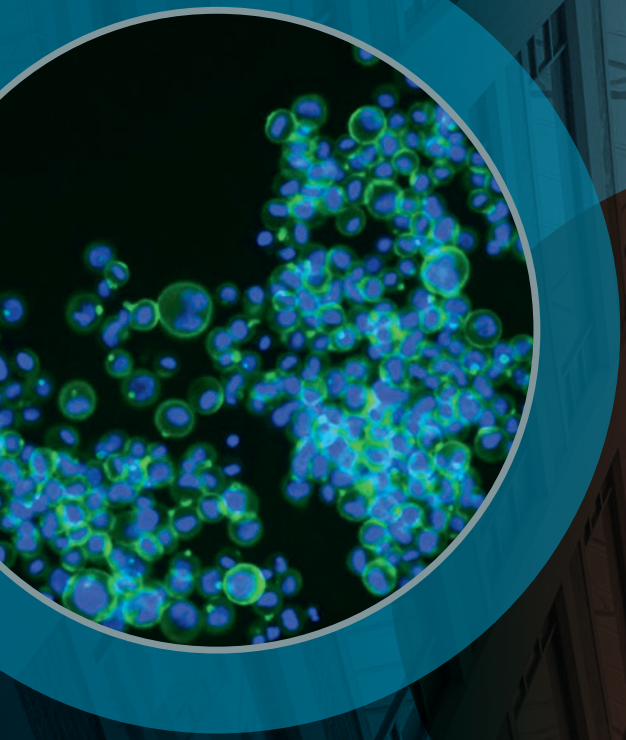


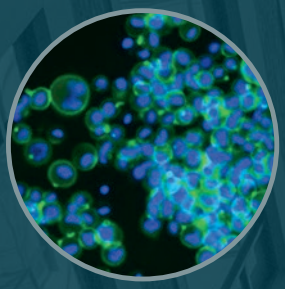
CENTER *for* CANCER RESEARCH

Annual Report 2019-2020

MASSACHUSETTS GENERAL HOSPITAL
CANCER CENTER

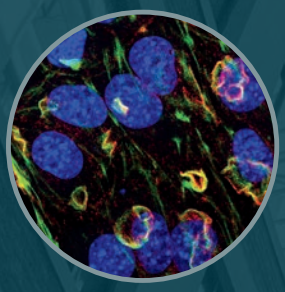
Microfluidic device for the generation of droplets containing mixed cell populations for long term culture.

Image courtesy of Rohan Thakur, Stott Laboratory



Ex vivo culture of circulating tumor cells from a breast cancer patient.

Image courtesy of Haber/Maheswaran Laboratory



EGF stimulation rapidly triggers actin/ERM- (green) and pAkt (red) rich macropinocytic cups on the surface of Nf2-/- cells.

Image courtesy of Christine Chiasson-MacKenzie, PhD, McClatchey Laboratory

Report design by Catalano Design; Cover background photo by Lee Hopkins, OLP Creative

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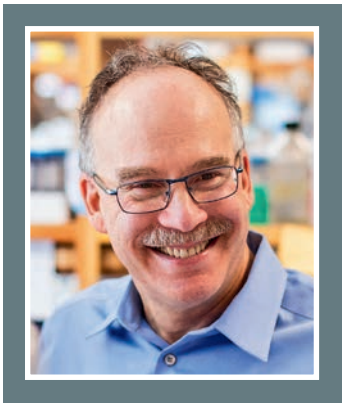
Reports from the Principal Investigators

Message from the Director



The Center for Cancer Research (CCR) is the major hub for basic and translational research within the Massachusetts General Hospital Cancer Center. The CCR includes 50 primary and affiliated faculty members, with multidepartmental Harvard Medical School appointments, including the Departments of Medicine, Pathology, Radiation Oncology, Surgery, Dermatology and Pediatrics. These scientists are pursuing every aspect of cancer research, from exploring cancer genetics, genomics, epigenetics and proteomics to developmental biology, cell signaling, molecular therapeutics, immunology, metabolism, cell cycle regulation, and microRNA biology. We occupy over 80,000 square feet of laboratory space in three Mass General research facilities, (Charlestown Navy Yard, Simches Research Building and the Jackson Building), and our laboratories include more than 500 postdoctoral fellows, graduate students and technicians. We host seminar series, an annual symposium, and a two-day retreat which provide opportunities for our investigators to discuss new ideas and spark productive collaborations.

Some of the CCR research highlights from the past year include:

- identifying T cell markers associated with positive response to checkpoint immunotherapy in melanoma
- targeting ATR response to DNA replication block in Myelodysplastic Syndrome
- defining the role of Merlin/ERM proteins in macropinocytosis and receptor signaling
- revealing new types of human dendritic cells contributing to immune responses
- defining the role of the histone deacetylase SIRT6 in orchestrating the Warburg effect
- identifying mutations caused by the APOBEC enzyme at DNA stem-loops, as drivers of recurrent mutagenesis
- using an RNA-based digital circulating tumor cell signature to predict drug response and early dissemination in prostate cancer
- linking changes in histone methylation to the initiation of gene amplification in cancer cells
- targeting RET fusions that mediate resistance to EGFR inhibition in lung cancer
- defining the scale of transcriptome-wide off-target hits using CRISPR-guided DNA base editing
- creating novel CAR-T cells secreting bispecific antibodies to target brain tumors

This year, we are delighted to introduce four new CCR faculty members: Esther Rheinbay, PhD (from the Broad Institute); Liron Bar-Peled, PhD (from the Scripps Research Institute); Russell Jenkins, MD, PhD (from the Dana-Farber Cancer Institute); and Robert Manguso, PhD (from the Broad Institute). These young investigators have already made important contributions in their respective fields and will further expand and strengthen our bioinformatics/computational biology, cell biology and cancer immunology programs.

Cancer immunology and immunotherapy was the main theme of the 2019 Jonathan Kraft Prize for Excellence

Daniel Haber photo by Scott Eisen

in Cancer Research and Symposium presented by the Mass General Center for Cancer Research. The Prize, which honors an extraordinary scientist who has made seminal contributions to cancer research, was presented to Carl June, MD from the University of Pennsylvania for his breakthrough contributions to the field of CAR-T cell therapy. He exemplifies the innovative scientist and thoughtful mentor that this award was intended to recognize, when established in 2014 by Robert Kraft to honor his son Jonathan's commitment to cancer research.

Our investigators have successfully competed for funding from NIH, NCI and many prestigious foundations including the Ambrose Monell Foundation, American Cancer Society, Breast Cancer Research Foundation, Cancer Research Institute, Conquer Cancer Foundation, Damon Runyon Cancer Research Foundation, Ellison Foundation, Gray Foundation, National Foundation for Cancer Research, Sontag Foundation, and V Foundation for Cancer Research, among other generous funders.

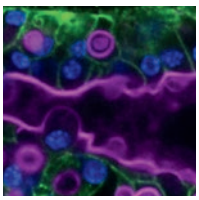
We are also grateful to the many individuals and families who so generously support our efforts, including donors who have established endowed chairs which are vital to the success of our investigators and the strength of our research program. During the past year Andrea McClatchey, PhD was appointed as the inaugural incumbent of the Poitras Family Endowed Chair in Oncology and David Sweetser, MD is the inaugural incumbent of the Leslie Meyer and Lewis Ball Holmes Chair in Genetics and Teratology.

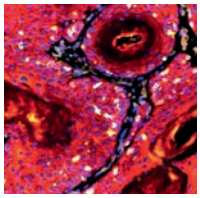
Our goal for the next year is to further advance our understanding of fundamental biological processes disrupted in cancer. We will continue our focus on developing new diagnostic and therapeutic tools that can alter the course of the disease at its earliest stages and provide improved clinical outcomes for patients treated at the Mass General Cancer Center and around the world. In addition, we will continue our commitment to fostering intellectual exchange and innovation and to training and supporting our students and postdoctoral fellows who represent the next generation of leaders in cancer research.

Daniel A. Haber

Daniel A. Haber, MD, PhD
Director, Massachusetts General Hospital Cancer Center







Kurt J. Isselbacher, MD — *In Memoriam*

On July 18, 2019, Dr. Kurt Isselbacher passed after a brief illness at the age of 93. The field of Gastroenterology lost one of its most influential and respected leaders and the Mass General Cancer Center lost its founder, whose insight and vision advanced cancer research at our institution and beyond.

Kurt was born in Wirges, Germany, where his father, Albert, was a merchant. Kurt's childhood changed dramatically after the infamous Kristallnacht pogrom in 1938, after which his family fled the Nazis and emigrated to the United States, settling in Portsmouth, New Hampshire.

An exceptionally bright student, Kurt enrolled at Harvard College and received his M.D. degree from Harvard Medical School, then completed a residency in Internal Medicine at Massachusetts General Hospital in 1953. He then spent three years as a Clinical Investigator at the National Institutes of Health, before returning to MGH and joining the Harvard faculty.

In 1956, at the age of 31, Kurt was invited by the Chief of Medicine, Walter Bauer, to lead the Gastroenterology Division. Under Kurt's direction for over 30 years, MGH Gastroenterology became a leading center in the country for training, research and clinical care of gastrointestinal diseases.

Kurt's own research focused on the mechanisms involved in the intestinal uptake and transport of sugars, amino acids and lipids. At NIH, he identified the enzymatic defect responsible for galactosemia, leading to the development of a specific test which is now used routinely to screen all newborns. He subsequently described the pathways of corticosteroid metabolism in the liver; mechanisms of intestinal fat and sugar absorption; metabolic defects that lead to alcohol-induced fatty liver; and malabsorption and immunologic defects associated with hepatic disease.

In 1987, the MGH Board of Trustees established the Cancer Center, appointing Kurt as its founding Director. Kurt created a research hub in the Charlestown Navy Yard, recruiting outstanding basic science faculty and fostering an environment of scientific excellence and vibrant collaborations. Kurt stepped down in 2003, succeeded as Cancer Center Director by Daniel Haber. He continued working

The field of Gastroenterology lost one of its most influential and respected leaders and the Mass General Cancer Center lost its founder, whose insight and vision advanced cancer research at our institution and beyond.

and mentoring faculty in the Cancer Center for the remainder of his life, and even at the age of 93, he regularly attended lab meetings and scientific lectures.

Kurt was repeatedly called upon to serve in leadership roles at MGH, Harvard and other national and international institutions. From 1966 to 1995, he served as Chairman of the Executive Committee of the Harvard Medical School Departments of Medicine. He served as the President of the American Gastroenterological Association, receiving the Distinguished Achievement Award and the Julius Friedenwald Medal; he received the prestigious Kober Medal from the Association of American Physicians. He received the John Phillips Memorial Award for Distinguished Achievement in Clinical Medicine from the American College of Physicians and the Bristol-Myers Squibb/Mead Johns Award for Distinguished Achievement in Nutrition Research. Kurt served as one of the editors of Harrison's *Principles of Internal Medicine*, the pre-eminent textbook in medical education. He was a member of the National Academy of Sciences, the National Research Council, and the American Academy of Arts and Sciences.

As a teacher and mentor, Kurt enriched the lives of both colleagues and trainees, setting the standard for clinical and scientific excellence, genuine compassion and inspiring leadership. He listened more than he spoke, and his advice was always thoughtful, kind and generous.

Kurt's passing was preceded by that of his beloved wife Rhoda and daughter Lisa. He is survived by his daughters Jody and Kate, and his son Eric, and their families. He has also touched the hearts of the entire faculty and trainees of the MGH Cancer Center and the Center for Cancer Research, which was his academic pride and joy. His vision continues to guide us as we remain true to his standard of scientific excellence combined with respect, friendship and collaboration.

He will be greatly missed.

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Jonathan Kraft Prize for Excellence in Cancer Research

Presented by the Massachusetts General Hospital Cancer Center

2020 *(to be presented in May 2020)*

Aviv Regev, PhD
Chair of the Faculty and Core Member, Broad Institute
Director, Klarman Cell Observatory, Broad Institute
Professor of Biology, MIT

2019

Carl H. June, MD
Professor in Immunotherapy
Director, Center for Cellular Immunotherapies
University of Pennsylvania Perelman School of Medicine

2018

Charles Swanton, MD, PhD
Professor and Chair, Personalized Cancer Medicine
University College London Cancer Institute, London, UK

2017

Kevan M. Shokat, PhD
Professor and Chair, Department of Cellular and Molecular Pharmacology, UCSF
Professor, Department of Chemistry, UC Berkeley

2016

Joan A. Steitz, PhD
Sterling Professor of Molecular Biophysics and Biochemistry
Yale School of Medicine

2015

C. David Allis, MD, PhD
Joy and Jack Fishman Professor, Laboratory of Chromatin Biology and Epigenetics, Rockefeller University

The Annual MGH Award in Cancer Research

In memory of Nathan and Grace Shiff

2014

Hans Clevers, MD, PhD
President of the Royal Netherlands Academy of Arts and Sciences
Professor of Molecular Genetics
University Utrecht, Netherlands

2013

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

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, Netherlands

Center for Cancer Research Faculty

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

Nicholas Dyson, PhD
Scientific Director
Mary B. Saltonstall Chair in Oncology
Professor of Medicine

Lee Zou, PhD
Associate Scientific Director
James and Patricia Poitras Chair in Cancer Research
Professor of Pathology

Nir Hacohen, PhD
Director, Center for Cancer Immunology
David P. Ryan Chair in Cancer Research
Professor of Medicine

Charlestown Laboratories

Martin Aryee, PhD
*Assistant Professor of Pathology**

Liron Bar-Peled, PhD
Assistant Professor of Medicine

Cyril Benes, PhD
Assistant Professor of Medicine

Priscilla K. Brastianos, MD
Assistant Professor of Medicine

Ryan Corcoran, MD, PhD
Associate Professor of Medicine

Shawn Demehri, MD, PhD
*Assistant Professor in Dermatology^*

Nicholas Dyson, PhD
Professor of Medicine

Andrew Elia MD, PhD
Assistant Professor of Radiation Oncology

David E. Fisher, MD, PhD
Professor and Chief of Dermatology

Gaddy Getz, PhD
Professor of Pathology

Timothy A. Graubert, MD
Professor of Medicine

Wilhelm Haas, PhD
Assistant Professor of Medicine

Daniel A. Haber, MD, PhD
Professor of Medicine

Nir Hacohen, PhD
Professor of Medicine

Aaron Hata, MD, PhD
Assistant Professor of Medicine

Jonathan G. Hoggatt, PhD
Assistant Professor of Medicine◊

Othon Iliopoulos, MD
Associate Professor of Medicine

Keith Joung, MD, PhD
*Professor of Pathology**

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Assistant Professor of Radiation Oncology#

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*Associate Professor of Pathology**

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Assistant Professor of Pathology

Shyamala Maheswaran, PhD
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Robert Manguso, PhD
Faculty Member†

Marcela V. Maus, MD, PhD
Assistant Professor of Medicine

Andrea I. McClatchey, PhD
Professor of Pathology

David T. Miyamoto, MD, PhD
Assistant Professor of Radiation Oncology#

Mo Motamedi, PhD
Assistant Professor of Medicine

Christopher J. Ott, PhD
Assistant Professor of Medicine

Shiv Pillai, MD, PhD
Professor of Medicine ◊◊

Luca Pinello, PhD
*Assistant Professor of Pathology**

Esther Rheinbay, PhD
Assistant Professor of Medicine

Miguel N. Rivera, MD
*Assistant Professor of Pathology**

Dennis Sgroi, MD
*Professor of Pathology**

Toshihiro Shioda, MD, PhD
Associate Professor of Medicine

David Spriggs, MD
Professor of Medicine†

Shannon Stott, PhD
Assistant Professor of Medicine

Mario L. Suvà, MD, PhD
*Assistant Professor of Pathology**

David T. Ting, MD
Assistant Professor of Medicine

Alexandra-Chloé Villani, PhD
Assistant Professor of Medicine°

Lee Zou, PhD
Professor of Pathology

Jackson Laboratories

Nir Hacohen, PhD
Professor of Medicine

A. John Iafrate, MD, PhD
*Professor of Pathology**

Russell W. Jenkins, MD, PhD
Assistant Professor of Medicine

Simches Laboratories

Nabeel Bardeesy, PhD
Associate Professor of Medicine

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

Leif Ellisen, MD, PhD
Professor of Medicine

Konrad Hochedlinger, PhD
*Professor of Medicine***

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

Raul Mostoslavsky, MD, PhD
Professor of Medicine

David A. Sweetser, MD, PhD
Assistant Professor of Pediatrics□

Shobha Vasudevan, PhD
Associate 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

^ Joint appointment with MGH Cutaneous Biology Research Center

Joint appointment with MGH Molecular Radiation Oncology Unit

◊ Joint appointment with MGH Transplantation Research Center

◊◊ Joint appointment with Ragon Institute of Harvard and MIT

□ Joint appointment with MGH Pediatric Hematology Oncology Unit

† Appointment process initiated

° Joint appointment with Center for Immunology and Inflammatory Diseases

REPORTS *from the* PRINCIPAL INVESTIGATORS

Martin Aryee, PhD

The Aryee laboratory develops analysis methods for studying the genetic and epigenetic basis of cancer and other diseases. Most of their work is focused on improving our understanding of how aberrations in the physical and chemical structure of DNA within the nucleus is linked to cancer and other common diseases. Projects range from basic biology, probing how DNA misfolds in cancer cells, to clinical applications aiming to develop blood tests for early detection of cancer. The lab also develops tools that aim to enable the safe translation of gene editing techniques such as CRISPR into human therapeutics.

Aryee Laboratory

Martin Aryee, PhD
Caleb Lareau*
Ayush Raman, PhD
Alejandro Reyes, PhD

*PhD Candidate

Tumor heterogeneity

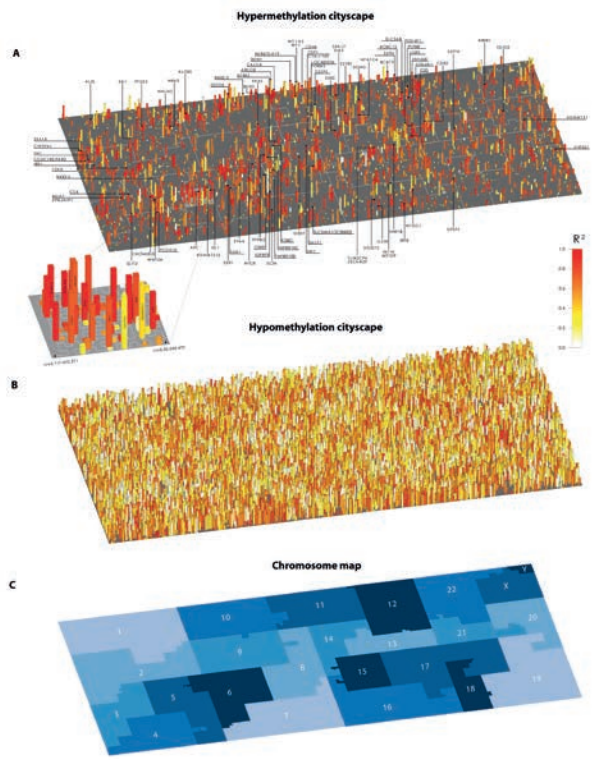
We develop statistical methods to improve our understanding of cell-to-cell variability and its relationship to cancer-related phenotypes. Much of this work relates to the computational and statistical challenges posed by single-cell transcriptome and epigenome data. The goal of these methods is to characterize 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 to 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 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.



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).

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:

Ligorio M, Sil S, Malagon-Lopez J, Nieman LT, Misale S, Di Pilato M, Ebright RY, Karabacak MN, Kulkarni AS, Liu A, Vincent Jordan N, Franses JW, Philipp J, Kreuzer J, Desai N, Arora KS, Rajurkar M, Horwitz E, Neyaz A, Tai E, Magnus NKC, Vo KD, Yashaswini CN, Marangoni F, Boukhali M, Fatherree JP, Damon LJ, Xega K, Desai R, Choz M, Bersani F, Langenbucher A, Thapar V, Morris R, Wellner UF, Schilling O, Lawrence MS, Liss AS, Rivera MN, Deshpande V, Benes CH, Maheswaran S, Haber DA, Fernandez-Del-Castillo C, Ferrone CR, Haas W, **Aryee MJ**, Ting DT. Stromal Microenvironment Shapes the Intratumoral Architecture of Pancreatic Cancer. *Cell*. 2019 Jun 27;178(1):160-175.

Ludwig LS, Lareau CA, Ulirsch JC, Christian E, Muus C, Li LH, Pelka K, Ge W, Oren Y, Brack A, Law T, Rodman C, Chen JH, Boland GM, Hacohen N, Rozenblatt-Rosen O, **Aryee MJ**, Buenrostro JD, Regev A, Sankaran VG. Lineage Tracing in Humans Enabled by Mitochondrial Mutations and Single-Cell Genomics. *Cell*. 2019 Mar 7;176(6):1325-1339.e22.

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Lareau CA, **Aryee MJ**. hichipper: a preprocessing pipeline for calling DNA loops from HiChIP data. *Nat Methods*. 2018 Feb 28;15(3):155-156.

Lareau CA, **Aryee MJ**. diffloop: a computational framework for identifying and analyzing differential DNA loops from sequencing data. *Bioinformatics*. 2018 Feb 15;34(4):672-674.

Liron Bar-Peled, PhD

Bar-Peled Laboratory

Liron Bar-Peled, PhD
Franziska Bemmann**
Ben Jiang*
Marion Schweiger*
Abby Smith
Tommy Weiss, PhD
Konstantin Wolf*
Mengyao Xu, MD
shared with David Spriggs lab
Junbing Zhang, PhD

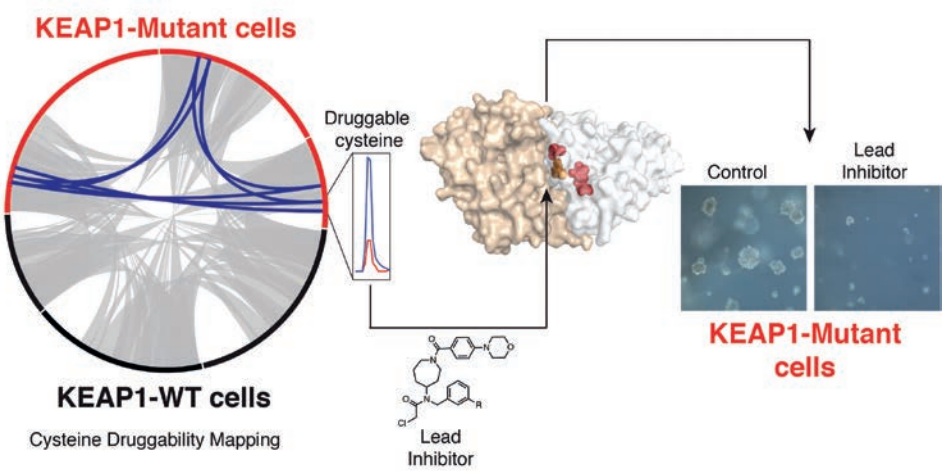
*Master's student
**Graduate student

Research in **the Bar-Peled laboratory** sits at the interface of cellular metabolism and signal transduction and focuses on understanding how cancer cells respond to altered metabolic states. Rapidly proliferating cancer cells are characterized by increased production of toxic metabolic byproducts known as reactive oxygen species (ROS) that at high levels potently block cancer cell growth. To neutralize high ROS levels, cancer cells activate the NRF2 pathway, which governs the cellular antioxidant response. While the NRF2 pathway is critical for cancer growth, the molecular mechanisms by which this pathway functions and provides cancer cells with a proliferative advantage remain poorly understood. By combining frontier molecular, chemical and proteomic approaches, research in our lab has revealed that NRF2 establishes a unique cellular environment that protects critical proteins required for cancer cell growth from inactivation by ROS. Our studies indicate that these ROS-regulated proteins are highly targetable by small molecule inhibitors and may be exploited to develop chemical tools to inactivate these dependencies in cancers.

