an intricate signaling network, called the checkpoint, to detect and signal problems in the genome. During cancer development, the activation of oncogenes and loss of tumor suppressors leads to genomic instability, rendering cancer cells addicted to specific checkpoint signaling proteins to survive. **The Zou laboratory** is particularly interested in understanding how the checkpoint detects DNA damage and genomic instability, and how the checkpoint can be targeted in cancer therapy. Our current studies are focused on the activation of ATR and ATM, the master sensor kinases of two major checkpoint pathways. Furthermore, we are developing new strategies to exploit the genomic instability and checkpoint addiction of different cancer cells in targeted cancer therapy.

Zou Laboratory

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Dominick Matos
David Moquin, PhD
Hai Dang Nguyen, PhD
Jian Ouyang, PhD
Ching-Shyi Wu, PhD
Stephanie Yazinski, PhD
Lee Zou, PhD

DNA damage sensing and checkpoint activation

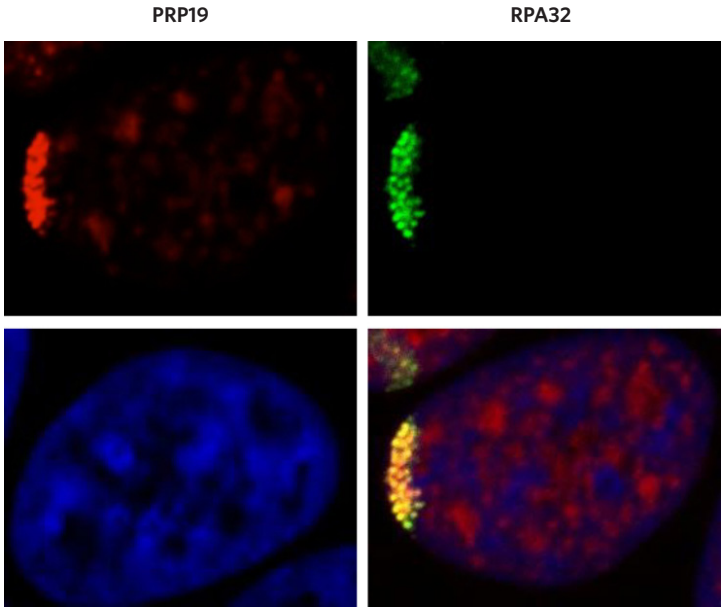
The ATM checkpoint is primarily activated by double-stranded DNA breaks, whereas the ATR checkpoint responds to a broad spectrum of DNA damage. To understand how ATM and ATR are activated, we sought to identify the key DNA structural elements that activate ATM and ATR as well as to identify the sensor proteins that recognize these DNA structures. We have developed unique biochemical systems to mechanistically dissect the process of ATR and ATM activation. Using both proteomic and genomic approaches, we have identified a number of key regulators of the ATR checkpoint. We are extending our studies to investigate how ATR and ATM are regulated in different biological contexts, such as in response to various types of oncogenic stress, in radioresistant cancer cells, and during cellular aging.

Checkpoint, telomeres and the cell cycle

Telomere is a special DNA-protein structure formed at the ends of chromosomes. Dysfunctional telomeres have been linked to both cancer and aging. We have developed new biochemical assays to understand the interplay between checkpoint sensors and telomere-capping proteins at telomeres. Using these assays, we found that the dynamics between the two protein groups is regulated during the cell cycle. Our recent studies have revealed that the regulation of ATR checkpoint at telomeres is altered in a subset of cancers, offering an opportunity for targeted cancer therapy.

Checkpoint signaling and epigenetic regulation

The signaling of DNA damage through the checkpoint pathway is generally viewed as a cascade of protein phosphorylation events. However, several other types of protein modifications—such as ubiquitylation, SUMOylation, methylation and acetylation—are also regulated by DNA damage. Furthermore, noncoding RNAs have also been implicated in the DNA damage response. We have recently identified a number of proteins



This image shows that the ubiquitin ligase PRP19 (red) colocalizes with the single-stranded DNA binding protein RPA (green) at sites of laser-induced DNA damage. Our recent studies revealed that a ubiquitin-mediated circuitry regulated by PRP19 plays an important role in activation of the ATR checkpoint.

that may link these regulatory mechanisms to DNA damage response. Our ongoing studies aim to elucidate how this network of regulatory events is integrated in the presence of DNA damage.

Checkpoint inhibition and targeted cancer therapy

While the checkpoint is often compromised in cancers, certain checkpoint proteins are uniquely required for the survival of cancer cells because of the elevated genomic instability within them. Interestingly, we found that due to the different oncogenic stress in cancer cells, different cancers are addicted to different checkpoint signaling proteins. We are investigating how oncogenic stress leads to checkpoint addiction, aiming to exploit the checkpoint addiction of cancer cells in targeted therapy.

Selected Publications:

Wu C, Ouyang J, Mori E, Nguyen HD, Marechal A, Hallet A, Chen DJ, and **Zou L**. SUMOylation of ATRIP Potentiates DNA Damage Signaling by Boosting Multiple Protein Interactions in the ATR Pathway. *Genes & Dev.* 28:1472-84, 2014.

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