Cancer cells display remarkable plasticity allowing them to adapt to ever changing environments. A key feature of this plasticity is their ability to rewire core metabolic networks to provide a steady source of energy and building blocks needed for rapid growth. This demand for energy produces byproducts, including ROS that alters the function of proteins, DNA and lipids, and if left unchecked, results in oxidative stress and impairs cancer cell viability. To counter a rise in oxidative stress, cells activate the NRF2 transcription factor leading to the expression of a vast network of antioxidant and detoxification genes that restore redox homeostasis. Multiple cancer cells, including ~30% of non-small cell lung cancers (NSCLCs) activate NRF2 through the genetic disruption of its negative regulator KEAP1. Despite its clear importance in cancer cell proliferation, we know remarkably little about how the NRF2/KEAP1 pathway functions within cancer cells or how ROS modification of proteins alters their function. Our long-term goal is to understand how cancer cells sense and respond to ROS and to pharmacologically modulate these pathways in cancers where they are deregulated.

Redox control pathways in Lung Cancer

Our recent studies focus on how the intracellular environment generated by NRF2 in NSCLCs is required for cancer cell proliferation. By employing a chemical proteomics platform (isoTOP-ABPP) that identifies changes in cysteine reactivity mediated by ROS, we demonstrated that NRF2 is required for the protection of dozens of proteins from ROS modification. We found that silencing NRF2 in NSCLCs reduced the reactivity of the catalytic cysteine of the glycolytic enzyme GAPDH without changing GAPDH protein abundance. Concomitant knockdown of NRF2 significantly reduced GAPDH enzyme activity and glycolytic flux, a metabolic pathway required to fuel cancer cell proliferation. These results illustrate how NRF2 can regulate enzyme and pathway activity, not through direct transcriptional control, but rather by fostering a favorable



(Left) A cysteine druggability map identifies proteins exclusively druggable in KEAP1-mutant NSCLC cells enabling the development of small molecule inhibitors that disrupt NR0B1 protein interactions (middle) and block KEAP1-mutant cell growth (right).

Images from Bar-Peled et al., 2017.

redox environment required for proper enzyme function. Current studies in our lab seek to elucidate how other proteins are post-translationally regulated by NRF2 and feedback into this pathway. To address these questions, we are studying the function of ROS-regulated sites on proteins as well as the identifying reactive metabolites that modify them.

Druggable co-dependencies

Our investigations suggest that the cellular state created by NRF2 may be exploited to develop inhibitors targeting proteins whose expression and function are stimulated by this environment. Because of their importance to protein function, cysteines are targeted by multiple clinically approved inhibitors. To identify pharmacological targets of the NRF2 pathway, we use powerful chemical proteomic platforms (cysteine druggability mapping) to identify the landscape of protein druggability (e.g. ligand-protein interactions) in genetically defined lung cancers. Our studies reveal that multiple proteins, including the orphan nuclear receptor NR0B1, are exclusively druggable in KEAP1-mutant, NRF2-activated cells. By developing a small molecule inhibitor that disrupts NR0B1 protein interactions we show that NR0B1 functions as a critical signaling node within the NRF2 pathway to support its pro-proliferative transcriptional output required for anchorage-independent growth. Recently we uncovered that cysteine residues that are sensitive to ROS modification are highly targetable by covalent inhibitors. Our current studies suggest that these sites may be exploited to develop inhibitors that target proteins required for the proliferation of NRF2- activated cancers.

Ongoing projects:

1. Determine how cancer proteomes respond to changes in the intracellular redox environment
2. Elucidate the role of NRF2-regulated reactive metabolites on protein function
3. Decipher how cells adapt to anchorage-independent growth
4. Identify druggable transcriptional dependencies in genetically-defined cancers

Selected Publications:

Chen AL, Lum KM, Lara-Gonzalez P, Ogasawara D, Cognetta AB 3rd, To A, Parsons WH, Simon GM, Desai A, Petrascheck M†, **Bar-Peled L**†, Cravatt BF†. (2019) Pharmacological convergence reveals a lipid pathway that regulates C. elegans lifespan. *Nat Chem Bio.* 15:453-462.

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Wang S, Tsun ZY, Wolfson RW, Shen K, Wyant GA, Plovanich ME, Yuan ED, Jones T D, Chantranupong L, Comb W, Wang T, **Bar-Peled L**, Zoncu R, Straub C, Kim C, Park J, Sabatini BL, and Sabatini DM. (2015) The amino acid transporter SLC38A9 is a key component of a lysosomal membrane complex that signals arginine sufficiency to mTORC1. *Science.* 347: 188-194.

Tsun ZY, **Bar-Peled L***, Chantranupong L*, Zoncu R, Wang T, Kim C, Spooner E., Sabatini DM. (2013). The Folliculin Tumor Suppressor is a GAP for the RagC/D GTPases That Signal Amino Acid Levels to mTORC1. *Molecular Cell* 52: 495-505.

Bar-Peled L*, Chantranupong L*, Cherniack AD, Chen WW, Ottina KA, Grabiner BC, Spear ED, Carter SL, Meyerson ML, and Sabatini DM. (2013). A tumor suppressor complex with GAP activity for the Rag GTPases that signal amino acid sufficiency to mTORC1. *Science* 340: 1100-1106.

Bar-Peled L, Schweitzer LD, Zoncu R., and Sabatini DM. Ragulator is a GEF for the Rag GTPases that signal amino acid levels to mTORC1 (2012). *Cell* 150: 1196-1208.

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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 and patient-derived models to define the role of key gene mutations that drive 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 states that lead to cancer initiation and maintenance. These studies are being used to inform improved therapeutic approaches.

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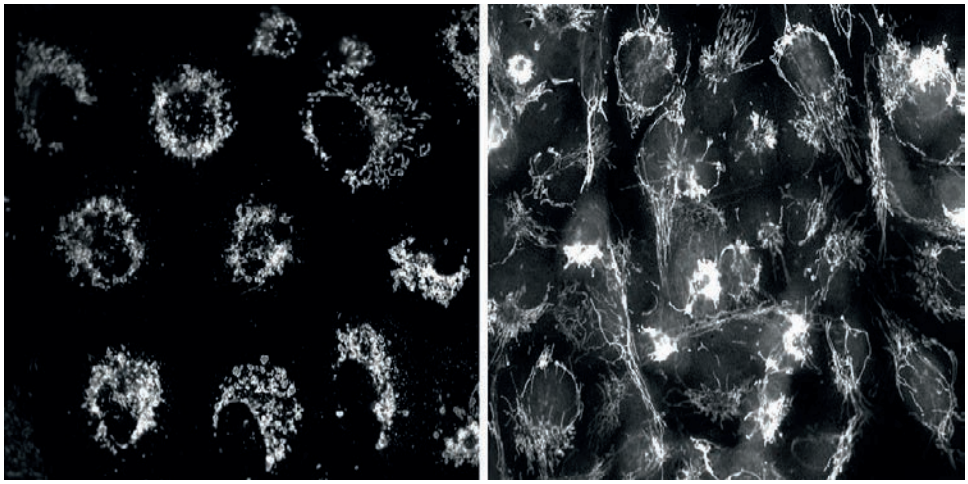
The Bardeesy lab focuses on defining the pathways driving the pathogenesis of pancreatic and biliary cancers. Our lab has developed a series of genetically engineered mouse models that has elucidated the functional interactions of major gene mutations associated with these diseases in humans. Specifically,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

Interplay between metabolism and chromatin regulation in pancreatic and biliary cancer

An important area of current focus in our lab is to elucidate the metabolic regulators of pancreatic cancer and biliary cancers, with particular attention paid to factors that subvert normal differentiation pathways and reprogram cancer cell epigenetics. We have linked mutations in LKB1/STK11 and other important genetic alterations to changes in metabolism that ultimately alter epigenetic states. Identifying these pathways has provided insights in mechanisms of cell transformation arising from these mutations and predict novel therapeutic vulnerabilities. In biliary cancer, there are recurrent mutations in the IDH1 and IDH2 genes. Mutant IDH proteins 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.

Genetic regulation of metabolic reprogramming in pancreatic cancer

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 pancreatic cancer, 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. Our recent studies demonstrate that distinct metabolic programs are activated in pancreatic cancer depending on which gene mutations are present. 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.



Genetic control of expression of the Mitochondrial Fission Factor (MFF) dictates mitochondrial architecture and metabolic phenotypes of cancer cells. The image shows mitochondrial staining (Mitotracker) of cancer cells which express high levels of MFF (left panel) or low levels of MFF (right panel). The MFF-high cancer cells show hyper-fragmented mitochondria compared to the fused mitochondrial network of MFF-low cancers. This differential control of mitochondrial dynamics results in distinct metabolic programs and vulnerabilities.

Understanding and targeting FGFR2-driven biliary cancer

Genetic alterations that activate Fibroblast Growth Factor 2 (FGFR2) signaling are common in biliary cancer and predict response to pharmacological inhibition of the FGFR in patients. However, tumor shrinkage is often modest and acquired resistance invariably arises. We are investigating oncogenic mechanisms controlled by FGFR2 in biliary cancer, including direct targets of FGFR2 signaling as well as downstream impact on cellular metabolism and differentiation. Additionally, we are investigating resistance mechanisms and approaches to prevent and overcome resistance.

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 and the use of this system in large-scale genetic and small-molecule screens to systematically define targetable vulnerabilities in molecularly defined subtypes of this cancer.

Selected Publications:

Goyal L, Shi L, Liu LY, Fece de la Cruz F, Lennerz JK, Raghavan S, Leschiner I, Elagina L, Siravegna G, Ng RWS, Vu P, Patra KC, Saha SK, Uppot RN, Arellano R, Reyes S, Sagara T, Otsuki S, Nadres B, Shahzade HA, Dey-Guha I, Fetter IJ, Baiev I, Van Seventer EE, Murphy JE, Ferrone CR, Tanabe KK, Deshpande V, Harding JJ, Yaeger R, Kelley RK, Bardelli A, Iafrate AJ, Hahn WC, Benes CH, Ting DT, Hirai H, Getz G, Juric D, Zhu AX, Corcoran RB, **Bardeesy N**. TAS-120 Overcomes Resistance to ATP-Competitive FGFR Inhibitors in Patients with FGFR2 Fusion-Positive Intrahepatic Cholangiocarcinoma. *Cancer Discov*. 2019 Aug;9(8):1064-1079.

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Kottakis F, Nicolay BN, Roumane A, Karnik R, Nagle, J Boukhali M, Hayward MC, Li YY, Chen T, Liesa M, Hammerman PS, Wong KK, Hayes DN, Shirihai OS, Dyson, NS, Haas W, Meissner A, **Bardeesy N***. LKB1 loss links the serine metabolism to DNA methylation and tumourigenesis. *Nature*. 2016 Nov 17;539(7629): 390-395.

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The Benes laboratory, also 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. We use a very large collection of previously established tumor cell lines derived from many different cancers as well as newly established lines from patients treated at MGH. To better understand why some patients respond more favorably than others to therapy, we use both cancer cells and other cells found in tumors and study their interplay.

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

Molecular Basis of Cancer Therapeutic Response

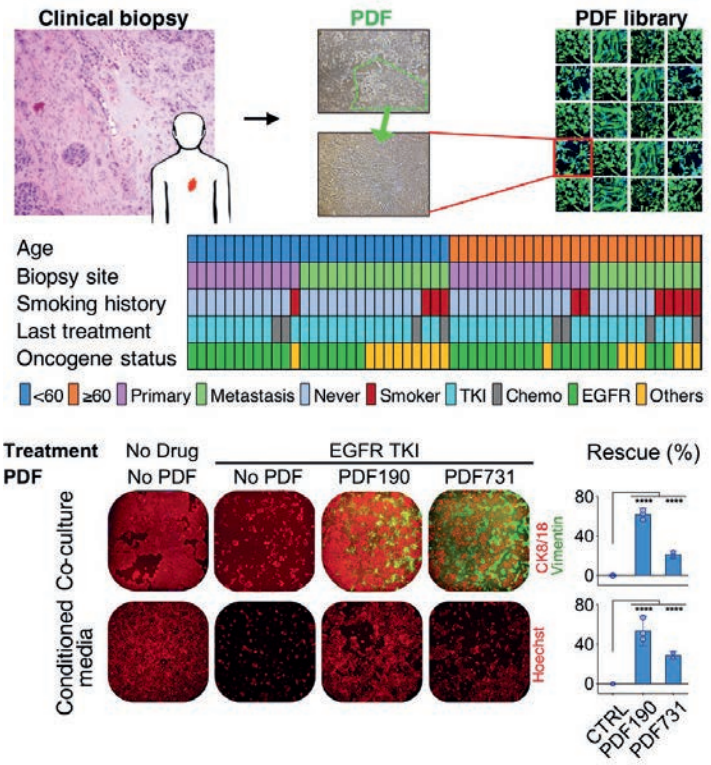
Clinical responses to anticancer therapeutics are often restricted to a subset of cases treated. In some instances, clear evidence is available that correlates clinical responses with specific tumor genotypes. Our goal is to identify tumor cell states that predict sensitivity to anticancer agents. To accomplish this goal, we use historically established cancer cell lines as well as cancer cells obtained from tumor biopsies and study their response to anticancer agents and their combinations using high-throughput approaches. We collaborate with multiple groups at MGH and beyond to identify new treatment options for rare cancers. We use molecular profiling at multiple levels including genetic, epigenetic and proteomic to discover the mechanistic basis of drug response and identify biomarkers predictive of response in patients.

Targeting the Tumor Microenvironment

Tumors contain fibroblasts, endothelial cells and immune cells among others. These cells and the extracellular material they produce constitute the tumor microenvironment. We study how the tumor microenvironment influences therapeutic response. In particular we culture cancer associated fibroblasts from tumor biopsies. Our living collection of Patient Derived Fibroblasts gives us insights into the functional diversity of fibroblasts in tumors, and how they influence cancer cells as well as immune cells. Through these studies we aim to design therapeutic strategies targeting the tumor as a whole by perturbing routes of communication and cooperation between the different cell types present in tumors.

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



Top: A collection of Patient Derived Fibroblasts (PDF) established from tumor biopsies of patients at the MGH. Fibroblasts were isolated from biopsies of a diverse population of non-small cell lung cancer patients. Bottom: PDFs impact the response to Epidermal Growth Factor Tyrosine Kinase Inhibitor (EGFR TKI) through secreted factors: cancer cells sensitive to EGFR inhibition are protected by PDFs in co-culture (top) as well as in the presence of culture media conditioned by PDFs (bottom). Cancer cells are labelled red and PDFs green.

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.

Selected Publications:

Amzallag A, Ramaswamy S, **Benes CH**. Statistical assessment and visualization of synergies for large-scale sparse drug combination datasets. *BMC Bioinformatics.* 2019 Feb 18;20(1):83.

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Kodack DP, Farago AF, Dastur A, Held MA, Dardaei L, Friboulet L, von Flotow F, ... **Benes CH**. Primary Patient- Derived Cancer Cells and Their Potential for Personalized Cancer Patient Care. *Cell Rep.* 2017 Dec 12;21(11):3298-3309.

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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 or not—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.

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 genome-wide views of chromatin structure, chromatin regulator binding and genome organization at single cell and single molecule resolution. Integrative analysis of such chromatin state maps yields detailed annotations of the locations and dynamics of functional elements in the human

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).

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:

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Puram SV, Tirosh I, Parikh AS, Patel AP, Yizhak K, Gillespie S, Rodman C, Luo CL, Mroz EA, Emerick KS, Deschler DG, Varvares MA, Mylvaganam R, Rozenblatt-Rosen O, Rocco JW, Faquin WC, Lin DT, Regev A, **Bernstein BE**. Single-Cell Transcriptomic Analysis of Primary and Metastatic Tumor Ecosystems in Head and Neck Cancer. *Cell*. 2017 Dec 14;171(7):1611-1624.e24.

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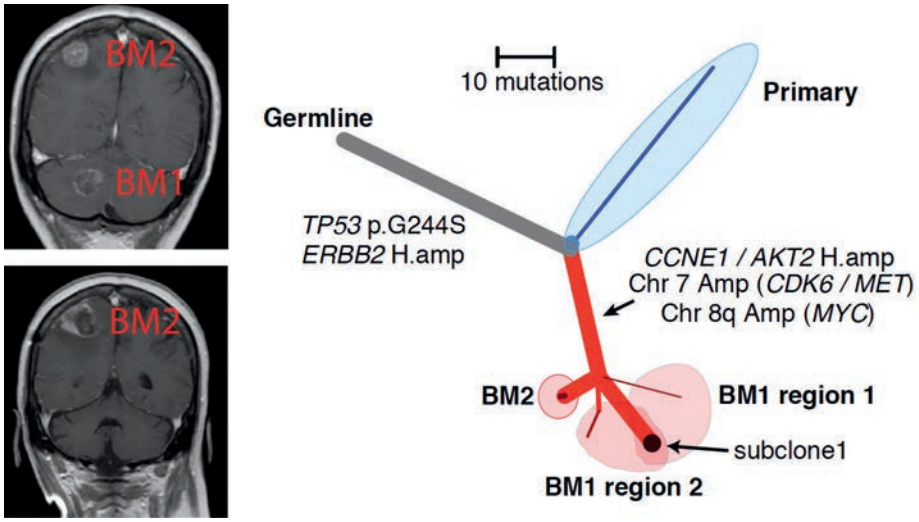
The Brastianos laboratory studies genomic drivers of human brain tumors. A lack of understanding of the molecular drivers of many brain tumors has hampered the development of novel therapies for many brain cancers. Our overarching objective is to characterize molecular drivers of both progression in primary brain tumors and brain metastases, and accelerate the development of novel therapeutic approaches for these diseases. We recently discovered clinically significant genetic drivers in meningiomas, craniopharyngiomas, hemangioblastomas, glioneuronal tumors and brain metastases. We are currently investigating the role of these genomic drivers as potential therapeutic targets in several national NCI-sponsored multi-center clinical trials. Additionally, we are expanding our in vitro and in vivo investigations to further elucidate the molecular evolution of the metastatic process to the central nervous system.

Characterizing Genomic Drivers of Craniopharyngiomas

Craniopharyngiomas are epithelial tumors that arise in the pituitary stalk along the path of the craniopharyngeal duct. There are two main subtypes of craniopharyngiomas, the adamantinomatous form that is more common in children, and the papillary form that predominantly occurs in adults. Craniopharyngiomas can cause profound clinical sequelae both through mass effect at presentation and through morbidity of treatment. No effective treatment besides surgery and radiation is known for craniopharyngiomas, and incomplete knowledge of the molecular mechanisms that drive craniopharyngiomas has limited the development of targeted therapies for this tumor. We recently comprehensively characterized the molecular drivers of craniopharyngiomas. We identified activating mutations in CTNNB1 in nearly all adamantinomatous craniopharyngiomas and recurrent mutations in BRAF (resulting in p.Val600Glu) in nearly all papillary craniopharyngiomas (Brastianos et al. *Nature Genetics* 2014). These findings have important implications for the diagnosis and treatment of these neoplasms. We recently treated a patient with multiple recurrent papillary craniopharyngioma with a BRAF and MEK inhibitor and achieved an exceptional therapeutic response. We have initiated a national multicenter trial in craniopharyngiomas (Alliance A071601) to investigate the role of targeted therapies in these tumors. Circulating biomarkers and genomic analysis of craniopharyngiomas will be employed to investigate mechanisms of resistance.

Identifying Molecular Drivers of Meningiomas

Meningiomas are the most common primary nervous system tumor with no known effective systemic therapy. Recently, we comprehensively characterized meningiomas. Through whole-genome, whole-exome and targeted sequencing, we have demonstrated that meningiomas harbor recurrent oncogenic clinically actionable mutations in AKT1 (E17K) and SMO (W535L) (Brastianos et al. *Nature Genetics* 2013). Notably, these mutations were present



Representative phylogenetic tree of a primary tumor and 2 anatomically distinct brain metastases. Different regions of the brain metastases shared the same amplifications in CCNE1, AKT2, CDK6, MET and MYC, which were not present in the primary tumor biopsy.

in therapeutically challenging tumors of the skull base. We also recently identified potential genetics drivers of progression in meningiomas (BAP1, TERT promoter mutations, DMD). Because therapeutic targets for SMO and AKT1 mutations are currently in clinical use in other cancers, we are now conducting a prospective national multicenter Phase 2 study (A071401) of targeted therapy in patients with recurrent or progressive meningiomas harboring clinically actionable mutations, respectively. The trial is activated at more than 400 sites throughout the US. We will be genomically characterizing prospectively collected samples to identify biomarkers of response and mechanisms of resistance.

Central Nervous System Metastasis Program

Brain metastases are a common complication of cancer, with a dismal prognosis. There is a limited understanding of the oncogenic alterations harbored by brain metastases and whether these are shared with their primary tumors or other metastatic sites. The objectives of the Central Nervous System Metastasis Program are to (1) identify novel therapeutic targets through comprehensive genomic characterization, (2) functionally characterize candidate drivers through in vitro and in vivo models of metastasis, and (3) accelerate the application of our scientific findings to the clinical setting. In collaboration with many national and international institutions, currently we are comprehensively characterizing the genomics of brain metastases to understand the molecular pathways that drive these tumors. We have demonstrated that brain metastases harbor clinically actionable drivers not detected in the primary tumors. We are evaluating the roles of these genetic alterations using various assays of metastasis. Based on this work, we have now initiated a national genomically guided brain metastasis trial (A071701). Our hope is that the findings from our genomic and functional investigations will allow us to develop more rational therapeutic approaches for this disease.

Selected Publications:

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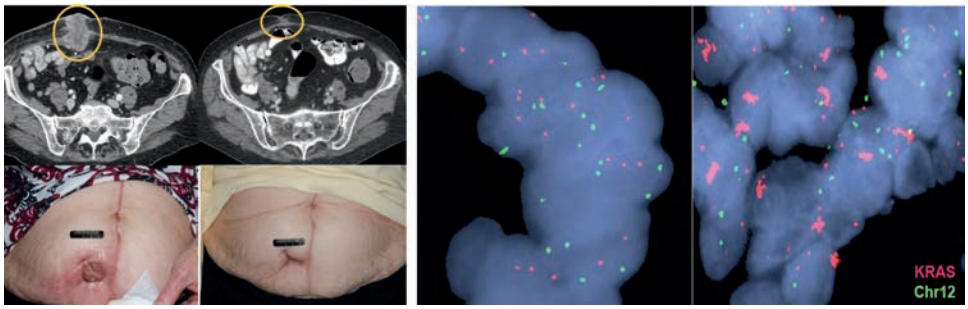
The Corcoran laboratory focuses on developing new and effective therapies for gastrointestinal cancers, including colorectal, pancreatic, stomach, and esophageal cancers, by targeting the specific survival signals that are active in a given patient's cancer. Our research utilizes targeted therapies, 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.

Targeted therapy strategies for gastrointestinal cancers

Historically, the standard clinical approach for patients with advanced cancers has been to treat all patients with the same tumor type with the same generalized chemotherapy strategy. However, even among patients with the same type of tumor, the genetic mutations driving tumor growth in each individual patient can be vastly different. As an alternative approach, by identifying the key gene mutations present in an individual patient's tumor, we can "personalize" therapy by matching each patient with specific therapies that target those mutations essential for tumor growth. Our laboratory focuses on developing targeted therapy strategies directed against specific mutations commonly found in gastrointestinal cancers, including cancers with BRAF and KRAS mutations. However, while targeted therapy strategies can lead to dramatic tumor responses, clinical benefit is often limited by the ability of tumor cells to evolve and develop resistance to therapy. By identifying and understanding the key signals driving resistance, our laboratory aims 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. While BRAF inhibitors have shown dramatic anti-tumor activity in melanomas harboring BRAF mutations, these agents are 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 found that reactivation of the MAPK signaling pathway (often mediated through EGFR), contributes to the relative insensitivity of BRAF mutant colorectal cancers to BRAF inhibition. However, we found that combining BRAF inhibitors with EGFR and/or MEK inhibitors can overcome resistance, leading to improved efficacy (*Cancer Discovery*, 2012). We have also identified multiple mechanisms of resistance that can arise to these newer BRAF inhibitor combinations, and are utilizing this information to develop therapeutic strategies to surmount resistance (*Cancer Discovery*, 2015; *Cancer Discovery*, 2018).



Pre-treatment Week 16 Pre-treatment Post-progression

Response and resistance in BRAF-mutant colorectal cancer. (Left) Example of a dramatic tumor response in a patient treated with the combination of a BRAF and a MEK inhibitor. (Right) KRAS amplification (red probes) can lead to BRAF inhibitor resistance in BRAF mutant colorectal cancer patients.

KRAS-mutant cancers

KRAS is the most commonly mutated oncogene in human cancer, mutated in ~20% of all cancers, including pancreatic (~90%) and colorectal cancers (~40%). 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 through 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. We have also identified adaptive feedback signals that impede the ability of MEK inhibitors to suppress MAPK signaling. We have expanded these approaches to identify other potentially effective targets 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 to bringing new therapeutic strategies into the clinic for evaluation in novel clinical trials. Based on our observations, we have launched several clinical trials of BRAF inhibitor combinations in BRAF-mutant colorectal cancers that are showing increased efficacy (*J Clinical Oncology*, 2015). We have also developed a clinical trial combining the BCL-XL/BCL-2 inhibitor navitoclax with the MEK inhibitor trametinib in KRAS-mutant cancers.

To guide our laboratory investigations, we are utilizing key clinical specimens, including tumor biopsies and patient-derived tumor models to understand how tumors become resistant to therapy. We also utilize serial blood collections for circulating tumor DNA analysis to monitor the tumor heterogeneity and clonal dynamics associated with the emergence of therapeutic resistance (*Cancer Discovery* 2015, *Nature Medicine* 2015, *Cancer Discovery* 2016, *Cancer Discovery* 2017, *Cancer Discovery* 2018.)

Selected Publications:

Parikh AR*, Leshchiner I*, Elagina L*, Goyal L, Levovitz C, Siravegna G , Livitz D, Rhrissorrakrai K, Martin EE, Van Seventer EE, Hanna M, Slowik K, Utro F, Pinto CJ, Wong A, Danysh BP, Fece de la Cruz F, Fetter IJ, Nadres B, Shahzade HA, Allen JN, Blaszkowsky LS, Clark JW, Giantonio B, Murphy JE, Nipp RD, Roeland E, Ryan DP, Weekes CD, Kwak EL, Faris JE, Wo JY, Aguet F, Dey-Guha I, Hazar-Rethinam M, Dias-Santagata D, Ting DT, Zhu AX , Hong TS, Golub TR, Iafrate AJ, Adalsteinsson VA, Bardelli A, Parida L, Juric D, Getz G, **Corcoran RB**. Liquid versus tissue biopsy for detecting acquired resistance and tumor heterogeneity in gastrointestinal cancers. *Nature Medicine*, in press (2019).

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Hazar-Rethinam M, Kleyman M, ... , Iafrate AJ, Van Allen EM, **Corcoran RB**. Convergent Therapeutic Strategies to Overcome the Heterogeneity of Acquired Resistance in BRAFV600E Colorectal Cancer. *Cancer Discovery*. 2018; 8: 417-427.

Strickler JH, Loree JM, Ahronian LG, Parikh AR, Niedzwiecki D, Pereira AAL, McKinney M, Korn WM, Atreya CE, Banks KC, Nagy RJ, Meric-Bernstam F, Lanman RB, Talasaz A, Tsigelny IF, **Corcoran RB**, Kopetz S. Genomic Landscape of Cell-Free DNA in Patients with Colorectal Cancer. *Cancer Discovery*. 2018; 8: 164-173.

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Russo M, Siravegna G, Blaszkowsky LS, ... , Iafrate AJ, Bardelli A, **Corcoran RB**. Tumor heterogeneity and lesion-specific response to targeted therapy in colorectal cancer. *Cancer Discovery*. 2016; 6: 147-53

*Denotes equal contribution

Shawn Demehri, MD, PhD

The focus of **the Demehri laboratory** is to determine the role of the immune system in regulating the early stages of cancer development in order to harness its anti-tumor potential for cancer prevention and treatment. To date, several cancer immunotherapies have been developed with proven efficacy against late-stage cancers; however, the role of the immune system in preventing the early development of cancer remains uncertain. The research in the Demehri laboratory is focused on identifying the immune mechanisms that drive an immune activation sufficient to prevent cancer formation from pre-cancerous lesions. This approach raises a great opportunity to discover novel immune pathways that can be leveraged in cancer therapy and prevention.

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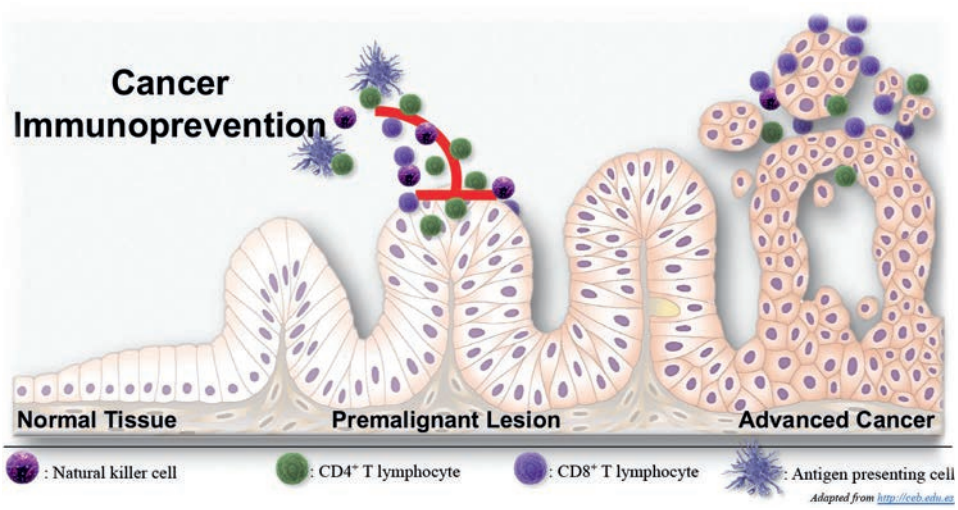
The field of cancer immunology has made substantial advances in recent years by deciphering the role of the tumor infiltrating CD8+ cytotoxic T lymphocytes (CTLs) in attacking cancer cells, which have led to promising new cancer immunotherapeutics. The current immunotherapeutic approaches, however, are largely designed to boost the anti-tumor immune response that has already formed against late-stage metastatic cancers. Therefore, the current cancer immunotherapies like immune checkpoint blockade, which rely on a pre-existing CTL infiltrate in the tumor for their effects, are proven ineffective to treat cancers that frequently lack a significant anti-tumor immune infiltrate, especially during the early in-situ phases of their development. In order to expand the potential of cancer immunotherapy, our laboratory studies the pathways that lead to immune system activation against early phases of cancer development. Devising a mechanism to activate the immune system against early-stage cancers has clear immunopreventive implications by directly blocking the cancer promotion and immunotherapeutic benefits by potentiating the immunity against late disease.

To pursue this goal, the Demehri laboratory is currently focused on three areas of research:

1) Mechanisms of CD4+ T cell activation against cancer. Our laboratory has studied the mechanism of thymic stromal lymphopoietin (TSLP) in evoking tumor suppression. TSLP is an epithelial-derived cytokine that plays a central role in stimulating CD4+ T helper 2 (Th2)-mediated allergic diseases like atopic dermatitis and asthma. We have shown that high TSLP levels establish a dominant anti-tumorigenic immune environment preventing cancer promotion. Currently, our team investigates the detailed mechanism of TSLP anti-tumor function against solid cancers and examines its application for the treatment of pre-cancerous skin and breast lesions in patients.

2) Mechanisms of natural killer (NK) cell recruitment and activation against cancer. NK cells are known for their potent anti-tumor properties. However, their role in controlling the cancer development in vivo remains unclear. Our laboratory is utilizing a virally encoded ligand for NK cells to determine the combination of signals necessary to activate NK cells against early



Immune Regulation of Early Cancer Development.

stages of carcinogenesis and to identify the mechanism of anti-tumor immunity mounted by the activated NK cells in order to block cancer promotion and progression.

3) Mechanisms of tumor promotion by the immune system. Although immune cells can mount anti-tumor immunity against cancer, they are also implicated in promoting cancer development under certain conditions. Chronic inflammation is one of the conditions that can predispose patients to cancer; however, the mechanism of such immune-mediated tumor promotion is unclear. To determine this mechanism, our laboratory studies skin and colorectal cancer development as ideal cancer models in which the spatial and temporal relationship between inflammation and cancer development can be determined with exceptional precision. We are currently investigating the immune mechanisms that promote skin cancer development in the context of chronic allergic contact dermatitis and cutaneous lupus and colorectal cancer development in the context inflammatory bowel disease.

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Rosenberg AR, Tabacchi M, Ngo KH, Wallendorf M, Rosman IS, Cornelius LA, **Demehri S**. Skin cancer precursor immunotherapy for squamous cell carcinoma prevention. *JCI Insight*. 2019;4(6).

Ameri AH, Moradi Tuchayi S, Zaalberg A, Park JH, Ngo KH, Li T, Lopez E, Colonna M, Lee RT, Mino-Kenudson M, **Demehri S**. IL-33/regulatory T cell axis triggers the development of a tumor-promoting immune environment in chronic inflammation. *Proceedings of the National Academy of Sciences of the United States of America*. 2019. Epub 2019/01/31.

Zaalberg A, MoradiTuchayi S, Ameri AH, Ngo KH, Cunningham TJ, Eliane JP, Livneh M, Horn TD, Rosman IS, Musiek A, Anadkat MJ, **Demehri S**. Chronic inflammation promotes skin carcinogenesis in cancer-prone discoid lupus erythematosus. *The Journal of investigative dermatology*. 2018 Jul 17. pii: S0022-202X(18)32328-5.

Cunningham TJ, Tabacchi, M, Eliane JP, Tuchayi SM, Manivasagam S, Mirzaalian H, Turkoz A, Kopan R, Schaffer A, Saavedra AP, Wallendorf M, Cornelius LA, and **Demehri S**. Randomized trial of calcipotriol combined with 5-fluorouracil for skin cancer precursor immunotherapy. *J Clin Invest*. 2017; 127(1): 106-116.

Demehri S, Cunningham TJ, Manivasagam S, Ngo KH, Moradi Tuchayi S, Reddy R, Meyers MA, DeNardo DG, and Yokoyama WM. Thymic stromal lymphopoietin blocks early stages of breast carcinogenesis. *J Clin Invest*. 2016; 126(4): 1458-70.

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Nicholas Dyson, PhD

Dyson Laboratory

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The Dyson laboratory studies the role of the retinoblastoma tumor suppressor (RB). RB is expressed in most cell types and its functions enable cells to stop dividing. RB is inactivated in many types of cancer. We have three main goals: we want to understand the molecular details of how RB acts, we want to know how the inactivation of RB changes the cell, and we are using these insights to target tumor cells.

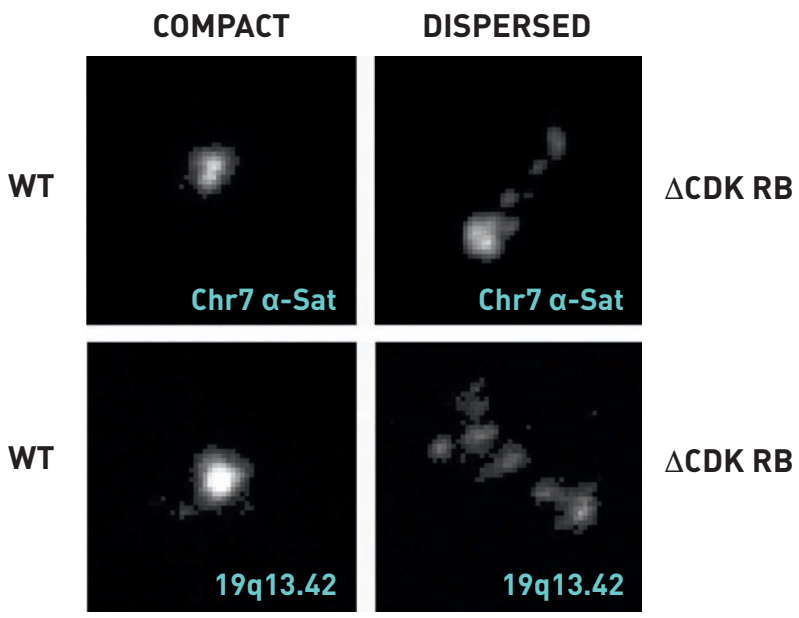
My laboratory investigates mechanisms that limit cell proliferation in normal cells and the ways that these controls are eroded in cancer cells. Our research focuses on RB, the protein product of the retinoblastoma susceptibility gene (*RB1*), and on E2F, a transcription factor regulated by RB. RB/E2F control the expression of a large number of genes that are needed for cell proliferation. This transcription 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 activities but one of its key roles is to limit the transcription of E2F targets. As a result, most tumor cells select for changes that compromise RB function. Our research program spans three areas of RB biology.

Dissecting the molecular functions of RB

RB's precise mechanism of action remains an enigma. RB has been linked to hundreds of proteins and has been implicated in many cellular processes. However, purification of endogenous RB complexes has been a major challenge and, consequently, it is uncertain which proteins physically interact with RB in any specific context. We solved this problem and, in collaboration with the Haas lab, have used Mass Spectrometry to take detailed snapshots of RB in action. We used this approach to test the hypothesis that RB's activity is tailored by mono-phosphorylation. Our data shows that the various mono-phosphorylated forms of RB interact with different cellular proteins, regulate different sets of genes and have distinct functional properties (Sanidas et al 2019).

Active RB alters the organization of chromosomal domains

ChIP-seq experiments revealed that RB does not simply act at a few cell cycle-regulated promoters but targets thousands of sites that are distributed in euchromatin and heterochromatin. We have taken advantage of Oligopaint/FISH technology to visualize the impact of active RB on the nuclear organization of relatively large chromosomal regions (1-2 MB) that contain RB binding sites but lack canonical E2F-regulated, cell cycle genes. Induced expression of ΔCDK-RB (an active mutant protein that is impervious to CDK regulation) caused major changes in the organization of four different regions. Changes were quantified in both euchromatin and heterochromatin, but were most obvious with heterochromatic probes that typically gave a tight focal signal in cycling or quiescent cells. Following ΔCDK-RB expression these focal signals became diffuse, dispersed and scattered into multiple punctas (see Figure). Similar changes occurred following long-term palbocyclib treatment and in IMR-90 cells induced to enter senescence. These changes were time-dependent, and wash-out experiments suggest that they correlate with irreversible cell cycle exit. Interestingly, analysis of a



Consistent with the idea that RB is a master regulator of cell proliferation and that its activity leads to major changes in transcription, the expression of active RB (ΔCDK-RB) leads to changes in the organization of large chromosomal domains. ΔCDK-RB was induced in RPE1 cells, a non-transformed cell line, and the organization of a 4MB heterochromatic region (α-satellite) of chromosome 7 and 2.3MB euchromatic region of chromosome 19 was detected by FISH.

panel of mono-phosphorylation RB mutants revealed that some RB forms strongly induce these changes in G1-arrested cells while others do not, even though all repress E2F-dependent transcription. We infer that unphosphorylated RB does not simply suppress E2F-dependent transcription but drives changes in the nuclear organization of large chromosomal regions.

Targeting tumor cells with RB1 mutations

Our long-term goal is to use information gleaned from molecular studies to improve cancer treatment. RB is functionally compromised in most types of cancer, but the specific mutation of the *RB1* gene is a hallmark of just three tumor types (retinoblastoma, osteosarcoma and small cell lung cancer (SCLC)). This implies that the complete elimination of RB function is especially important in these tumors. Together with Dr. Anna Farago, our clinical collaborator, and with help from members of the Haber/ Maheswaran laboratories we have generated an extensive panel of patient derived xenograft (PDX) models of SCLC. These PDX models accurately reflect the genomic features and the drug sensitivities of the tumors from which they were derived (Drapkin et al 2018). We are using this panel of models to compare the effectiveness of different therapies, and to understand which SCLC tumors will respond best to each type of treatment (Farago et al 2019).

Selected Publications:

Farago AF, Yeap BY, Stanzione M, Hung YP, Heist RS, Marcoux JP, Zhong J, Rangachari D, Barbie DA, Phat S, Myers DT, Morris R, Kem M, Dubash TD, Kennedy EA, Digumarthy SR, Sequist LV, Hata AN, Mahreswaran S, Haber DA, Lawrence MS, Shaw AT, Mino-Kenudson M, **Dyson NJ**, Drapkin BJ. Combination Olaparib and Temozolomide in relapsed small cell lung cancer. *Cancer Discovery*. 2019; Aug15.

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Andrew Elia, MD, PhD

Elia Laboratory

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In response to DNA damage from environmental or endogenous sources, cells evoke an elaborate signaling network known as the DNA damage response (DDR). This response functions to preserve genomic integrity, which is necessary for normal development and the prevention of cancer. **The Elia laboratory** studies the DNA damage response, with current projects focusing on DDR pathways regulated by ubiquitin-dependent signaling and DDR pathways that promote the stabilization and repair of stalled replication forks. We utilize innovative proteomic and genetic approaches to investigate these processes. Our ultimate goal is to understand how DDR disruption influences cancer progression and can be exploited to target tumors with specific DNA repair defects.

DNA damage response

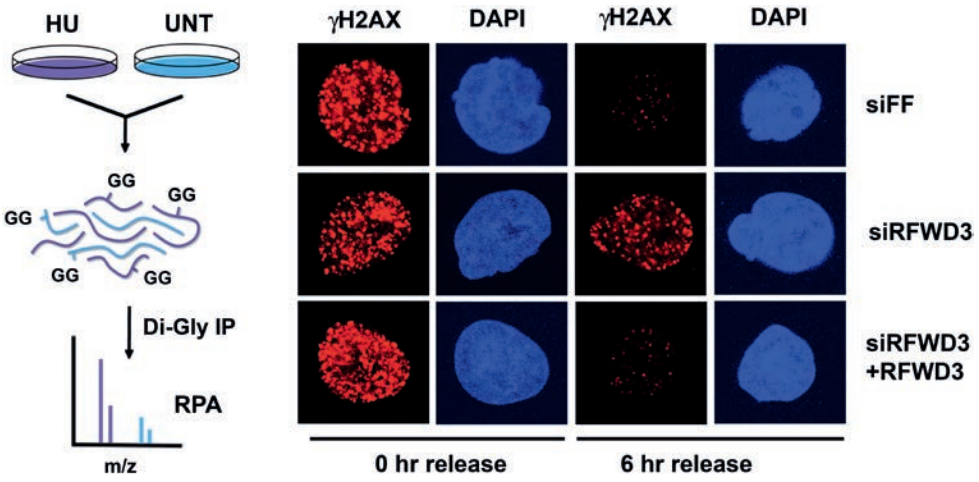
DNA within cells is under continual assault from metabolic and environmental sources. In response to the ensuing damage, cells activate a signaling network called the DNA damage response (DDR). Defects in this response can lead to numerous hereditary cancer syndromes and can underlie the genomic instability which is a hallmark of many sporadic cancers. The DDR promotes genomic integrity by targeting hundreds of factors in diverse pathways ranging from DNA replication and repair to cell-cycle arrest, senescence, and immune regulation. While much is known about these core pathways, the complex regulatory events coordinating them are less well understood. Our lab aims to elucidate biochemical and genetic relationships between DDR factors to understand how they are integrated and collectively regulated.

Quantitative proteomics in ubiquitin signaling

Execution of the DDR relies upon a dynamic array of protein modifications, with phosphorylation playing a historically central role. It is now evident that the DDR also depends on ubiquitin signaling. Numerous ubiquitin ligases have been implicated in the response, yet finding their substrates by simple binding techniques can be difficult due to weak substrate interactions. To circumvent this problem, we have pioneered a quantitative proteomic approach to globally profile ubiquitination. Initially, we used this approach to identify substrates of Cullin-RING ubiquitin ligases (*Cell* 2011), which are involved in numerous DNA repair processes. Subsequently, we used it to uncover novel ubiquitination events directly stimulated by DNA damage (*Mol Cell* 2015a), demonstrating the vast breadth of ubiquitin signaling in the DDR. We are continuing to use innovative proteomic approaches to characterize novel and poorly understood ubiquitin ligases in DNA damage signaling pathways.

Replication stress and cancer

Replication fork collapse can induce chromosome instability and mutagenic events that cause cancer. Organisms have therefore evolved pathways to stabilize stalled replication forks and to repair collapsed forks through processes such as homologous recombination (HR). Multiple



(Left) Quantitative proteomics identifies RPA ubiquitinaton mediated by the ubiquitin ligase RFWD3, which is mutated in the cancer predisposition syndrome Fanconi anemia. (Right) Depletion of RFWD3 inhibits the repair of collapsed replication forks, as demonstrated by delayed resolution of γH2AX foci six hours after release from hydroxyurea-induced replication fork stalling and collapse.

factors involved in HR and replication fork stabilization, such as BRCA1 and BRCA2, are mutated in hereditary cancer syndromes, highlighting the importance of these pathways. We have demonstrated that the ubiquitin ligase RFWD3, which is mutated in the cancer predisposition syndrome Fanconi anemia, ubiquitinates the single-stranded DNA binding protein RPA to promote homologous recombination at stalled replication forks and replication fork restart (*Mol Cell* 2015b). We are currently studying RFWD3 function in the replication stress response and elucidating novel mechanisms of replication fork stabilization and repair.

Targeted cancer therapy

Defects in the DNA damage response can render tumors dependent upon specific DNA repair pathways for survival. Moreover, targeted modulation of the DDR can affect tumor sensitivity to genotoxic chemotherapy and radiation. Increased understanding of DNA repair pathways will lead to enhanced opportunities for developing therapies that target cancers with DNA repair defects, and for improving the efficacy of genotoxic treatments. We are employing methods to translate our work to the development of such therapies.

Selected Publications:

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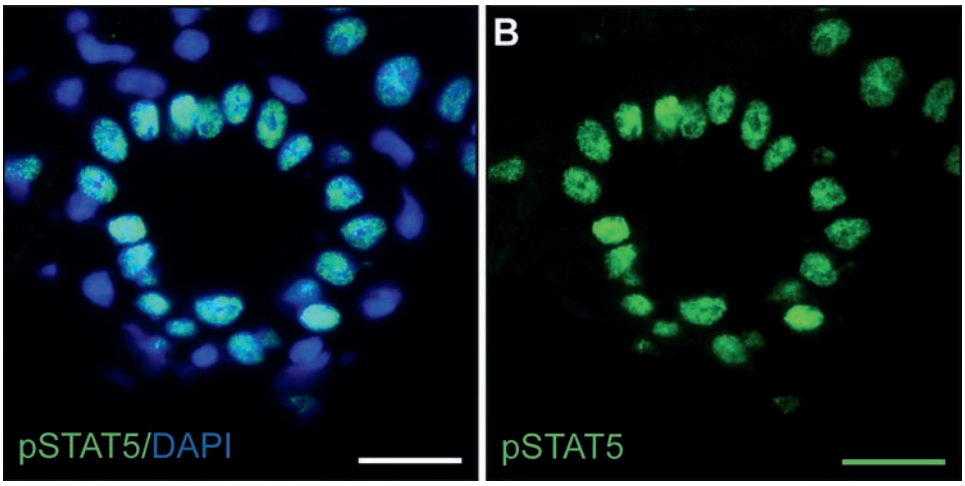
Recent progress in cancer treatment has been made possible through new insights into the key genes and pathways that underlie most malignancies. Understanding how these central players trigger the early, stepwise progression of cancer will be essential to moving beyond incremental steps and toward revolutionary advances in cancer treatment and prevention. **The Ellisen laboratory** is broadly interested in identifying such genetic abnormalities, understanding how they influence the biology of cancer cells, and discovering how 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 molecular analysis of patient tumor samples conducted in partnership with collaborators in the fields of molecular diagnostics and computational biology. Our discoveries in the basic laboratory and through tumor analysis have already been translated to clinical trials that seek to identify new predictive markers, and new prevention and therapeutic strategies for breast and other cancers.

Our group is broadly interested in how genetic abnormalities in breast cancer and related malignancies influence tumor biology, and how that biology can, in turn, be exploited to therapeutic advantage. We address these questions through basic research studies of key cancer drivers including DNA repair defects through BRCA1/2 and related pathways, and transcriptional reprogramming through the p53 gene family. Supporting and complementing these studies are sophisticated analyses of patient-derived precancerous and cancerous tissues. Recent innovative tissue-based studies have led to our discovery of novel cancer drivers, and have provided a unique window on early cancer pathogenesis, intratumoral heterogeneity and tumor progression. Our discoveries in the basic laboratory and through human tumor analysis are being applied in ongoing clinical trials that seek to identify predictive markers of response to specific therapeutics 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 family 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. As a transcription factor and key nodal point for integrating cellular responses to DNA damage, p53 regulates genes involved in diverse cellular processes including cell cycle progression, apoptosis and angiogenesis. Through analysis of two p53-related genes, p63 and p73, we and others have defined a functional network through which these factors interact in human tumorigenesis. We have further defined a tissue-specific role for p63 as the enforcer of an epigenetically-controlled stem/progenitor state. Tumor-selective deregulation of p63 and its associated chromatin remodeling factors



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.

reprograms the transcriptome and thereby promotes proliferation, inhibits differentiation, and contributes to immune evasion. 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. Collectively, this work serves as a paradigm for analysis of transcriptional reprogramming in cancer, while potentially providing new therapeutic possibilities for multiple treatment-refractory malignancies.

BRCA1/2, hereditary cancer predisposition and prevention

Germline mutations in the DNA repair genes BRCA1 and BRCA2 confer dramatically elevated risk of cancers of the breast, ovary, and pancreas, yet the precise pathogenesis of BRCA1/2-associated cancer remains to be elucidated. Together with an international team of collaborators we are carrying out systematic studies of early events that give rise to these cancers, in part through detailed molecular analysis of normal and pre-cancerous tissues from BRCA1/2 mutation carriers. Defining the altered signaling and early cooperating events in this context is likely to reveal new markers of breast cancer predisposition and new targets for prevention. For example, our recently-published single-cell genome analysis has revealed extensive chromosomal damage in BRCA1/2-mutant breast tissues that precedes any histological abnormalities. This seminal finding implies the existence of early cellular defects and associated vulnerabilities that could be exploited for cancer prevention in this setting.

Novel drivers of aggressive breast cancer subtypes

Our recent work employing advanced tumor molecular diagnostics has revealed gene fusions as novel drivers of an aggressive breast cancer subset. In a distinct aggressive breast cancer, triple-negative breast cancer (TNBC), extensive intratumoral heterogeneity is itself a driver that we have characterized through single-cell genomic and transcriptomic analysis. Our longstanding work on the biology of TNBC is supported by the institution-wide Triple-Negative Breast Cancer Program, which integrates basic research, translational and clinical studies together with human tumor propagation and high-throughput drug screening, all focused on overcoming drug resistance and improving outcomes for patients with TNBC.

Selected Publications:

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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 redhair, 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.

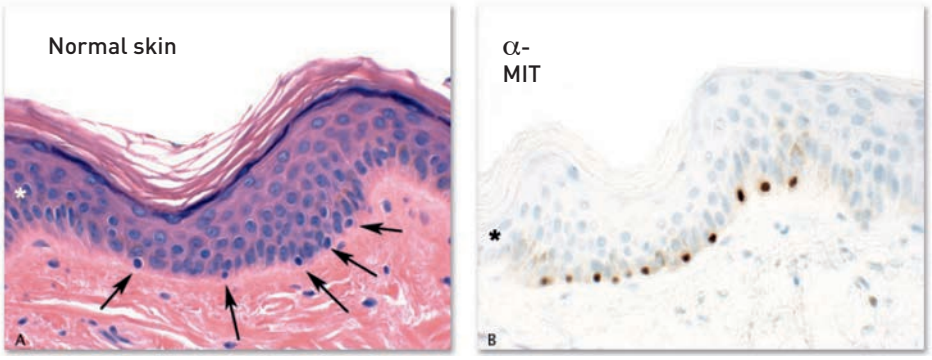
Our group studies cell death/proliferation signals in relation to development and disease, particularly in cancer of pigment cells (melanoma) and tumors of childhood. We attempt to understand critical modes of cell homeostasis with a goal of molecular targeted therapy as well as prevention of melanoma 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, and clinical analyses of novel melanoma treatments. We also study the role of UV in pigmentation responses and carcinogenesis.

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



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.

preclinical 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.

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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 cancer (starting from normal cells and progressing to premalignancy, primary cancer, and emergence of resistance), comparing events at the DNA, RNA, and protein levels between one or more tumor and normal 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 drive cancer progression, drive resistance, or increase its risk as well as identifying molecular subtypes of the disease, their markers, and relationship to clinical variables. Recently, the Getz lab is also studying the tumor and its immune microenvironment using both bulk and single-cell RNA-sequencing (RNA-seq) data. In addition to developing tools for high throughput analysis of cancer data and experimentally testing the findings, the Getz lab develops computer platforms that enable large-scale analytics and visualization.

Characterizing the Cancer Genome

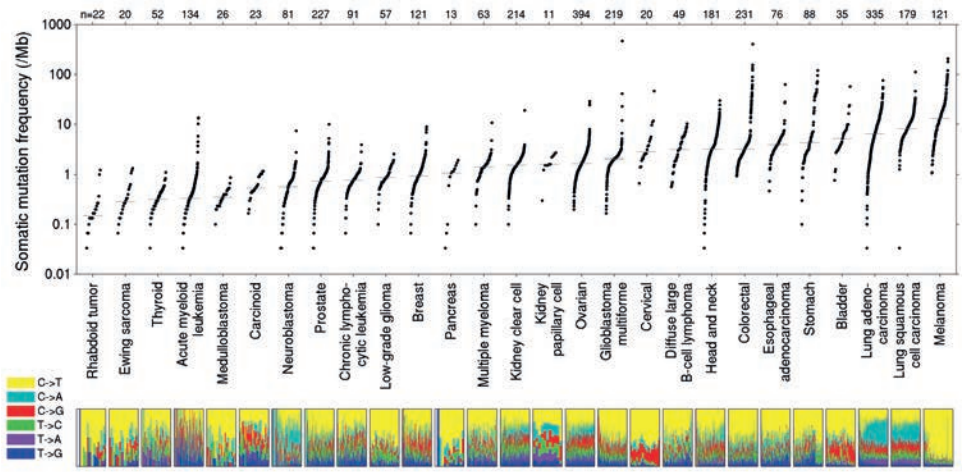
Cancer is a disease of the genome driven by a combination of possible germline risk-alleles, together with a few 'driver' somatic mutations that increase fitness and promote clonal expansion. Mutations occur at all levels and scales, including (i) DNA point mutations; (ii) small insertions and deletions; (iii) larger genomic rearrangements and copy-number alterations; and (iv) epigenetic, transcriptional, and proteomic changes. To generate a comprehensive list of all germline and somatic events that occurred during (and prior to) cancer development, we are developing and applying highly sensitive and specific tools to detect these events in sequencing data. The complexity of the underlying cancer genomes requires state-of-the-art statistical and machine learning approaches to most efficiently extract the signal from the noise.

Detecting Cancer-Associated Genes

After detecting genomic events, we search for genes (and pathways) that show significant signals of positive selection (e.g., the number of mutations exceeds what is expected by chance) across a cohort of samples by constructing a detailed statistical model of the background mutational processes and detecting genes that deviate from it. We developed tools to discover genes significantly gained or lost (GISTIC), and genes with increased density or irregular mutational patterns (MutSig, CLUMPS). In these analyses, correctly modeling the heterogeneity of mutational processes across patients, sequence contexts, and the genome is critical. We are constantly improving methods and working towards a unified method for all types of alterations. We also discovered drivers in non-coding regions of the genome in breast cancer (e.g., hotspot mutations in FOXA1 promoter that likely alter its expression) and, more recently, across cancer, as part of a large international effort.

Heterogeneity and Clonal Evolution of Cancer

Cancer samples are heterogeneous: non-cancer cells intermingle with a cancer cell



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).

population that typically contains multiple subclones. Since cancer is a dynamic system, these subclones may represent (i) remaining cells of less-fit clones not yet overtaken by the expanding the most-fit clone, (ii) interacting subclones that co-evolved and have reached an equilibrium, or (iii) a combination of both. We have developed tools (ABSOLUTE, PhylogicNDT) to characterize the heterogeneity and dynamics of cancer using copy-number, mutational, and other data measured on bulk samples and single cells. These tools can analyze multiple samples per patient to infer clonality of mutations, number of subclones, and subclonal evolution over time or space. We previously demonstrated that subclonal driver mutations are associated with outcome, emphasizing the importance of including clonal information in clinical trials. By analyzing RNA-seq, we recently showed that most healthy adult tissues contain genetic clones with somatic mutations, some in known cancer-associated genes.

Mutational Processes

Processes that damage, repair, replicate, and deliberately alter DNA create mutations. Mutation data can thus be used to study these processes, understand their mutational "signatures," infer their molecular mechanisms, and identify alterations associated with their activity. By studying asymmetries in mutational processes, we detected a mechanism that acts on the lagging DNA strand during replication and a new mutational process that generates mutations on the non-transcribed strand. We also used the association between a mutational signature and homologous recombination (HR) defects to show that epigenetic silencing of RAD51C within the HR pathway is an important mechanism for HR deficiency in breast cancer. With international collaborators, we are mapping all common mutational signatures affecting single- and di-nucleotide substitutions as well as small insertions and deletions (indels). We also study indels that occur at microsatellites and, in particular, tumors that have microsatellite instability (MSI) that may benefit from immune checkpoint inhibitor treatment (e.g., anti-PD1). We are developing a method to computationally detect the presence of MSI tumors from cell-free DNA (cfDNA) containing DNA shed from tumor cells, easily obtained from non-invasive blood biopsies.

Selected Publications:

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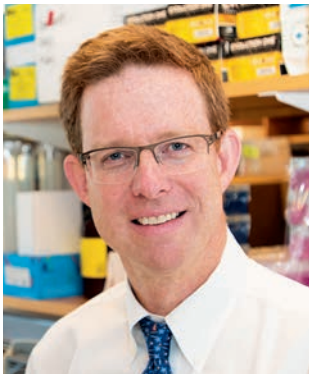

Graubert Laboratory

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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.

Clonal heterogeneity of myelodysplastic syndromes

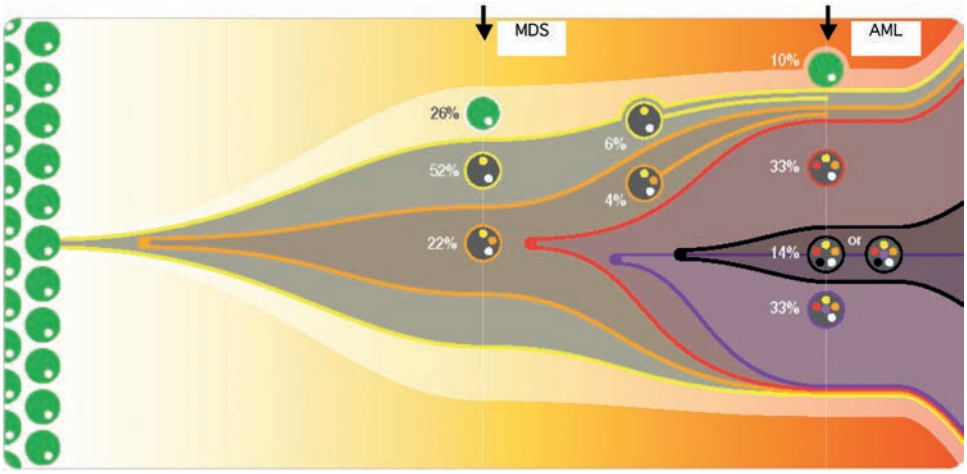
Myelodysplastic syndromes are the most common form of acquired bone marrow failure in adults. Despite the ineffective hematopoiesis that is characteristic of this disease in its early stages, we found through whole genome sequencing that nearly all cells in the bone marrow of these patients are clonally 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, the functional consequences of these mutations for blood cell development, and vulnerabilities created by splicing gene mutations that provide opportunities for novel therapies.

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, Mendelian disorders. Three



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.

genes (*RUNX1*, *GATA2*, *CEBPA*) explain fewer than half of these Mendelian cases. The genetic basis in the majority of families 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:

Nguyen HD, Zou L, and **Graubert TA**. Targeting R-loop Associated ATR Response in Myelodysplastic Syndrome. *Oncotarget*. 2019 Apr 5; 10(27):2581-2582.

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Shirai CL, White BS, Tripathi M, Tapia R, Ley JN, Ndonwi M, Kim S, Shao J, Carver A, Saez B, Fulton RS, Fronick C, O'Laughlin M, Lagisetti C, Webb TR, **Graubert TA**, Walter MJ. Mutant U2AF1-Expressing Cells Are Sensitive to Pharmacological Modulation of the Spliceosome. *Nat. Communications*, 2017 Jan 9, 8:14060.

Wilhelm Haas, PhD

Haas Laboratory

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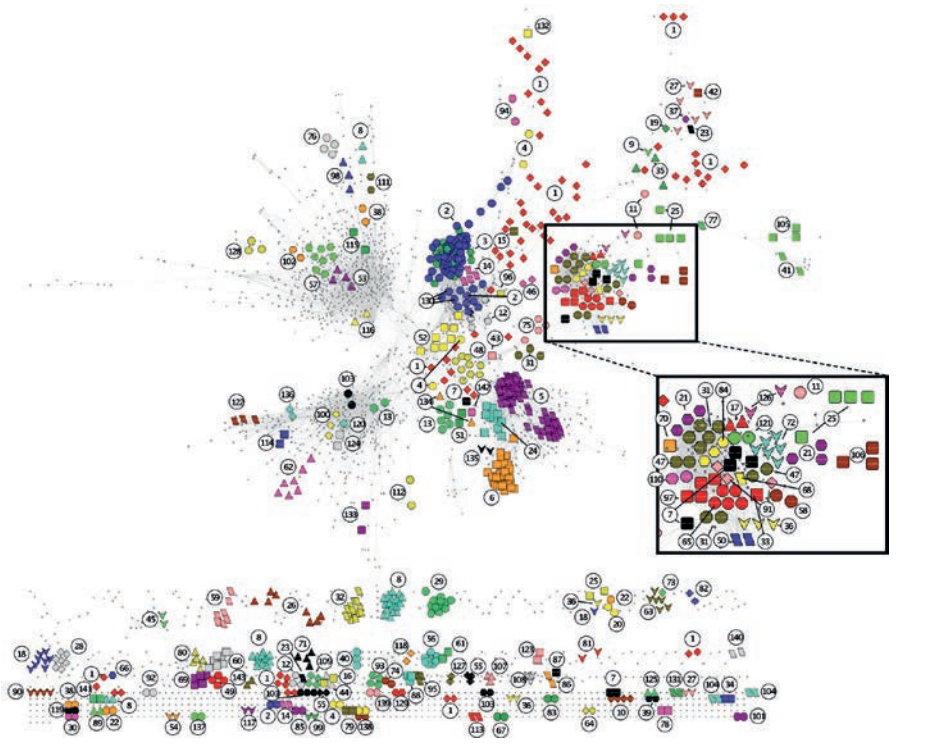
The Haas laboratory uses quantitative mass spectrometry-based proteomics to characterize cancer cells and their vulnerabilities in a comprehensive proteome-wide manner. This is fueled by recent discoveries that have enhanced the depth and throughput of proteomics in quantifying proteins and their post-translational 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. We are specifically interested in mapping changes in the global landscape of protein-protein interactions - the interactome - that occur in cancer cells, and we have shown that dysregulations in the interactome are enabling the prediction of cancer vulnerabilities. We believe that our proteomics technologies have the potential to become a powerful tool in basic and clinical cancer research and may be used to diagnose cancer, predict its susceptibility, and monitor its progression.

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 number of proteins expressed in mammalian cells. Yet, the proteome holds enormous potential to improve our understanding of the basic principles underlying cancer to revolutionize the 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 and the interactome – the global map of protein-protein interactions – has the potential to help us refine our approaches to drug design.

The core technology used in our research group is high-throughput quantitative proteomics enabled through multiplexed mass spectrometry. This technology allows us to map the proteome of a cancer cell line or tumor tissue at high throughput. Analyzing the proteome maps across a panel of cancer cell lines, we recently made the observation that the concentration of proteins in known complexes are accurately correlated across all analyzed cell lines. We showed that protein co-regulation analysis allows the genome-wide mapping of protein-protein interactions with an accuracy ten-times larger than when using co-expression



A Map of Protein-Protein Interactions Identified Using the IMAHP Technology Based on Protein Concentration Co-Regulation across Cancer Cell Lines.

analysis based on RNAseq data. We further found that deviations from co-regulation of two interacting proteins in specific cancer cell lines reflect perturbed cellular circuitry, and it remarkably predicts sensitization to therapeutics targeting regulatory modules in the associated pathway. We have termed this approach to fast, in-depth characterization of protein-protein interaction landscapes the Interactome MApping by High-throughput quantitative Proteome analysis (IMAHP) technology. This novel method has been developed in collaboration with the laboratory of Dr. Cyril Benes at the MGH Cancer Center. It enables an interactome-wide mapping of protein-protein interaction dysregulation and inferred cancer vulnerabilities of any cancer sample based on a proteome map that is acquired at high throughput.

We are further interested in the development and application of high-throughput proteomics methods to globally map protein phosphorylation dynamics in cancer samples and to use the data to specifically identify new kinase targets as cancer vulnerabilities.

Our goals are to apply this technology to (i) identify novel cancer vulnerabilities that direct new treatment strategies, to (ii) map cancer vulnerability dynamics, such as those occurring in the development of therapy resistance, to identify novel targets that enable to overcome the treatment resistance, and to (iii) use our technology in a clinical setting for mapping tumor vulnerabilities to inform treatment strategies in a patient-specific manner.

Selected Publications:

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Xin Hong, PhD
Elad Horwitz, PhD
Maria Kessler**
Laura Libby
Doug Micalizzi, MD
Stefanie Morgan, PhD
Benjamin Nicholson
Brittany Reeves
Joanna Vuille**
Benjamin Wesley
Devon Wiley
Ben Wittner, PhD

* Co-directed with Shyamala Maheswaran, PhD

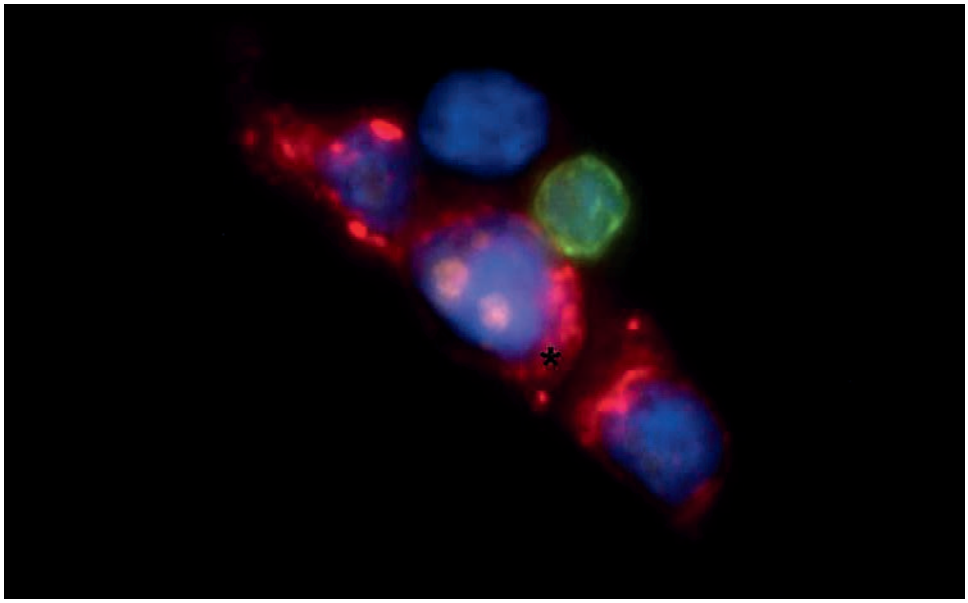
** Graduate students

The Haber laboratory focuses on understanding mutations that are acquired by tumors and render them susceptible to specific targeted drug therapies. In 2004, we identified mutations in the EGFR gene in lung cancers which confer dramatic sensitivity to drugs that specifically inhibit that pathway. This finding triggered the application of targeted therapies in lung cancer, and more generally pointed to the critical importance of mutational analysis for treatment selection in common epithelial cancers. Since then, we have collaborated with the bioengineering team led by Dr. Mehmet Toner, the molecular biology group of Dr. Shyamala Maheswaran, and the MGH Cancer Center clinical disease centers to develop, characterize and apply microfluidic devices to isolate rare circulating tumor cells (CTCs) in the blood of patients with cancer. Using these technologies, our lab seeks to explore 1) blood-based early detection of cancer, 2) noninvasive monitoring of cancer for the emergence of drug resistance, and 3) understanding mechanisms of tumor cell dissemination and metastasis, with the ultimate goal of suppressing blood-borne spread of cancer.

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 early detection of invasive cancer, 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 circumvent mutations that alter drug binding affinity. Following these efforts to monitor the emergence of drug resistance mutations, we established collaborations 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 depletion of antibody-tagged leukocytes, thereby enriching for intact CTCs without selection bias. 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 in lung cancer and measurements of androgen receptor (AR) activity in prostate cancer. We have applied next generation single-molecule RNA sequencing and RNA-in-situ hybridization to characterize the heterogeneous expression profiles of individual CTCs in breast, prostate and pancreatic cancers, as well as melanoma and glioblastoma. To facilitate CTC



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

quantitation and provide the sensitivity and specificity required for early cancer detection, we have established a droplet digital PCR readout for CTC-derived RNA, with promising applications in the early detection of liver cancer.

In addition to noninvasive detecting and monitoring of cancer, CTCs provide a window to study the process of blood-borne metastasis. We demonstrated treatment-associated epithelial-to-mesenchymal transitions (EMT) within CTCs from women with breast cancer. 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. We successfully established long-term *in vitro* cultures of CTCs from patients with estrogen-receptor (ER)-positive breast cancer, identifying treatment-associated mutations in the estrogen receptor (ESR1), as well as acquired mutations in druggable 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. In cultured CTCs from women with advanced ER+ breast cancer, we documented dramatic plasticity, with a proliferative HER2-expressing subpopulation interconverting spontaneously with a drug-resistant Notch1-expressing subset. Using mouse reconstitution models, we demonstrated the consequences of this phenotype switch for both tumorigenesis and drug response. Ongoing studies are directed at using patient-derived CTCs and mouse models to understand key steps in cancer metastasis, including the shift from cell quiescence to proliferation, viability during blood-borne transit, and resistance to targeted and immune therapies.

Selected Publications:

Zheng Y, Comaills V, Burr R, Boulay G, Miyamoto DT, Wittner BS, Emmons E, Sil S, Koulopoulos MW, Broderick KT, Tai E, Rengarajan S, Kukarni AS, Shioda T, Wu CL, Ramaswamy S, Ting DT, Toner M, Rivera MN, Maheswaran S*, **Haber DA***. COX-2 mediates tumor-stromal prolactin signaling to initiate tumorigenesis. *Proc Natl. Acad. Sci USA* 16: 5223-5232, 2019.

Jordan NV, Bardia A, Wittner BS, Benes C, Ligorio M, Zheng Y, Yu M, Sundaresan TK, Licausi JA, Desai R, O'Keefe RM, Ebright RY, Boukhali M, Sil S, Onozato ML, Iafrate AJ, Kapur R, Sgroi D, Ting DT, Toner M, Ramaswamy S, Haas W, Maheswaran S*, **Haber DA***. HER2 expression identifies dynamic functional states within circulating breast cancer cells. *Nature.* 537(7618):102-106, 2016.

Miyamoto DT, Zheng Y, Wittner BS, Lee RJ, Zhu H, Broderick KT, Desai R, Fox DB, Brannigan BW, Trautwein J, Arora KS, Desai N, Dahl DM, Sequist LV, Smith MR, Kapur R, Wu C-L, Shioda T, Ramaswamy S, Ting DT, Toner M, Maheswaran S*, **Haber DA***. RNA-Seq of single prostate CTCs implicates noncanonical Wnt signaling in antiandrogen resistance. *Science.* 349 (6254): 1351-6, 2015.

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.

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The Hacohen laboratory consists of immunologists, geneticists, biochemists, technologists, physicians and computational biologists working together to develop new and unbiased strategies to understand basic immune processes and immune-mediated diseases, with an emphasis on the innate immune system and personalized medicine. We address three key questions in immunology (1) how are immune responses against cancer initiated, maintained and evaded? (2) what are the immune circuits that sense and control pathogens, such as viruses and bacteria? (3) how does immunity against the body develop, in particular, in patients with autoimmune lupus? In addition to discovering and studying specific molecular and cellular mechanisms, we also address how and why the immune response (to tumors, pathogens or self) varies so dramatically across individuals. Finally, we are adapting our unbiased analytical strategies into real-world therapeutics, having initiated clinical trials (with our collaborator Dr. Catherine Wu), in which patients are vaccinated against their own tumors with a fully personal vaccine that is designed based on a computational analysis of their personal tumor genome.

Initiators, resistors and targets of tumor immunity

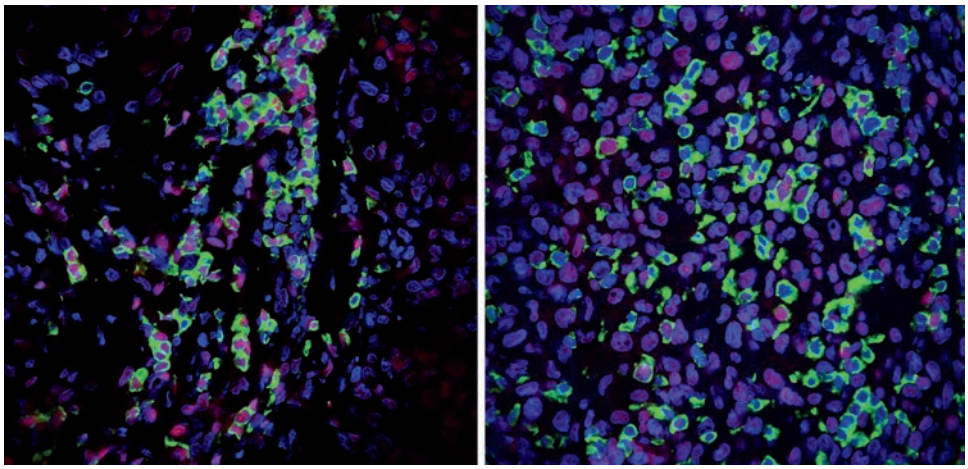
While cancer immunology has been deeply studied in animal models, there remain many open questions in human tumor immunology due to lack of tools to investigate human samples. We have developed genetic and genomics approaches to explain the large variance in anti-tumor immunity across people, and to discover how tumors evolve to resist productive immunity. We've identified somatic mutations in tumors that are associated with anti-tumor immunity in patients (Rooney et al., *Cell* 2015), discovered mutations in β2m in patients resistant to checkpoint therapy (Sade-Feldman et al., *Nat Comm* 2017) and found that TCF7+ T cells are associated with a response to anti-PD-1 immunotherapy in melanoma (Sade-Feldman et al., *Cell* 2019). We have also developed new methods to predict which tumor antigens are presented (Abelin et al., *Immunity* 2017, Sarkizova et al., submitted), which are now being used to develop novel therapeutic approaches and targets for immunotherapy, such as a personal tumor vaccine targeting multiple HLA-associated neoantigens in human tumors (together with Dr. Catherine Wu at DFCI, Ott et al., *Nature* 2017, Keskin *Nature* 2018).

Genes and networks underlying innate immunitys

We've used genome-wide CRISPR libraries to discover mammalian genes mediating the sensing of pathogens (Parnas et al., *Cell* 2015), impacting HIV infection (Park et al., *Nat Gen* 2017) and affecting influenza infection and dendritic cell biology (ongoing projects). We have also characterized innate myeloid cells (DCs and monocytes) in human blood as part of the human Immune Cell Atlas (Villani et al., *Science* 2017).

Genetic basis for inter-individual variations in immune responses

We have also developed genomic strategies



Immunofluorescence staining of T cells found in human melanoma biopsies from a patient who responded (left) and a patient who did not respond (right) to checkpoint anti-PD-1 therapy. Staining: nuclei (blue), CD8 (green) and TCF7 (red).

to analyze human immune responses and explain immune phenotypes with germline genotypes. We characterized the genetic basis for inter-individual variation in the innate immune response to viruses and bacteria (Lee et al., *Science* 2014; Raj et al., *Science* 2014; Ye et al., *Science* 2014). For example, we found that common alleles of IRF7 tune the strength of an individual's anti-viral response, and that genetic control of splicing is prevalent and important for the immune response (Ye et al., *Genome Res* 2018). Building on these studies, we have recently developed and are now using systematic methods to analyze the role of genetic and non-genetic variations in human immunity.

Drivers of autoimmunity

Deficiencies in nucleases that degrade DNA lead to accumulation of self DNA, activation of innate immune responses and development of autoimmune disorders, including systemic lupus erythematosus and Aicardi-Goutières syndrome in humans, and autoimmune arthritis, nephritis and myocarditis in mice. We have been interested in understanding how autoimmunity develops upon triggering of innate immunity by self DNA (rather than pathogen-derived DNA). In studying this question, we made the surprising observation that immunostimulatory DNA can arise from host damaged DNA that is exported from the nucleus to the lysosome (Lan et al., *Cell Rep* 2014). We hypothesize that this cellular process is a source of inflammation in autoimmunity, cancer, chemotherapy and aging (Lan et al., *Aging Cell* 2019). To deepen our understanding of pathways that drive autoimmunity, we have been analyzing immune responses in lupus nephritis patients, with an emphasis on cellular and molecular analysis of kidney biopsies and blood samples from lupus patients (Arazi et al., *Nat Imm* 2019).

Selected Publications:

Arazi A, Rao DA, Berthier CC, Davidson A, Liu Y, Hoover PJ, Chicoine A, Eisenhaure TM, Jonsson AH, Li S, Lieb DJ, Zhang F, Slowikowski K, Browne EP, Noma A, Sutherby D, Steelman S, Smilek DE, Tosta P, Apruzzese W, Massarotti E, Dall'Era M, Park M, Kamen DL, Furie RA, Payan-Schober F, Pendergraft WF 3rd, McInnis EA, Buyon JP, Petri MA, Putterman C, Kalunian KC, Woodle ES, Lederer JA, Hildeman DA, Nusbaum C, Raychaudhuri S, Kretzler M, Anolik JH, Brenner MB, Wofsy D, **Hacohen N**, Diamond B; Accelerating Medicines Partnership in SLE network. The immune cell landscape in kidneys of lupus nephritis patients. *Nature Immunology* 2019. Jul;20(7):902-914.

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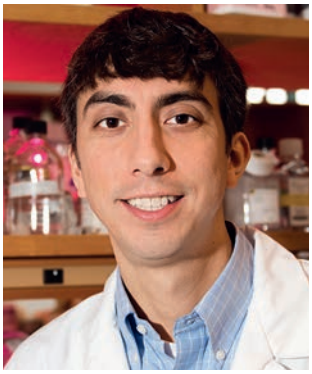
Ott P ... **Hacohen N** & Wu CJ. An immunogenic personal neoantigen vaccine for patients with melanoma. *Nature*. 2017 Jul 13;547(7662):217-221.

Villani A-C, Satija R, Reynolds G, Sheknar K, Fletcher J, Sarkizova S, Griesbeck M, Butler A, Zheng S, Lazo S, Jardine L, Dixon D, Stephenson E, McDonald D, Filby A, Li W, De Jager PL, Rozenblatt-Rosen O, Lane AA, Haniffa M, Regev A, **Hacohen N**. Single-cell RNA-seq reveals new types of human blood dendritic cells, monocytes and progenitors. *Science*. 2017 Apr 21;356(6335).

Lee MN*, Ye C*... **Hacohen N**. Common genetic variants modulate pathogen-sensing responses in human dendritic cells. *Science*. 2014 Mar 7;343(6175):1246980.

*Equal contribution

Aaron Hata MD, PhD



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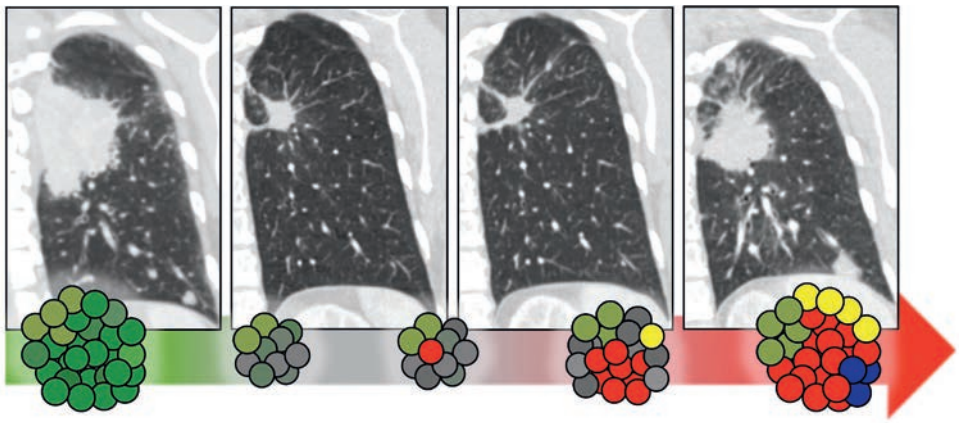
The research goal of **the Hata laboratory** is to advance targeted therapies to benefit patients with lung cancer. Our research focuses on understanding the biological underpinnings of sensitivity and resistance of oncogene-addicted lung cancers (those with activating genetic alterations EGFR, ALK, KRAS, etc.) to small molecule inhibitors of growth and survival signaling pathways. Our studies are highly translational, integrating assessment of clinical specimens with generation and analysis of patient-derived cell culture and mouse tumor xenograft (PDX) models, and are performed in close collaboration with clinicians in the MGH Thoracic Oncology group. This has enabled us to identify a number of promising therapeutic approaches for overcoming mechanisms of intrinsic and acquired drug resistance. More recently, we have begun to focus on understanding how cancer cells adapt and evolve during the course of therapy in order to identify vulnerabilities of persistent drug tolerant cancer cells that can be exploited to prevent resistance from developing. Our ultimate goal is to translate these findings into clinical trials.

Mechanisms of acquired drug resistance to targeted therapies

Lung cancers that harbor activating EGFR mutations and ALK-translocations are exquisitely sensitive to small molecule EGFR and ALK tyrosine kinase inhibitors, respectively. However, even though most patients experience dramatic responses, drug resistance invariably develops leading to disease relapse. Similar patterns of sensitivity and acquired resistance are also observed in other subsets of oncogene-addicted lung cancers treated with molecularly targeted therapies (e.g. ROS1 translocations, RET fusions, BRAF mutations, MET exon 14 skipping mutations). We work closely with oncologists in the MGH Center for Thoracic Cancers to identify and characterize mechanisms of acquired resistance in lung cancer patients treated with targeted therapies. By analyzing tumor biopsies or tumor DNA isolated from blood, we are often able to detect mutations and other genomic alterations that cause drug resistance. We also culture tumor cells from biopsies as cell lines or PDX models in order to functionally interrogate pathways that contribute to drug resistance. These models also allow us to test novel therapies and select the most promising for clinical trials.

Targeting apoptotic regulators to overcome intrinsic resistance to targeted therapies

Despite the success in targeting oncogenic kinases such as EGFR and ALK, effective therapies for KRAS mutant lung cancers have remained elusive to date. The recent discovery of covalent inhibitors of the KRAS G12C oncoprotein have renewed hope that effective targeted therapies for this subset of lung cancer may be within reach. Work by our group and others has suggested that the many KRAS mutant lung cancers may exhibit decreased oncogenic dependency and a dampened apoptotic response that may lead to intrinsic resistance to KRAS targeted therapy. To overcome this limitation, we are exploring the use of BH3 mimetics that



EGFR mutant lung cancers can develop acquired resistance to EGFR inhibitors (e.g. acquisition of the gatekeeper EGFR^{T790M} mutation) by selection of pre-existing EGFR^{T790M} cells, or via evolution of initially EGFR^{T790M}-negative drug tolerant cells that then develop the mutation during the course of treatment. EGFRi denotes EGFR inhibitor treatment, such as gefitinib or erlotinib. Reproduced from Hata and Niederst, et al. Nature Medicine 2016.

inhibit pro-survival BCL-2 family proteins such as MCL-1 and BCL-XL to increase sensitivity to inhibitors of KRAS-driven signaling pathways. In addition, we are focused on understanding how apoptotic dependencies may be shaped by the interplay between primary oncogenic driver and co-occurring genetic alterations in order to rationally deploy BH3 mimetic drug combination strategies in the clinic.

Tumor adaptation and evolution during treatment

The identification of secondary drug resistance mutations in EGFR and ALK patients progressing on first-generation TKIs has led to the development of next-generation TKIs to overcome them. However, acquired resistance develops to these new agents as well. To halt this perpetual cycle of drug resistance, novel strategies designed to alter the evolution of resistance mechanisms are needed. We recently demonstrated that genomic mechanisms of resistance can arise via evolution of drug tolerant clones that survive initial therapy and then acquire a secondary genomic alteration. This suggests that drug tolerant cells that survive initial treatment may comprise a cellular reservoir from which heterogeneous mechanisms of resistance may arise. We have ongoing efforts focused on characterizing persistent tumor cells that survive during drug treatment in both experimental models and patients. By identifying targetable vulnerabilities of these cells, we hope to develop novel therapeutic strategies that will disrupt this perpetual cycle of acquired resistance.

Patient-specific experimental modeling of oncogene addicted lung cancers

To facilitate our studies on drug sensitivity and resistance, we have developed a robust infrastructure for generating patient-derived cell lines and mouse patient-derived xenograft (PDX) models from lung cancer patients treated at the MGH Cancer Center. This effort is enabled by a close collaboration with clinicians in the MGH Center for Thoracic Cancers, Interventional Radiology, Interventional Radiology and Thoracic Surgery, and a team of dedicated research assistants and laboratory technicians. These models have allowed us to identify novel mechanisms of acquired resistance in EGFR and ALK lung cancers and test potential new therapies to overcome them.

Selected Publications:

Raoof S*, Mulford IJ*, Frisco-Cabanos H, Nangia V, Timonina D, Labrot E, Hafeez N, Bilton SJ, Drier Y, Ji F, Greenberg M, Williams A, Katterman K, Damon L, Sovath S, Rakiec DP, Korn JM, Ruddy DA, Benes CH, Hammerman PS, Piotrowska Z, Sequist LV, Niederst MJ, Barretina J, Engelman JA, **Hata AN**. Targeting FGFR overcomes EMT-mediated resistance in EGFR mutant non-small cell lung cancer. *Oncogene*. 2019 Jul 19.

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Piotrowska Z*, Isozaki H*, ... , **Hata AN***, Sequist LV*. Landscape of acquired resistance to osimertinib in EGFR-mutant NSCLC and clinical validation of combined EGFR and RET inhibition with osimertinib and BLU-667 for acquired RET fusion. *Cancer Discovery*. 2018 Dec;8(12):1529.

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Hata AN, ... , Engelman, JA. Tumor cells can follow distinct evolutionary paths to become resistant to epidermal growth factor receptor inhibition. *Nature Medicine*. 2016; 22:262-9

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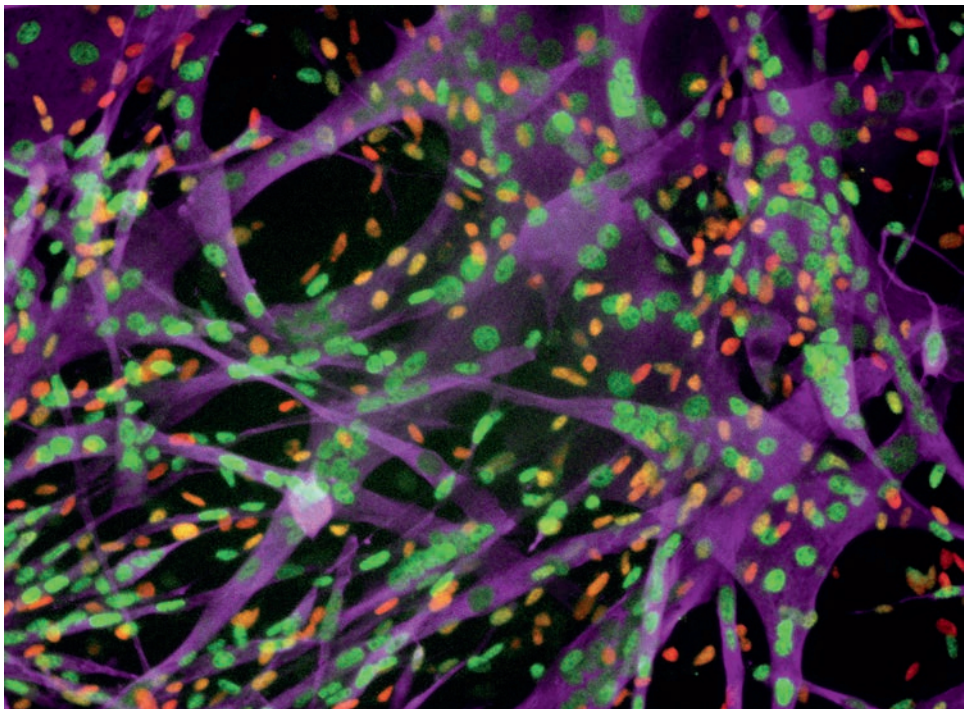
The Hochedlinger laboratory explores the molecular mechanisms underlying pluripotency, which is the ability to produce all mature cell types of the body. Previous groundbreaking discoveries have shown that adult cells can be reprogrammed into pluripotent stem cells by activating a handful of embryonic 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 allowed us to elucidate basic mechanisms that maintain cellular identity and prevent aberrant cell fate change. Our ultimate goal is to utilize these mechanistic insights for the development of new strategies to treat cancer and other complex diseases.

The Hochedlinger lab is studying the mechanisms underlying cell fate transitions by using transcription-factor-mediated conversion of somatic cells into induced pluripotent stem (iPSCs) as a tractable tool. iPSCs are typically derived by viral 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 due to the low efficiency of reprogramming and the heterogeneity of cell cultures. 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 rare intermediate cell populations that are poised to become iPSCs, and we are currently using these populations to understand the transcriptional, epigenetic and proteomic changes in cells undergoing reprogramming. Additionally, our lab has conducted unbiased shRNA screens for barriers to reprogramming, uncovering new mechanisms that safeguard somatic cell identity. For example, we identified components of chromatin assembly (CAF-1), protein sumoylation (SUMO-2, UBC9) and alternative polyadenylation of RNA (NUDT21) as novel safeguard mechanisms and we are currently exploring the underlying mechanisms as well as their role in tissue homeostasis and cancer. More recently, we discovered that MAPK signaling is critical to preserve the epigenetic and genomic stability as well as full the developmental potential of mouse pluripotent stem cells. Mechanistically, we showed that MAPK signaling is critical to fine-tune global DNA methylation levels and maintain genomic imprinting. Importantly, we extended these observations to human cells, allowing us to provide more stable and thus safer embryonic stem cell and iPSC models.

We hypothesized that the manipulation of safeguard mechanisms we previously identified in the context of iPSC reprogramming might endow somatic cells



Induced myogenic progenitor cells (iMPCs) derived from fibroblasts. Immunostaining for markers of muscle stem cells (Pax7, red) and differentiated cells (MyoD, green; MyHC, purple)(see Bar-Nur et al., Stem Cell Reports 2018 May 8;10(5):1505-1521).

Image: Ori Bar-Nur, PhD

with increased plasticity and could facilitate the derivation of adult stem cell types that have been difficult to capture using conventional approaches. Indeed, we recently provided proof-of-principle evidence for this idea by showing that pharmacological inhibition of defined safeguard mechanisms, together with overexpression of the muscle-specific transcription factor MYOD, reprograms fibroblasts to muscle stem cell-like cells. The reprogrammed cells share key molecular and functional characteristics with *bona fide* muscle stem cells including dependence on PAX7, self-renewal, differentiation and the ability to engraft in the muscles from a dystrophic mouse model. Ongoing efforts include dissection of the underlying mechanisms and an attempt to recapitulate these findings in human cells.

Selected Publications:

Di Stefano B, Ueda M, Sabri S, Brumbaugh J, Huebner AJ, Sahakyan A, Clement K, Clowers KJ, Erickson AR, Shioda K, Gygi SP, Gu H, Shioda T, Meissner A, Takashima Y, Plath K, **Hochedlinger K**. Reduced MEK inhibition preserves genomic stability in naive human embryonic stem cells. *Nat Methods*. 2018 Aug 20.

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Choi J, Huebner AJ, Clement C, Walsh RM, Savol A, Lin K, Gu H, Di Stefano B, Brumbaugh J, Kim SY, Sharif J, Rose CM, Mohammad A, Odajima J, Charron J, Shioda T, Gnirke A, Gygi SP, Koseki H, Sadreyev R, Xiao A, Meissner A & **Hochedlinger K**. Prolonged Mek1/2 suppression impairs the developmental potential of embryonic stem cells. *Nature*. 2017 Aug 10;548(7666):219-223.

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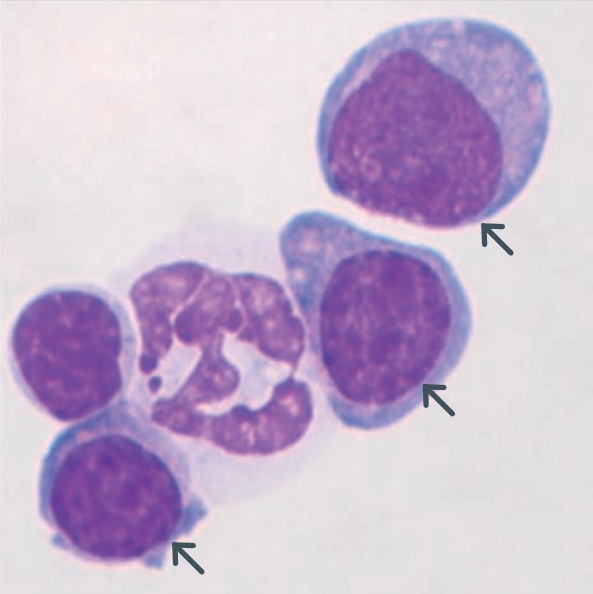
The Hock laboratory explores the molecular basis of blood cell formation and the pathogenesis of leukemia and lymphoma. Specifically, we study the transcription factors that regulate gene activity during normal blood cell development and how the transcriptional apparatus goes awry in cancer. For example, 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.

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



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 a novel experimental model generated in the lab.

Deciphering the molecular events leading to acute lymphoblastic leukemia of childhood

About one in 2000 children develops 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. We are 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.* 2014; 211, 909–927.

Qin J, Whyte WA, Anderssen E, Apostolou E, Chen H, Akbarian S, Bronson RT, Hochedlinger K, Ramaswamy S, Young RA, and **Hock H**. The Polycomb Group Protein L3mbtl2 Assembles an Atypical PRC1-family Complex with Essential Roles in Pluripotent Stem Cells and Early Development. *Cell Stem Cell*. 2012; 11, 319-332, 2012.

Hock H. A complex Polycomb issue: the two faces of EZH2 in cancer. *Genes Dev*. 2012; 26, 751-755.

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Schindler JW, Van Buren D, Foudi A, Krejci O, Qin J, Orkin SH, **Hock H**. TEL-AML1 corrupts hematopoietic stem cells to persist in the bone marrow and initiate leukemia. *Cell Stem Cell*. 2009; Jul 2, 5(1):43-53.

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*. 2009; Sep, 41(9):968-76.

Jonathan G. Hoggatt, PhD

The Hoggatt laboratory is broadly interested in the stem cell niche regulatory mechanisms that govern tissue regeneration, particularly regulation by macrophages, and we have a specific interest in translational science for bone marrow transplantation and other treatments. Our laboratory identified a unique "highly engraftable" hematopoietic stem cell that we are currently investigating which has applications for further probing of stem cell niche biology, and clinical applications in transplantation, gene therapy, and other fields. We have also developed unique genetic mouse models allowing us to dynamically explore macrophage heterogeneity in a variety of disease settings.

Hoggatt Laboratory

Bin-Kuan Chou, PhD
Sana Shareef
Jonathan G. Hoggatt, PhD

Macrophage Regulation of Tissue Regeneration

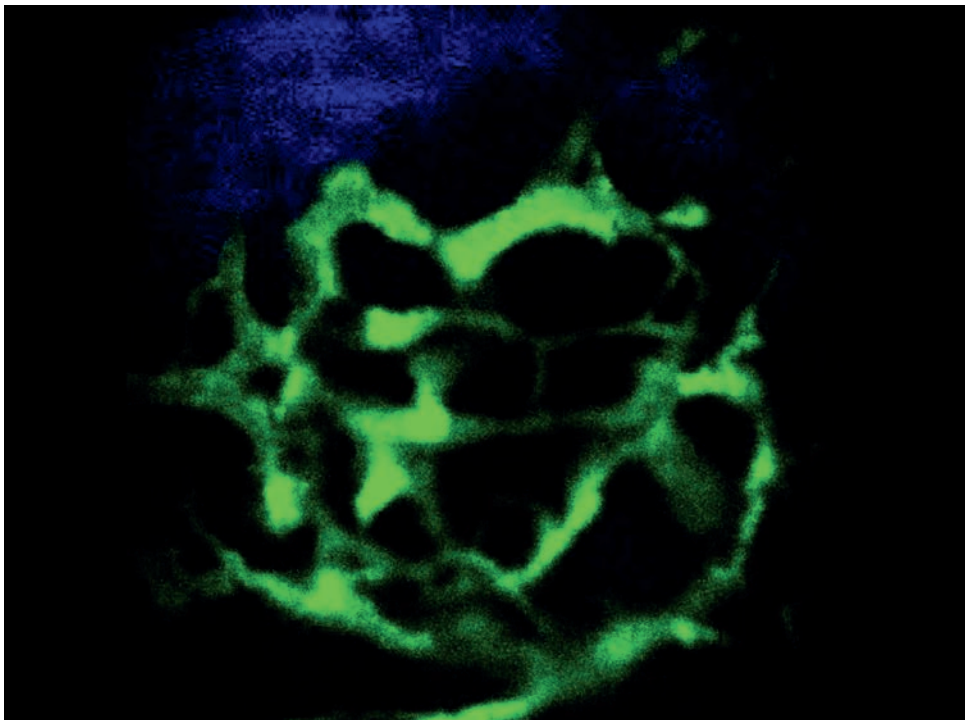
Macrophages are ancient cells of the innate and adaptive immune system. My old microbiology textbook defines macrophages as "scavengers and sentries – routinely phagocytizing dead cells and debris, but always on the lookout, ready to destroy invaders, and able to call in reinforcements when needed." Our laboratory believes they are so much more.

Tissue resident macrophage populations exist in virtually every tissue, whether they are Kupffer cells in the liver, alveolar macrophages in the lung, microglia in the brain or Langerhans cells in the skin. Some of these macrophages have been recently reported to specify hepatic progenitor cell fate, regulate epithelial progenitor niches in the colon and drive oligodendrocyte differentiation during remyelination in the central nervous system. After depletion of macrophages, an adult salamander is unable to regenerate an amputated limb. However, when macrophage levels were allowed to replenish, full limb regeneration capacity of failed stumps was restored upon re-amputation. Macrophages, therefore, may be a common cellular regulator across a diverse repertoire of stem cell niches. The problem that exists today is that macrophages are extraordinarily diverse and plastic, necessitating the need to identify specific subsets responsible for stem cell and tissue regeneration, in both homeostatic and disease scenarios.

We have created a unique mouse model that allows tracking of macrophages with deferring embryonic origins with specific, genetic-fluorescent markers, aiding in de-convoluting this heterogeneous cell population. Our laboratory is exploring several clinically relevant applications for stem cell transplantation, and will broadly use these macrophage tools and knowledge to delineate macrophage regenerative signals in multiple tissue stem cell niches, organ transplantation, and disease.

Hematopoietic Stem Cell Biology

Hematopoietic stem cell (HSC) transplantation is used to treat a number of malignant and non-malignant diseases. Over the last decade, there has been increasing evidence that the HSC pool is heterogeneous in function; with identification of HSCs with differing lineage outputs, kinetics of repopulation, length of life-span, and perhaps differences amongst HSCs contributing to homeostatic blood production from those that are the engraftable units in



Shown are sinusoidal vessels (green) within the calvaria bone of mice during live, in vivo imaging of the hematopoietic stem cell niche.

transplantation. Delineating the mechanisms of these functional differences has the potential to increase the efficacy of stem cell transplantation.

Currently, there are no great methods for prospectively isolating differing HSC populations to study heterogeneity; much of the data that has been acquired is based on clonal tracking, single cell transplantation, etc. We have developed a rapid mobilization regimen as a new method to acquire HSCs. Fifteen minutes after administering a single subcutaneous injection in mice, stem cell mobilization to the blood is greater than five days of granulocyte-colony stimulating factor (G-CSF) treatment; the current gold standard for hematopoietic mobilization. Surprisingly, when equivalent numbers of highly-purified HSCs from the blood of mice treated with the rapid regimen versus G-CSF were subsequently competitively transplanted into lethally irradiated recipients, the HSCs mobilized by the rapid regimen substantially outperformed those mobilized by G-CSF. The rapid regimen mobilizes a "highly engraftable" hematopoietic stem cell (heHSC) compared to those mobilized by G-CSF.

Much like panning for gold, we have used the differential mobilization properties of our regimen and G-CSF as a "biologic sieve" to isolate the heterogeneous HSC populations from the blood. Our laboratory will continue to leverage this approach to analyze the transcriptomic and epigenetic differences between the two populations of HSCs to determine the specific gene(s) that account for the heHSC phenotype, and to further explore the biologic potential of this new population of stem cells. These efforts have the potential to substantially increase our knowledge of heterogeneity and increase efficacy of HSC based clinical therapies.

Selected Publications:

Czechowicz A, Palchaudhuri R, Scheck A, Hu Y, **Hoggatt J**, Saez B, Pang WW, Mansour MK,Tate TA, Chan YY, Walck E, Wernig G, Shizuru JA, Winau F, Scadden DT, Rossi DJ. "Selective hematopoietic stem cell ablation using CD117-antibody-drug-conjugates enables safe and effective transplantation with immunity preservation." *Nature Communications*, 2019; Feb6;10(1):617-628.

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Hoggatt J, Kfoury Y and Scadden DT. Hematopoietic Stem Cell Niche in Health and Diseases. *Annual Review of Pathology*, 2016;11:555-581.

Hoggatt J**†, Hoggatt AF**†, Tate TA, Fortman J, Pelus LM**. Bleeding the Laboratory Mouse: Not All Methods are Equal. *Experimental Hematology*, 2016: Feb;44(2):132-137.

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A. John Iafrate, MD, PhD

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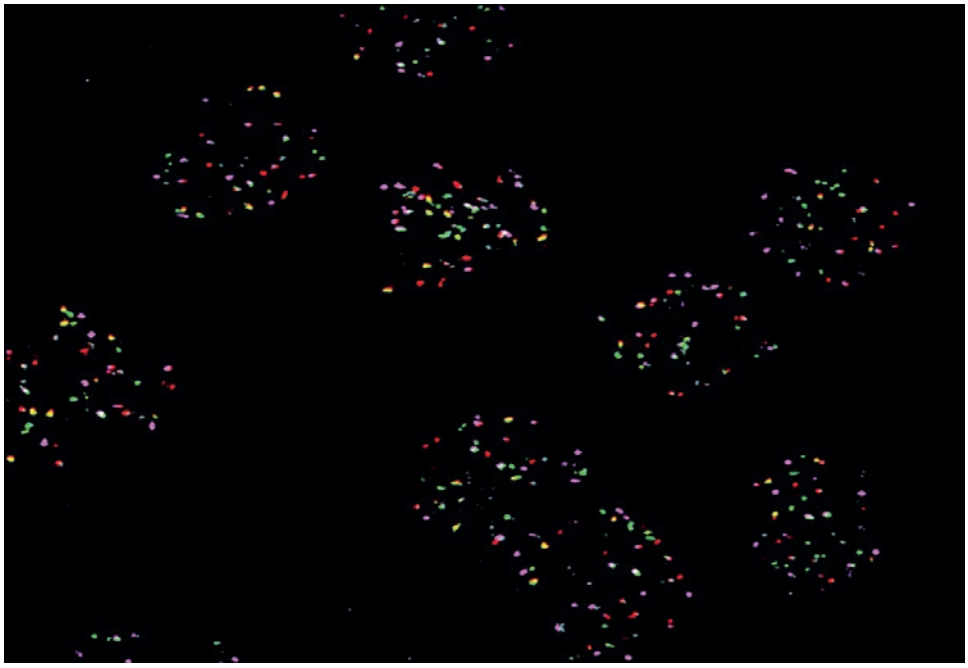
The Iafrate laboratory has focused efforts on developing highly complex molecular analyses of tumor genetics using novel technologies. We have a strong interest in the clinical implementation of genetic screening technologies that can help direct targeted therapies, focusing on lung, breast and brain tumors. Our recent contributions in the treatment of a subset of non-small cell lung carcinoma (NSCLC) with rearrangements of the ALK tyrosine kinase, rearrangements of the ROS1 tyrosine kinase and MET exon 14 skipping with a small molecule kinase inhibitor (crizotinib), underscore the promise of personalized cancer care (1, 2). We currently are focusing on detecting tumor DNA in blood samples ("liquid biopsies") to allow for efficient and convenient tracking of cancer progression. In additional we are developing new techniques to allow for early detection of cancers by detecting tumor-specific DNA in circulation.

We have developed and deployed next generation sequencing to detect chromosomal rearrangements in tumor tissue, with on-going studies that assess the relative sensitivity in much larger clinical cohorts. The method we have developed, termed "anchored multiplex PCR" or AMP, is an efficient target enrichment technology, allowing for 100s of targets to be simultaneously analyzed from small tissue samples (3). We have used AMP to screen thousands of tumor samples, and have uncovered numerous novel driver fusion genes. Our lab is now focused on modeling novel fusions in vitro and developing therapeutic approaches to screening these fusions. We have also initiated studies of tumor heterogeneity; these efforts focus on gene amplification of receptor tyrosine kinases in glioblastoma (4). 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 different subpopulations within a particular tumor shared other gene mutations, indicating that 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. Our lab has developed novel highly-multiplexed FISH technology to address how many genes show copy number heterogeneity, and to study the spatial distribution of such populations (5), see image. We are exploring the therapeutic implications of such driver gene heterogeneity in cell line model systems of glioblastoma using genome-wide CRISPR knock out screens.

More recently we have adapted the AMP sequencing technology in other areas, including (1) mapping off-target rates for CRISPR-CAS genome editing; (2) sequencing and mapping the distribution of IgH and TCR rearrangements in tumor samples; and (3) ultra-high sensitive mutation calling in



Multiplex FISH to detect copy number changes in circulating tumor cells.

circulating tumor cells and cell free plasma samples. Using AMP we have developed tissue-specific cell-free DNA (cfDNA) panels to examine the most important cancer genes in common tumors, including lung, melanoma, breast and colon cancer. Such panels are allowing us to track, with a simple blood draw, the tumor burden in patients. We are able to use cfDNA analysis in metastatic patients to see if they are responding to therapy, and also can track the development of resistance mutations. This allows a real-time dynamic optimization of therapy. Most recently we have developed a methylation-based sequencing assay to allow efficient analysis of tumor-specific methylation patterns in cfDNA samples. We hope that such an approach can be a lot more sensitive in the detection of small amounts of circulating tumor DNA, allowing potential early detection of tumors before they are clinically symptomatic. In addition, the methylation patterns are actually specific to the type of tumor the DNA is derived from, potentially allowing us to determine the actual anatomic site of origin.

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Snuderl M, Fazlollahi L, Le LP, Nitta M, Zhelyazkova BH, Davidson CJ, Akhavanfard S, Cahill DP, Aldape KD, Betensky RA, Louis DN, **Iafrate AJ**. Mosaic amplification of multiple receptor tyrosine kinase genes in glioblastoma. *Cancer Cell*. 2011; 20:810-7.

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Cheng J, Cao Y, MacLeay A, Lennerz JK, Baig A, Frazier RP, Lee J, Hu K, Pacula M, Meneses E, Robinson H, Batten JM, Brastianos PK, Heist RS, Bardia A, Le LP, **Iafrate AJ**. Clinical Validation of a Cell-Free DNA Gene Panel. *J Mol Diagn*. 2019; 21(4): 632-645.

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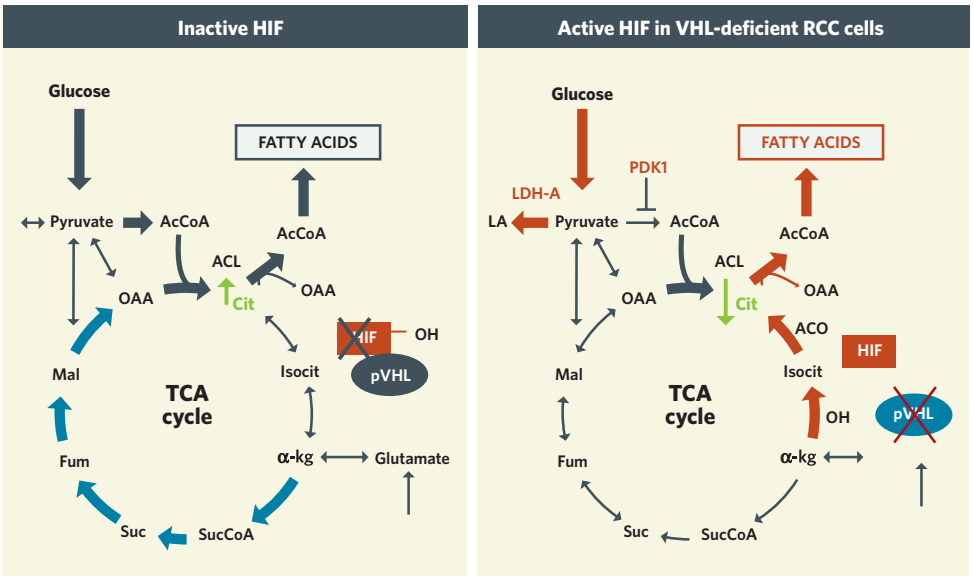
The Iliopoulos laboratory works on the main mechanisms underlying the reprogramming of cancer cell metabolism and cancer angiogenesis with the goal to develop mechanism-based strategies for selectively killing cancer cells. We use Renal Cell Carcinoma (RCC) as a model disease of altered cancer metabolism and angiogenesis mechanisms. Cancer cells transform their metabolism to adapt to the needs of fast growth and to compete with the surrounding normal cells for nutrients and oxygen. In addition to a reprogrammed metabolism, cancer cells stimulate the growth of new blood vessels that bring blood to them, a phenomenon known for many years as "cancer angiogenesis". The laboratory identifies and validates therapeutic targets that disrupt these processes.

Discovery and development of hypoxia inducible factor 2a (HIF2a) inhibitors for treatment of renal cell carcinoma and other HIF2a-dependent cancers

We screened libraries of chemical compounds and discovered chemical molecules that significantly and specifically decrease the expression of HIF2a (Zimmer M. et al. *Molecular Cell* 2008; 32(6): 838-48). We used these HIF2a inhibitors as chemical biology probes and discovered that they suppress the expression of HIF2a by activating IRP1. We thus proved a crosstalk between the iron and oxygen sensing mechanisms within the cell. We demonstrated that the HIF2a inhibitors discovered are "active" and that they reverse the consequences of VHL protein loss (Metelo AM. *Journal Clinical Investigation* 2015; 125(5): 1987-97). Our chemical HIF2a inhibitors are very promising agents for treating RCC.

Targeting the metabolic reprogramming of RCC and HIF2a expressing tumors; from the lab to the bedside

We used metabolic flux analysis to show that hypoxic cells use glutamine as a carbon source for anabolism. We showed that low oxygen levels or HIF2a expression reprogrammed cells to use glutamine in a "reverse" TCA cycle to produce the metabolites required for anabolic reactions, a process called Reductive Carboxylation. These observations provided insights into a mechanism by which hypoxic and HIF2a expressing cancer cells compensate for the Warburg phenomenon (Metallo et al. *Nature* 2012; 481(7381): 380- 4). We delineated the mechanism driving Reductive Carboxylation and proved that reductive carboxylation does not only happen in cultured cells, but can also be detected in human RCC tumors growing as xenografts in mice. We therefore provided for the first time, in vivo evidence for the utilization of glutamine in tumors through reductive carboxylation (Gameiro et al. *Cell Metabolism* 2013; 17(3): 372-385). Recently, we showed that inhibition of Glutaminase 1 (GLS1) decreases significantly the intracellular pyrimidines and results in DNA replication stress in HIF-hypoxia driven cancer cells. Treatment of cancer cells with GLS1 and PARP inhibitors resulted in dramatic suppression of RCC in xenograft models (*J Clin Invest*. 2017; 127(5): 1631-1645).



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

We brought these fundamental observations of my laboratory on glutamine metabolism to the clinic. We initiated a Phase 1 trial with Glutaminase 1 (GLS1) inhibitors for patients with RCC and triple negative breast cancers nationwide. We are now opening a new clinical trial of GLS1 inhibitor CB-839 and PARP inhibitor combination treatment for patients with RCC, prostate, triple negative and ovarian cancer.

Modeling Renal Cell Carcinoma in the zebrafish

Zebrafish with homozygous inactivating mutations in VHL gene recapitulate aspects of the human VHL disease, including abnormal proliferation of their kidney epithelium. We are using the zebrafish as a model system to model the diverse pathways that lead to renal cell carcinoma development.

Selected Publications:

Okazaki A, Gameiro PA, Christodoulou D, Laviollette L, Schneider M, Chaves F, Stemmer-Rachamimov A, Yazinski SA, Lee R, Stephanopoulos G, Zou L, **Iliopoulos O**. Glutaminase and poly(ADP-ribose) polymerase inhibitors suppress pyrimidine synthesis and VHL-deficient renal cancers. *J Clin Invest*. 2017; 127(5): 1631-1645. (Research Highlights "Targeting metabolism in RCC" in *Nature Reviews Nephrology*. 2017; 13, 320.

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Jenkins Laboratory

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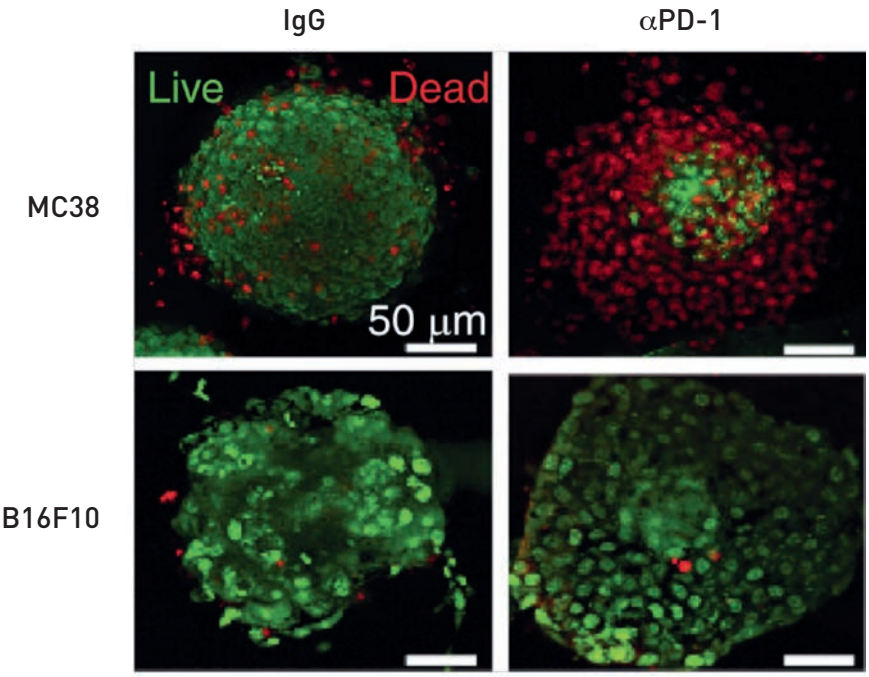
Immunotherapy has transformed the treatment of metastatic melanoma and other cancers, allowing a new avenue of therapeutic options and prolonging lives of many patients. Unfortunately, while immunotherapy is highly effective in some patients, it does not work for every patient and there are no available tests to determine whether or not a patient will respond to immunotherapy before treatment begins. To understand why immunotherapy works for some patients and not others, **the Jenkins laboratory** uses sophisticated tools and techniques to study and investigate the complex and dynamic interactions between cancer cells and the immune system. Our solution to this problem involves a specialized 3-dimensional culture of a patient's own tumor enabling researchers to examine interactions between tumor cells and immune cells. The integration of this novel approach with other emerging technologies is helping us navigate the complex landscape of the tumor immune microenvironment and learn which patients will respond to immunotherapy as well as how to effectively treat cancer patients that do not respond immunotherapy alone.

Precision cancer medicine currently focuses on knowledge of the cancer mutation repertoire and the tailored application of drugs that target altered genes or pathways in individual patients, such as use of BRAF inhibitors in patients with BRAF mutant melanoma. Immune checkpoint inhibitors targeting the PD-1/PD-L1 pathway have shown dramatic and durable clinical responses in melanoma and others cancers, but robust predictive biomarkers are lacking and innate resistance is common. Thus, a critical need exists for more sophisticated ex vivo functional testing modalities that recapitulate human tumor biology to predict response to targeted and immune-based therapies and to develop personalized treatment plans in real-time.

Major focus areas of the Jenkins lab include (1) identifying and characterizing mechanisms of response and resistance to PD-1 blockade, (2) discovering novel therapeutic strategies to overcome resistance to PD-1 blockade, and (3) using the MDOTS/PDOTS as a functional precision medicine platform for the development of novel combinations, and ultimately, personalized immunotherapy to tailor immunotherapy treatment to individual patients. Improved understanding of the response to immune checkpoint inhibitors within the tumor microenvironment will facilitate efforts to identify predictive biomarkers/models for immune checkpoint blockade in real-time, as well as future efforts to screen for therapeutic combinations that enhance the response to immune checkpoint blockade, and may ultimately provide a platform for the 'personalization' of immunotherapy.

Our novel approach for evaluating ex vivo response to PD-1 blockade utilizes murine- and patient-derived organotypic tumor spheroids (MDOTS/PDOTS) cultured in a 3-dimensional microfluidic system. Our study which was recently published



Live/Dead analysis (Acridine Orange – Green-Live; Propidium Iodide – Red-Dead) of murine-derived organotypic tumor spheroids (MDOTS) from PD-1 sensitive (MC38) and resistant (B16F10) syngeneic mouse models treated ex vivo with IgG or anti-PD-1 (10 μg/mL) for 6 days in 3D microfluidic culture (ref: Jenkins et al. Cancer Discovery 2018).

in *Cancer Discovery* (Jenkins et al., *Cancer Discovery* 2018; PMID: 29101162), has shown that organotypic tumor spheroids isolated from fresh mouse and human tumor samples retain autologous lymphoid and myeloid cell populations, including antigen-experienced tumor infiltrating CD4 and CD8 T lymphocytes, and respond to PD-1 blockade in short-term *ex vivo* culture. Furthermore, we have demonstrated that tumor killing was recapitulated *ex vivo* using MDOTS derived from the anti-PD-1 sensitive MC38 syngeneic mouse cancer model, whereas relative resistance to anti-PD-1 therapy was preserved in the CT26 and B16F10 syngeneic models. Our focused evaluation of rational therapeutic combinations to enhance response to PD-1 blockade using *ex vivo* profiling of MDOTS revealed TBK1 inhibition as a novel strategy to enhance sensitivity to PD-1 blockade, which effectively predicted tumor response *in vivo*. Our findings demonstrated the feasibility of *ex vivo* profiling of PD-1 blockade and offer a novel functional approach for the selection of immunotherapeutic combinations. The ultimate goals of these efforts are to identify and characterize novel features of response/resistance to PD-1 blockade and to identify novel therapeutic strategies to overcome resistance to anti-PD-1 therapy, ultimately to bring forward into human clinical trials.

Selected Publications:

Sade-Feldman M, Yizhak K, Bjorgaard SL, Ray JP, de Boer CG, **Jenkins RW**, Lieb DJ, Chen JH, Frederick DT, Barzily-Rokni M, Freeman SS, Reuben A, Hoover PJ, Villani AC, Ivanova E, Portell A, Lizotte PH, Aref AR, Eliane JP, Hammond MR, Vitzthum H, Blackmon SM, Li B, Gopalakrishnan V, Reddy SM, Cooper ZA, Paweletz CP, Barbie DA, Stemmer-Rachamimov A, Flaherty KT, Wargo JA, Boland GM, Sullivan RJ, Getz G, Hacohen N. Defining T Cell States Associated with Response to Checkpoint Immunotherapy in Melanoma. *Cell*. 2018 Nov 1;175(4):998-1013.

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Cañadas I, Thummalapalli R, Kim JW, Kitajima S, **Jenkins RW**, et al. Tumor innate immunity primed by specific interferon-stimulated endogenousa retroviruses. *Nat Med*. 2018 Aug;24(8): 1143-1150.

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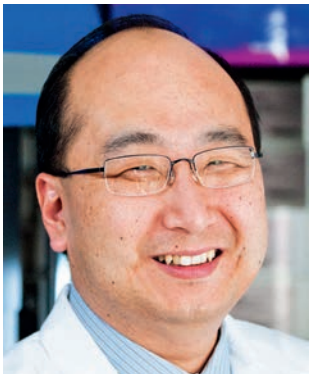
J. Keith Joung, MD, PhD



The Joung laboratory is developing strategies to reprogram the genomes and epigenomes of living cells to better understand biology and treat disease. We have developed and continue to optimize molecular tools for customized genome editing including engineered zinc finger, transcription activator-like effector (TALE), and RNA-guided CRISPR-Cas-based systems. These platforms 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 lead to more efficient gene therapy capable of correcting disease-related mutations in human cells.

Joung Laboratory

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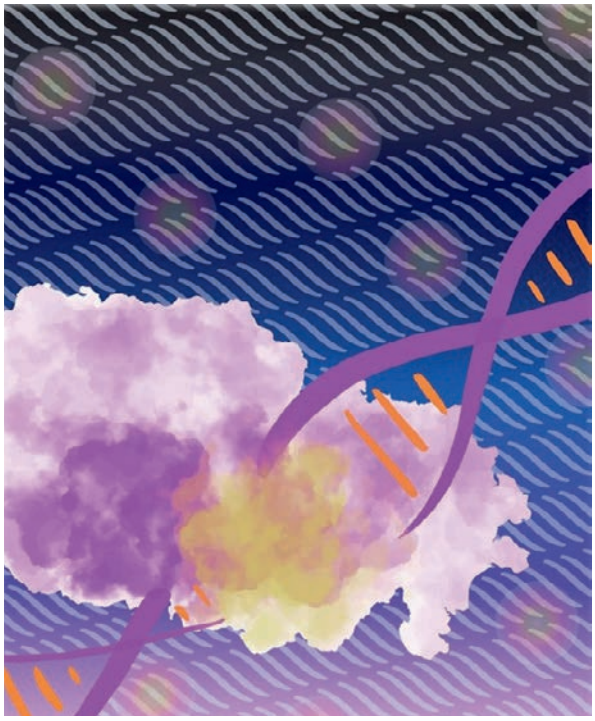
The Joung Laboratory develops technologies for genome and epigenome editing of living cells and organisms using engineered zinc finger, transcription activator-like effector (TALE), and RNA-guided CRISPR-Cas9-based systems and explores their applications for biological research and gene therapy.

Genome Editing Using Targeted Nucleases and Base Editors

Genome editing technology using CRISPR-Cas nucleases was named "Breakthrough of the Year" for 2015 by *Science* magazine. We and our collaborators were the first to demonstrate that these nucleases can function *in vivo* (Hwang & Fu et al., *Nat Biotechnol*. 2013) to modify endogenous genes in zebrafish embryos and the first to show that they can induce significant off-target mutations in human cells (Fu et al., *Nat Biotechnol*. 2013). We have led the field in development of unbiased, genome-wide strategies for profiling the specificities of CRISPR-Cas nucleases including the widely used cell-based GUIDE-seq method (Tsai et al., *Nat Biotechnol*. 2015) and the *in vitro* CIRCLE-seq method (Tsai et al., *Nat Biotechnol*. 2017). We have recently shown that CIRCLE-seq can be used to identify Cas9-induced off-targets *in vivo* (Akcakaya & Bobbin et al., *Nature*, 2018). In addition, we have engineered "high-fidelity" Cas9 variants (Kleinstiver & Pattanayak et al., *Nature* 2016) and Cas9 variants with novel DNA binding specificities (Kleinstiver et al., *Nature* 2015; Kleinstiver et al., *Nat Biotechnol*. 2015; Kleinstiver et al., *Nat Biotechnol*. 2019). More recently, we have developed a novel CRISPR base editor architecture that shows improved precision and reduced off-target effects (Gehrke et al., *Nat Biotechnol*. 2018) and described and minimized base editor-induced transcriptome-wide RNA off-target mutations (Grunewald et al., *Nature* 2019; Grunewald et al., *Nat Biotechnol*. 2019).

Epigenome Editing Using Targeted Transcription Factors

We have also performed work showing that the Transcription Activator-Like Effector (TALE) and CRISPR-Cas platforms can also be utilized to create artificial transcription



Schematic illustration of RNA off-target edits induced by CRISPR DNA base editors.

factors that can robustly alter expression of endogenous h*uman genes (Maeder et al., Nat Methods* 2013a; Maeder et al., *Nat Methods* 2013b). 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). More recently, we have shown that the CRISPR-Cpf1(Cas12a) platform can be modified to engineer robust transcriptional activators that can efficiently increase endogenous gene expression in human cells (Tak et al., *Nat Methods* 2017).

Selected Publications:

Grünewald J, Zhou R, Garcia SP, Iyer S, Lareau CA, Aryee MJ, **Joung JK**. CRISPR DNA base editors with reduced RNA off-target and self-editing activities. *Nat Biotechnol*. 2019 Sept; 37(9):1041-1048.

Grünewald J, Zhou R, Garcia SP, Iyer S, Lareau CA, Aryee MJ, **Joung JK**. Transcriptome-wide off-target RNA editing induced by CRISPR-guided DNA base editors. *Nature*. 2019 May; 569(7756): 433-437.

Kleinstiver BP, Sousa AA, Walton RT, Tak YE, Hsu JY, Clement K, Welch MM, Horng JE, Malagon-Lopez J, Scarfò I, Maus MV, Pinello L, Aryee MJ, **Joung JK**. Engineered CRISPR-Cas12a variants with increased activities and improved targeting ranges for gene, epigenetic and base editing. *Nat Biotechnol*. 2019 Mar; 37(3): 276-282.

Akcakaya P, Bobbin ML, Guo JA, Malagon-Lopez J, Clement K, Garcia SP, Fellows MD, Porritt MJ, Firth MA, Carreras A, Baccega T, Seeliger F, Bjursell M, Tsai SQ, Nguyen NT, Nitsch R, Mayr LM, Pinello L, Bohlooly-Y M, Aryee MJ, Maresca M, **Joung JK**. In vivo CRISPR editing with no detectable genome-wide off-target mutations. *Nature*. 2018 Sep;561(7723):416-419.

Gehrke JM, Cervantes O, Clement MK, Wu Y, Zeng J, Bauer DE, Pinello L, **Joung JK**. An APOBEC3A-Cas9 base editor with minimized bystander and off-target activities. *Nat Biotechnol*. 2018 Nov; 36(10): 977-982..

Tak YE, Kleinstiver BP, Nuñez JK, Hsu JY, Horng JE, Gong J, Weissman JS, **Joung JK**. Inducible and multiplex gene regulation using CRISPR-Cpf1-based transcription factors. *Nat Methods*. 2017; 14(12):1163-1166.

Li Lan, MD, PhD

Lan Laboratory

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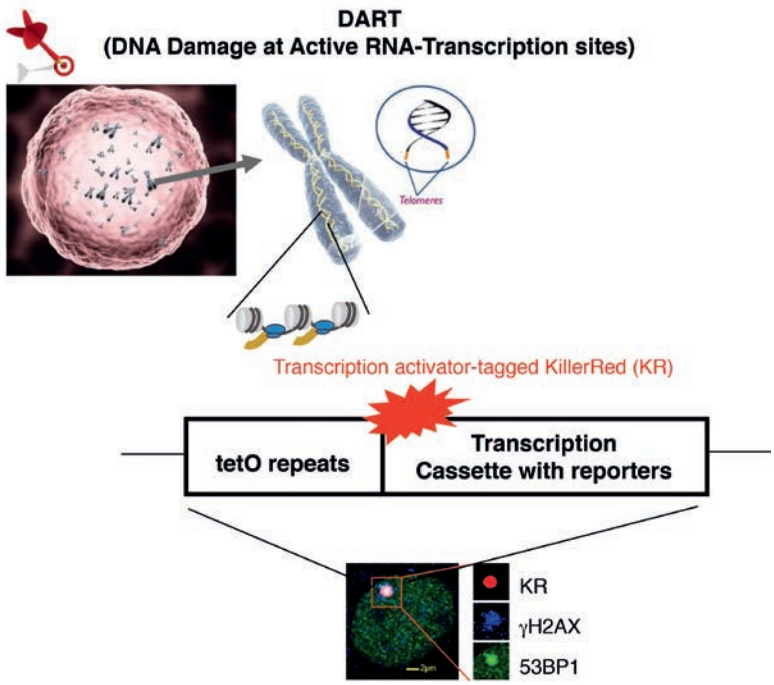
Oxidative DNA damage is a major source of genomic instability during tumorigenesis and aging. The main research interests of **the Lan laboratory** are centered on the mechanisms by which human cells maintain genomic stability against oxidative stress. With a strong appreciation for how human health conditions, especially cancer and neurological maladies, are connected to the loss of genome integrity, ranging from intrinsic genetic predispositions to environmental factors that inflict DNA damage, my lab has developed the first single-cell assay to interrogate the molecular mechanisms of oxidative DNA damage response at specific loci in the genome. By combining this innovative assay with state-of-the-art imaging techniques, we have opened new avenues to understanding the oxidative DNA damage response in different chromosomal environments.

The ongoing research of my lab is focused on transcription-coupled oxidative DNA damage response and cancer. A growing body of evidence suggests that oxidative stress plays an important role in tumorigenesis, aging, and neurodegenerative diseases. Oxidative stress caused by environmental insults and endogenous metabolites induces DNA base modifications and strand breaks. DNA strand breaks have detrimental effects not only on actively proliferating cells, but also on slowly proliferating cells and terminally differentiated cells. At active transcription sites, RNA Polymerase II can bypass DNA base modifications, but not strand breaks. Given the heterogeneity of cancer cells in tumors, it is critical to understand how dividing and non-dividing cells respond to oxidative DNA damage. One of the main research interests of the Lan laboratory is to understand how oxidative DNA damage response is differentially regulated in transcribed and un-transcribed regions, and in dividing and non-dividing cells. We discovered a novel mRNA-dependent and R loop-mediated homologous recombination (HR) mechanism that specifically promotes repair in the transcribed genome. Thus, our work has revealed an unexpected role for mRNA in HR. Importantly, we show that this mRNA-mediated HR mechanism is able to operate even in G0/G1 cells, challenging the current view that HR only occurs during the S/G2 phase of the cell cycle. Our findings may likely lead to a new paradigm in DNA repair, and to a better understanding of how actively proliferating and slowly proliferating cancer cells respond to oxidative damage. In the near future, we plan to address several important questions on this new pathway that we discovered: (1) Whether and how is the RNA-mediated HR pathway distinct from the canonical HR pathway? (2) How is repair "channeled" into the RNA-mediated HR pathway in transcribed regions? (3) Is the RNA-mediated HR pathway important for tumor suppression? In our ongoing studies, we are exploring the function of RNA modifications in the RNA-mediated HR pathway, and are using advanced super-resolution imaging techniques (STORM and PALM) to study DNA-RNA structural changes at specific sites of DNA damage within the genome. We are also using the zebrafish



The Lan laboratory developed the DNA Damage at RNA Transcribed sites (DART) method to precisely introduce oxidative DNA damage at specific transcribed loci in a dose-dependent manner. This is achieved by site-specific positioning of the photo-excitable and ROS-releasing protein KillerRed (KR). This unique method provides a tool to understand how oxidative DNA damage response is differentially regulated in transcribed and un-transcribed regions, and in dividing and non-dividing cells.

model to assess the functional significance of RNA-mediated HR in vivo. Going forward, we would like to expand our studies to investigate the status of this new RNA-mediated HR repair pathway in cancer cells, its potential function in tumor suppression, and its value as a therapeutic target.

A second research priority of my lab is to understand how telomeres respond to oxidative DNA damage. Telomere dysregulation is a major source of genomic instability and a potential target for cancer therapy. Due to G/C-rich telomeric repeats, telomeres are particularly vulnerable to oxidative stress. Interestingly, telomeres are protected by specific "capping" proteins, making DNA damage response at telomeres significantly different from elsewhere in the genome. More specifically, we are investigating whether and how oxidative damage at telomeres triggers telomere attrition, senescence, and the promotion of tumorigenesis. My lab has established a new method to introduce oxidative damage to telomeres in a highly controlled manner, allowing us, for the first time, to specifically follow the oxidative damage response at telomeres. In several projects, we have investigated how HR factors are regulated by shelterin proteins at telomeres during the oxidative damage response. The recruitment of repair factors to telomeres is coordinately regulated by poly-ADP-ribosylation, phosphorylation, SUMOylation, and ubiquitylation of TRF1 to protect cancer cells from telomere damage. Our future goal is to investigate whether and how the mechanisms orchestrating oxidative damage response at telomeres may contribute to the suppression of tumorigenesis and aging, and how we can exploit this specific vulnerability of cancer cells in therapy.

Selected Publications:

Teng Y, Yadav T, Duan M, Tan J, Xiang Y, Gao B, Xu J, Liang Z, Liu Y, Nakajima S, Shi Y, Levine AS, Zou L, **Lan L**. ROS-Induced R Loops Trigger a Transcription-Coupled but BRCA1/2-Independent Homologous Recombination Pathway through CSB. *Nature Communications*. 2018 Oct 8;9(1):4115.

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Tan R, Nakajima S, Wang Q, Sun H, Xue J, Wu J, Hellwig S, Zeng X, Yates N, Smithgall TE, Lei M, Jiang Y, Levine AS, Su B, **Lan L**. Nek7 protects telomeres from oxidative DNA damage by phosphorylation and stabilization of TRF1. *Mol Cell*. 2017 Mar 2;65(5):818-831.e5.

Gao Y, Li C, Wei L, Teng Y, Nakajima S, Ma H, Spagnol ST, Leger B, Wan Y, Dahl KN, Liu Y, Levine AS, **Lan L**. SSRP1 cooperates with PARP and XRCC1 to facilitate single strand break repair through chromatin priming. *Cancer Res*. 2017 May 15;77(10):2674-2685.

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David M. Langenau, PhD

Most pediatric patients whose sarcoma or leukemia recurs will succumb to their disease. The focus of **the Langenau laboratory** 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 utilized 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.

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Identifying molecular pathways that drive progression and relapse in pediatric cancer

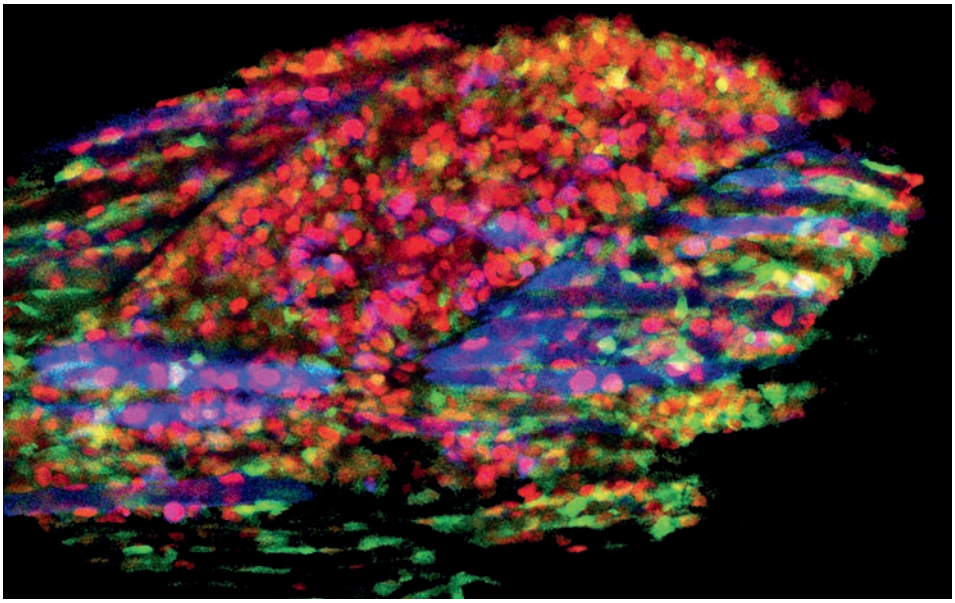
The Langenau laboratory research focus is to uncover progression and relapse mechanisms in pediatric cancer. Utilizing zebrafish models of T-cell acute lymphoblastic leukemia (T-ALL) and embryonal rhabdomyosarcoma (ERMS), we have undertaken chemical and genetic approaches to identify novel modulators of progression, therapy-resistance, 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 relapse disease are largely unresponsive to additional therapy and have a very poor prognosis. Ultimately, 70% of children and 92% of adults will die of relapse T-ALL, underscoring the clinical imperative for identifying the molecular mechanisms that cause leukemia cells to re-emerge at relapse. Utilizing a novel zebrafish model of relapse 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 exerts important roles in regulating human T-ALL proliferation, apoptosis and response to therapy. Discovering new relapse-driving oncogenic pathways will likely identify 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 that mimics the molecular underpinnings of human ERMS. We used fluorescent



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.

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+/vangl2+ self-renewing cancer stem cell drives continued tumor growth at relapse and is molecularly similar to a non-transformed, 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 ERMS cells. Using genetic approaches, we have also identified important roles for Myod, Myf5, Myogenin, Vangl2, and Notch1 in driving continued RMS growth.

Zebrafish Avatars of Human Cancer

The Langenau Lab has generated a number of immunocompromised zebrafish strains that efficiently engraft zebrafish, mouse, and human tumors. These models are amenable to real-time imaging of cancer hallmarks at single cell resolution and have been used in preclinical modeling experiments to identify drug combinations for the treatment of human rhabdomyosarcoma. These models are now being assessed for engraftment of a wider array of human cancers, ES and iPS cells, and regenerative tissues.

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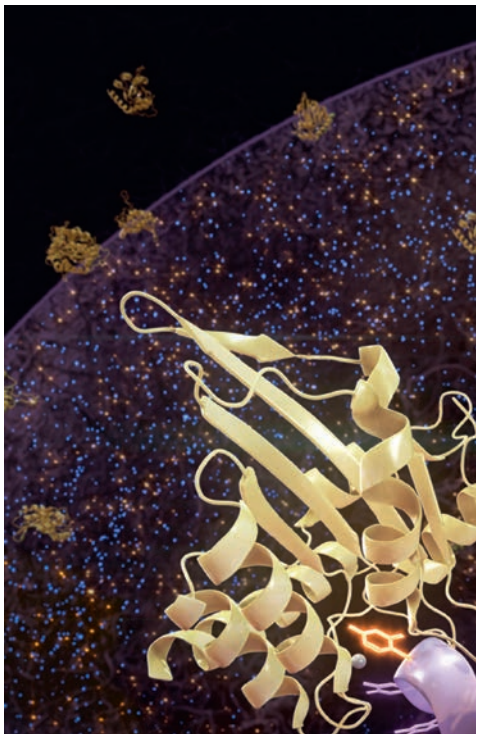
Cancer results from alterations to DNA that lead to the activation of oncogenes or the inactivation of tumor suppressors. **The Lawrence laboratory** focuses on understanding the many ways this can happen, using computation as a powerful microscope to study the processes of DNA damage and repair, gene expression and genome replication, and cancer driver genes. Over our lifetimes, DNA slowly accumulates mutations due to environmental toxins and radiation, as well as from naturally occurring copying errors. The vast majority of mutations have little or no effect on a cell, but out of all possible mutations, a few may hit exactly the right place in the genome, where they can act as a "driver mutation," pushing the cell toward aggressive growth and tumor formation. Sequencing the DNA in a tumor reveals not only its driver mutations, but also all the other "passenger mutations" that were present in the tumor-initiating cell. We seek insights about cancer from both driver and passenger mutations.

Tumor DNA Sequencing

High-throughput DNA sequencing is a workhorse of biomedical research. There are many challenges in processing the raw DNA sequencing reads from a patient's resected tumor or biopsy material, aligning them accurately to the reference human genome, and then scanning for loci where the tumor DNA differs from the patient's bulk "normal" DNA (e.g. from a blood draw). Distinguishing true somatic mutations from sequencing or alignment artifacts can be tricky, especially for subclonal events present in only a fraction of tumor cells. We are refining a "panel of normals" (PoN) approach, which combats stochastic artifacts seen in the patient's tumor sample, and not in the patient's normal sample but widespread however in many other patients' normal samples. We are continually discovering new artifact modes, making this a highly challenging and unpredictable area of research. Isolating true somatic mutations is crucial for downstream analyses of mutational signatures and driver events.

Analyzing Mutational Signatures

Cancers vary over many orders of magnitude in their total background mutation burden, ranging from very quiet tumor types such as leukemias and childhood tumors, which may have fewer than 10 somatic mutations in their exome, to carcinogen-associated tumor types such as lung cancer and melanoma, which may have over 1000. Mutations have many causes, and each mutagen can leave a telltale signature. For instance, spontaneous deamination of methylated CpG's causes the transition mutations that dominate many tumor types. Mutagens in tobacco smoke cause G-to-T transversions. Ultraviolet radiation causes C-to-T at dipyrimidines. Agitated APOBEC enzymes cause mutations at C's preceded by T. Loss of mismatch repair causes microsatellite instability (MSI), marked by expansion and contraction of simple-sequence repeats, as well as characteristic types of single-base changes. Tumors carrying mutations in the proofreading exonuclease domain of polymerase epsilon (POLE) tend to accrue C-to-A mutations at the trinucleotide TCT. Very rare "MSI+POLE" cancers show the highest yet known somatic mutation burdens, with upwards of 10,000 coding mutations per patient. Patients



The mutational landscape of a cancer cell across size regimes. At the smallest scale, local DNA trinucleotide sequences (lower-left foreground) correlate with the "mutational signatures" induced by various mutagens. At the largest scale (background of image), chromatin is organized into multi-megabase domains comprising Compartment B (tightly packed, gene-poor DNA lining the nuclear periphery) and Compartment A (gene-rich open DNA in the nuclear interior). Mutations induced by APOBEC enzymes (yellow points) are distributed equally across the two compartments, but most other types of mutations (blue points) are concentrated in Compartment B. Between the large and small extremes lies the "mesoscale" regime, where genomic features like hairpin-forming ability are determined. DNA exposed in a hairpin loop is vulnerable to attack by the enzyme APOBEC3A (center), giving rise to highly recurrent passenger mutations in cancer.

affected by MSI and/or POLE mutagenesis are known to experience better clinical outcomes, probably thanks to their high neoantigen loads which attract a powerful immune response. Our most recent research has focused on a less well-studied signal in somatic mutation datasets, mutational asymmetries between the two DNA strands. These illuminate transcriptional or "T-class" mutational patterns, associated with exposure to tobacco smoke, UV radiation, and a yet-unknown agent in liver cancer, as well as replicative or "R-class" patterns, associated with MSI, APOBEC, POLE, and a yet-unknown agent in esophageal cancer.

APOBEC Mutations and Mesoscale Genomic Features

Statistical approaches for distinguishing driver mutations from passenger mutations have relied on the gold standard of recurrence across patients. Seeing exactly the same DNA base-pair mutated recurrently across patients has been taken as proof that the mutation must be under functional selection for contributing to tumor fitness. The assumption is that mutational processes, being essentially random, are unlikely to hit the exact same base-pair over and over again. Our recent discoveries about APOBEC mutagenesis have cast doubt on this assumption. We have shown that APOBEC3A has a very strong preference for mutating cytosines presented in a short loop at the end of a strongly paired DNA hairpins. DNA hairpins occupy the genomic "mesoscale" regime, being larger than the trinucleotides that define mutation signatures, yet smaller than chromatin topological domains. Our results indicate that there are multiple routes to cancer mutational hotspots. Driver mutation hotspots in oncogenes can rise to prominence through positive selection, and are not restricted to the "favorite" sites of any particular mutagen. In contrast, special DNA sites (like hairpins) that happen to be optimal substrates for a mutagen (like APOBEC) can give rise to "passenger hotspot mutations" that owe their prevalence to substrate optimality, not to any effects on tumor fitness. These findings mean we need to be careful not to assume that all mutation hotspots are cancer drivers.

Selected Publications:

Buisson R, Langenbucher A, Bowen D, Kwan EE, Benes CH, Zou L*, **Lawrence MS***. Passenger hotspot mutations in cancer driven by APOBEC3A and mesoscale features. *Science*. 2019 Jun 28; 364(6447):eaaw2872.

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** Graduate students

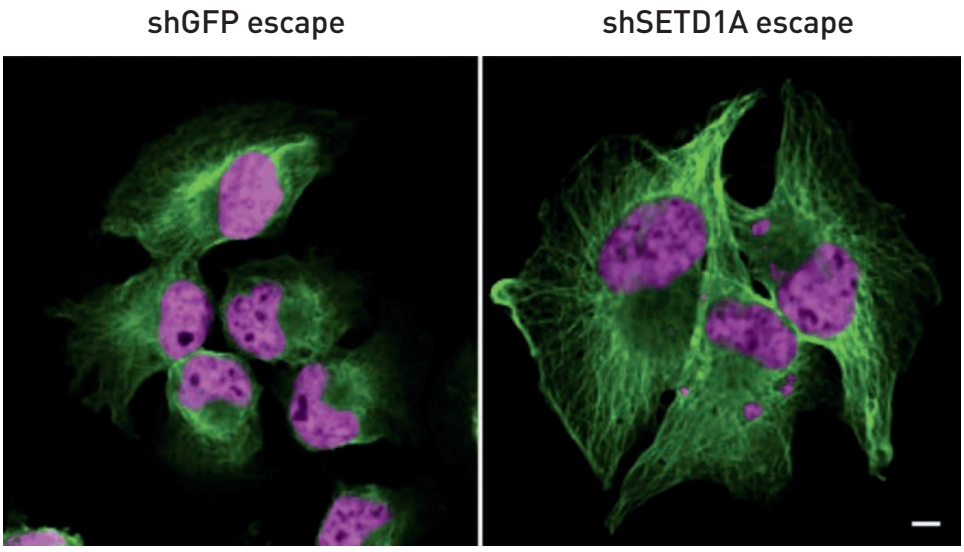
Metastasis, the leading cause of cancer-related deaths, is governed by multiple steps, which are not well understood. Using cell culture and mouse models, as well as patient-derived tumor tissues and tumor cells circulating in the blood (Circulating Tumor Cells/CTCs), **the Maheswaran laboratory** has uncovered novel tumor cell characteristics that promote metastasis in breast cancer patients. Our findings show that cancer cells exist in multiple cellular states, each state exhibiting different characteristics. As such, each breast cancer patient harbors a mixture of tumor cells with different functional properties. We intend to define the functional and molecular properties of different subclasses of tumor cells and their contribution to metastasis, tumor evolution and drug sensitivity using appropriate experimental models and patient-derived samples. These findings will provide insight into the contribution of heterogeneous cancer cell populations to metastasis and their significance as biomarkers and therapeutic targets.

Mechanisms of Breast Cancer Metastasis

The research in my laboratory is focused on defining the molecular mechanisms that drive breast cancer progression and metastasis. Cancer, initially confined to the primary site, eventually spreads to distal sites, including lung, liver, bone and brain, by invading into the bloodstream. Upon reaching these distal sites, the tumor cells continue to grow and evolve well after removal of the primary tumor resulting in overt metastasis and disease recurrence, the leading causes of cancer-related deaths. Using cell culture and mouse models, patient derived tissues, and circulating tumor cells (CTCs) enriched from the blood of women with breast cancer, we characterize the contribution of oncogenic-and tumor-microenvironment-derived signals to cellular states including: epithelial to mesenchymal plasticity, senescence, and how these aspects of tumor heterogeneity influence cancer progression and therapeutic responses.

Metastasis through the Prism of Circulating Tumor Cells

I am also collaborating with Drs. Daniel Haber and Mehmet Toner to define cancer biology across several tumor types including breast, prostate, liver, and lung cancers as well as melanoma using CTCs isolated from the blood of cancer patients. CTCs represent an extremely rare population of cells in the blood and their isolation presents a tremendous technical challenge. The CTC-iChip developed in Dr. Toner's laboratory enables enrichment of live CTCs through selective removal of blood components; red and white blood cells as well as platelets. Characterizing CTCs has far-reaching implications for both clinical care and defining cancer biology. They enable real time monitoring of tumor cells during disease progression and therapeutic responses, and could possibly be used for early detection of disease. Viable CTCs cultured from patients provide tremendous insight into the molecular heterogeneity



Confocal images of cells stained with tubulin (green) and DAPI (magenta) show that SETD1A-KD cells escaping senescence harbor chromosome segregation defects visualized as micronuclei (circled). The scale bar represents 50 µm.

and cellular plasticity of tumors that govern differential biological characteristics and responses to therapy. Characterization of CTCs ties in well with the overall goal of the lab to study cancer metastasis.

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The Manguso lab is working to improve the efficacy of cancer immunotherapy. We use a range of approaches including mouse models, functional genomics, cellular immunology, and single-cell profiling to understand how cancers evade the immune system. Our lab has pioneered the use of *in vivo* genetic screens with CRISPR to identify new immunotherapy targets and resistance mechanisms. Using these approaches, we identified the tyrosine phosphatase PTPN2, a critical regulator of immunotherapy sensitivity in tumor cells. We also identified the dsRNA-editing enzyme ADAR1 as a checkpoint that regulates the sensing of self-dsRNA by tumor cells. Our results indicate that there are dozens of ways that cancers can be targeted by the immune system, and we are working to understand the new mechanisms revealed by our studies. In the long term, these approaches will enable a new understanding of how the immune system interacts with cancerous tissue and how the interaction can be manipulated to destroy tumors.

Over the last decade, critical discoveries in immunology and cancer biology have revealed how tumors are shaped by the immune system and how they evolve to evade it. We now know that disrupting immune checkpoints such as CTLA-4 and PD-1/PD-L1 can lead to T cell-mediated elimination of tumors. However, there is still a critical unmet need, as the vast majority of patients with cancer do not benefit from current immunotherapies. Our most pressing challenge is to discover the next generation of immunotherapies that can bring clinical benefit to the majority of patients.

To discover immunotherapy targets and resistance mechanisms in high throughput, we have developed an *in vivo*, CRISPR-based genetic screening system to identify genes that regulate tumor cell sensitivity to immunotherapy (Manguso et al, *Nature* 2017). We genetically modify mouse cancer cell lines that can be transplanted into animals and used as immunotherapy models. After delivery of Cas9 and libraries of single guide RNAs (sgRNAs), we implant pools of modified tumor cells into animals that are treated with immunotherapy. In a single experiment we can determine genes that, when deleted, increase or decrease sensitivity to immunotherapy (Figure 1). This strategy has enabled the rapid and simultaneous identification of new targets and resistance mechanisms that are potent regulators of anti-tumor immunity.

This powerful, unbiased discovery system allows us to identify targets and resistance mechanisms with no previously identified roles in immunotherapy. Three examples illustrate the power of this system for discovery: 1) we found that deletion of the phosphatase PTPN2 enhanced tumor cell sensitivity to immunotherapy. While PTPN2 was known to negatively regulate T cell receptor activation, our screens determined that it is also the most potent suppressor of interferon-gamma sensing in tumor cells; 2) we discovered that the non-classical MHC-I gene HT-T23/Qa-1 (HLA-E) is a

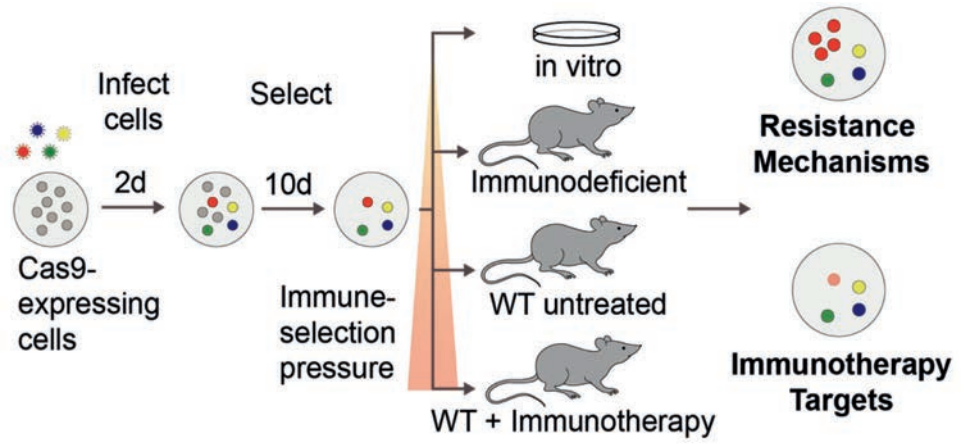

Diagram of in vivo CRISPR screening system. Pools of Cas9-expressing, sgRNA library-transduced tumor cells are implanted into either wild-type or immunocompromised mice. After 2 weeks, tumors are harvested and genomic DNA is extracted from tumor tissue. Next generation sequencing of the sgRNA library is used to identify resistance mechanisms or immunotherapy targets.

major immune checkpoint that limits anti-tumor immunity by T cells and NK cells; 3) our screens identified that deletion of ADAR1, an adenosine deaminase acting on RNA unmasks endogenous dsRNA that can be recognized by the cytosolic pattern recognition receptors PKR and MDA5, and can overcome resistance to immunotherapy caused by loss of antigen presentation (Ishizuka & Manguso et al, *Nature* 2018). Previously, these genes were not known or prioritized targets in immuno-oncology, but our unbiased approach enables discoveries that would have otherwise been unlikely.

We have demonstrated that *in vivo* CRISPR screens are a powerful way to discover new targets and probe the interaction of tumor cells with the host immune system. We can now broadly apply these genetic tools to advance our understanding of how immunotherapy works, why it may fail, and how we can improve it. Ongoing projects in the lab include:

1. Discover novel immunotherapy targets and mechanisms of resistance across several well-characterized mouse cancer models
2. Identify pathways that can overcome acquired resistance to immunotherapy
3. Understand how we can manipulate antigen presentation to enhance immunotherapy

These projects will define new ways to generate anti-tumor immune responses, reveal pathways that can be targeted to enhance these responses across cancer types, and anticipate and overcome the mechanisms by which tumors will become resistant. More broadly, these studies will improve our understanding of how tumors evolve under the selective pressure of immune surveillance and enable the development of more effective therapeutics.

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Marcela V. Maus, MD, PhD

Using the immune system as a cancer treatment has the potential to induce long-term, durable remissions, and perhaps even cures for some patients. The T cells of the immune system are able to specifically kill the target cells they recognize. T cells are also able to persist in the body for many years, and form immune 'memory,' which enables the possibility of long-term protection. **The Maus laboratory** is interested in using genetic engineering techniques to re-direct T cells to find and kill tumor cells, while sparing healthy tissues. We aim to develop new ways to design molecular receptors to target T cells to liquid and solid tumors; use T cells as delivery vehicles for other drugs, and use drugs to help T cells work against tumors; and understand how T cells can work as "living drugs" to treat patients with cancer.

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Immune therapies that engage T cells have the potential to induce long-term durable remissions of cancer. In hematologic malignancies, allogeneic hematopoietic stem cell transplants can be curative, in part due to T-cell mediated anti-tumor immunity. In solid tumors, checkpoint blockades with anti- CTLA-4 or anti-PD-1 monoclonal antibodies can mediate long-term responses by releasing T cells from tightly controlled peripheral tolerance. Chimeric antigen receptors (CARs) are synthetic molecules designed to re-direct T cells to specific antigens. Re-directing T cells with CARs is an alternative method of overcoming tolerance, and has shown great promise in the clinical setting for B cell malignancies such leukemia and lymphoma. However, successful application of this form of therapy to other cancers is likely to require refinements in the molecular and clinical technologies.

The goal of the Maus lab is to design and evaluate next generation genetically-modified (CAR) T cells as immunotherapy in patients with cancer.

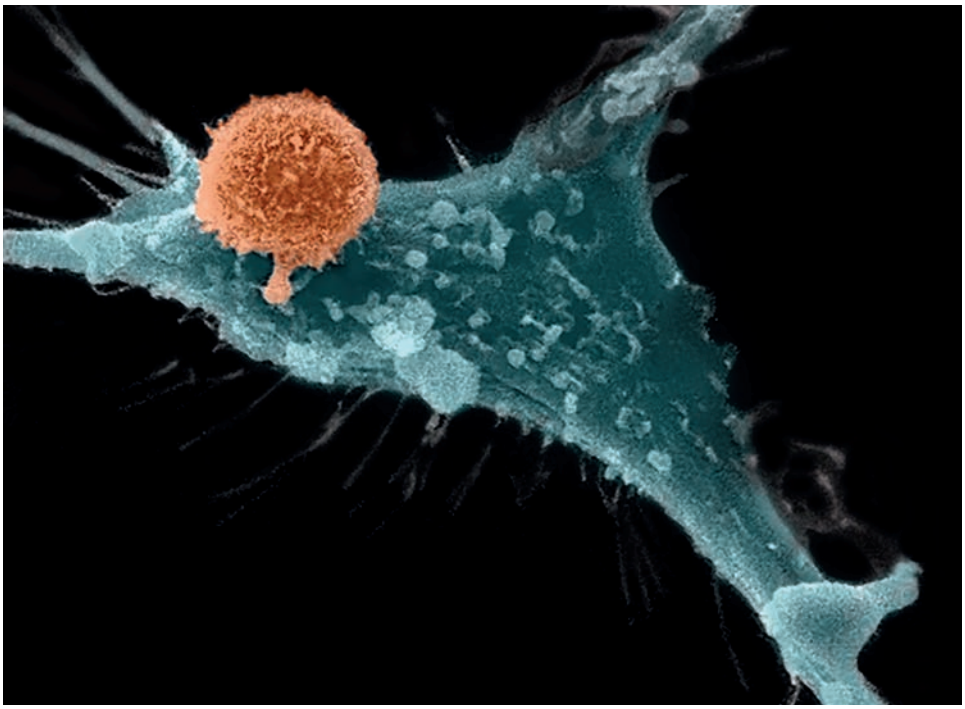
Specifically, next generation T cells that the Maus lab intends to develop includes CAR-T cells that:

1. **Contain molecular improvements in receptor design to enhance specificity, potency, and safety.**

   Most chimeric antigen receptors used to re-direct T cells to a new target are based on enforcing expression of either murine single-chain antibody fragments, natural ligands, or natural T cell receptors. However, novel types of antigen receptors are in development and could be exploited to re-direct T cells such that they can distinguish between antigen expressed on the tumor and the same antigen expressed in healthy tissues. In liquid tumors, it will also be important to improve the safety of CAR T cells, while in solid tumors, the focus is on increasing their potency.

2. **Are administered in combination with other drugs delivered either (a) systemically or (b) as payloads attached to T cells to sensitize tumors to T cell mediated killing and/or potentiate T cell function.**

   Some recently developed targeted therapies have effects on T cells or



CAR-T Cell Targeting a Glioblastoma Cell Expressing EGFRvIII, Scanning Electron Micrograph; Credit: Bryan D. Choi, Mark B. Leick, and Marcela V. Maus.

tumor cells that potentiates the tumor-killing effects. Alternatively, T cells can be chemically or genetically loaded with drugs to potentiate T cell function, such as cytokines or antibodies to checkpoint inhibitors. In this case, re-directed T cells could be used as a delivery mechanism to target an otherwise toxic drug specifically to the tumor.

3. **Have additional modifications that make CAR T cells (a) resistant to inhibitory mechanisms, (b) imageable, or (c) more feasible to manufacture and administer.**

   Control of T cell function is a complex process orchestrated by a variety of molecules, some of which deliver inhibitory signals. Tumors often express ligands to inhibit T cell function. Using a single vector, genetically modified T cells can be re-directed not only to recognize a new antigen on tumor cells, but also to be resistant to the inhibitory tumor micro-environment.

4. **We aim to understand the basic biology and mechanisms that drive engineered T cell function.**

   The MGH Cellular Immunotherapy Program directed by Dr. Maus aims to generate a pipeline of genetically engineered CAR T cells to use as "living drugs" in patients with cancer. The program is composed of a "research and discovery" arm, "a regulatory/ translational" arm to be able to test genetically-modified T cells in human subjects, and a "clinical/ correlative" sciences arm of immune profiling to examine the engraftment, persistence, and bioactivity of T cell products infused into patients. The Immune Monitoring Laboratory is directed by Dr. Kathleen Gallagher.

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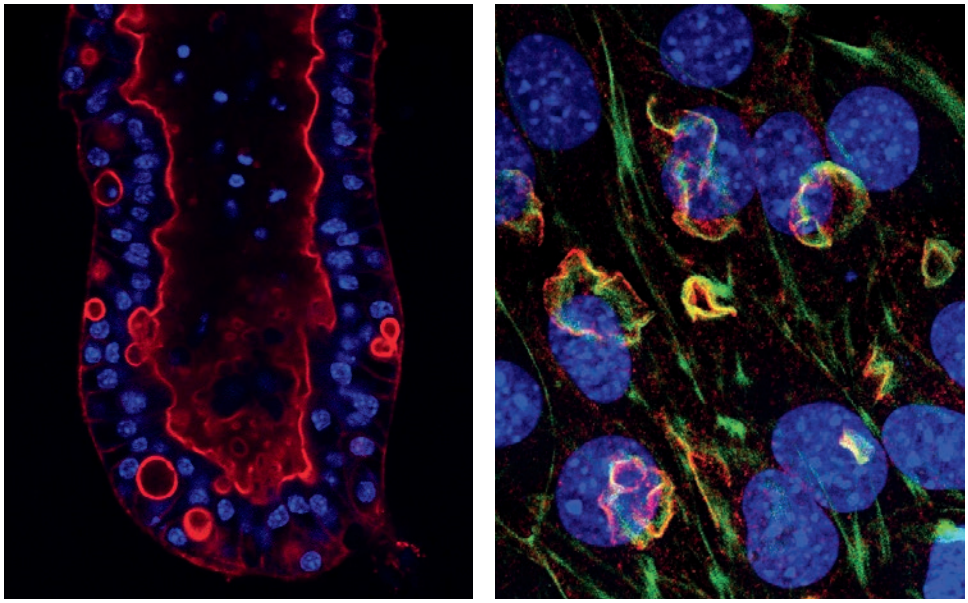
The McClatchey laboratory focuses on understanding how cells organize their outer surface – an important cellular compartment created by the interface between the cell membrane and underlying cortical cytoskeleton. This compartment governs the shape, identity and behavior of individual cells, as well as how they interact biochemically and mechanically with the extracellular environment. Normal cells modulate the features of the membrane:cytoskeleton interface to carry out key developmental processes and build functioning tissues. On the other hand, cancer cells exploit this compartment to interact inappropriately with other cells and with their environment during tumor initiation, invasion and metastasis. Our research stems from a longstanding quest to understand the molecular basis of a familial cancer syndrome caused by mutation of the *neurofibromatosis type 2 (NF2)* tumor suppressor gene. The *NF2*-encoded protein, Merlin, and closely related ERM proteins (Ezrin, Radixin and Moesin) are central architects of the cell cortex that have important roles in development and in many human cancers.

Understanding morphogenesis and tumorigenesis

The vast array of forms and functions exhibited by different cell types is enabled by the organization of specialized domains within the cell cortex such as the neuronal growth cone, immunological synapse and microvillus-studded apical surfaces of epithelial cells. Indeed, epithelial cells work together to establish discrete basal, lateral and apical surfaces as they organize into three dimensional structures that carry out organ-specific functions, such as the tubular networks of the lung, kidney, breast and liver. The spatial organization of cortical domains provides an essential layer of regulation to both biochemical and adhesive receptors on the cell surface, thereby limiting both proliferation and migration of cells in mature tissues. Alterations in the exquisite organization of epithelial structures are the earliest evidence of a developing tumor and signatures of tumor invasion and metastasis. The assembly of cortical domains requires the coordination of processes occurring at the plasma membrane and underlying cytoskeleton, and in particular, the formation of protein complexes that position membrane receptors, control their abundance and activity, and link them to the cortical cytoskeleton, which they modulate. The overarching goal of my laboratory is to understand how the dynamic organization of this cellular compartment contributes to morphogenesis and tumorigenesis. We have focused particular attention on the neurofibromatosis type 2 (NF2) tumor suppressor and closely related ERM proteins (Ezrin, Radixin and Moesin) - membrane:cytoskeleton linking proteins that simultaneously influence membrane complexes and the cortical actomyosin cytoskeleton, with the goals of delineating the molecular function of Merlin, identifying therapeutic targets for familial and sporadic *NF2*-mutant tumors and broadly examining the roles of Merlin/ERMs in development and cancer.



Left: Biliary cells form tubes with an actin- and ERM-rich (red) apical surface; Image credit: Evan O'Loughlin, PhD Student. Right: EGF stimulation rapidly triggers actin/ERM- (green) and pAkt (red) rich macropinocytic cups on the surface of Nf2-/- cells (the nucleus is stained blue in both images). Image credit: Christine Chiasson-MacKenzie, PhD.

Using mouse and bioengineered tissue culture models, we have identified important functions for Merlin and the ERM proteins in morphogenesis and tumorigenesis in many tissues. Cellular and molecular studies reveal that these phenotypes are driven by key, interdependent roles for Merlin and the ERM proteins in governing the dynamic and mechanical properties of the cortical cytoskeleton and, in particular, the inter-relationship between receptor tyrosine kinases (RTKs) and cortical cytoskeleton. Ongoing projects focus on the function of Merlin/ERMs and the membrane:cytoskeleton interface in establishing normal tissue architecture and contributing to tumor initiation and progression in biliary and mammary epithelial tubes, and in Schwann cell:axon relationships; complementary studies focus on how dynamic membrane:cytoskeleton remodeling of the cell surface triggers macropinocytosis, a form of bulk endocytosis that is exploited by some tumors for nutrient scavenging and a preferred conduit for the entry of many therapeutics into tumor cells. Thus far, our studies have provided novel insight into how the organization of the cell cortex governs the individual and collective behavior of cells and drives morphogenetic processes, how defective cortical organization contributes to tumor initiation and progression, and yielded unexpected therapeutic targets and avenues of translation for cancer therapy.

It is increasingly clear that cancer fundamentally reflects the aberrant re-enactment of developmental processes. We believe that the continued partnering of discovery-based science and translational studies will lead to novel therapeutic avenues while continuing to advance our understanding of the basic cellular activities that contribute to many human cancers.

Selected Publications:

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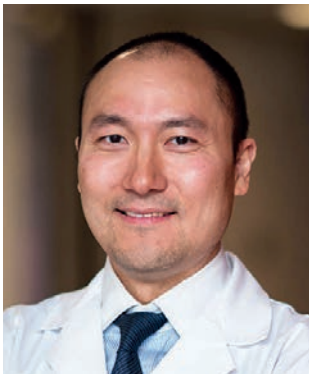
David T. Miyamoto, MD, PhD



The Miyamoto laboratory focuses on the discovery and development of novel biomarkers to guide the personalized treatment of patients with prostate and bladder cancer. We focus on two general classes of biomarkers, namely those based on the molecular profiles of tumor biopsies, and those based on circulating tumors cells (CTCs) in the blood that can be sampled non-invasively and repeatedly. By analyzing these patient-derived specimens, we have identified new molecular predictors of response to therapy and potential mechanisms of treatment resistance. Our overall aim is to develop tools for "real-time precision medicine" to probe the molecular signatures of cancers as they evolve over time, and to guide the precise and rational selection of appropriate therapies for each individual patient with prostate or bladder cancer.

Miyamoto Laboratory

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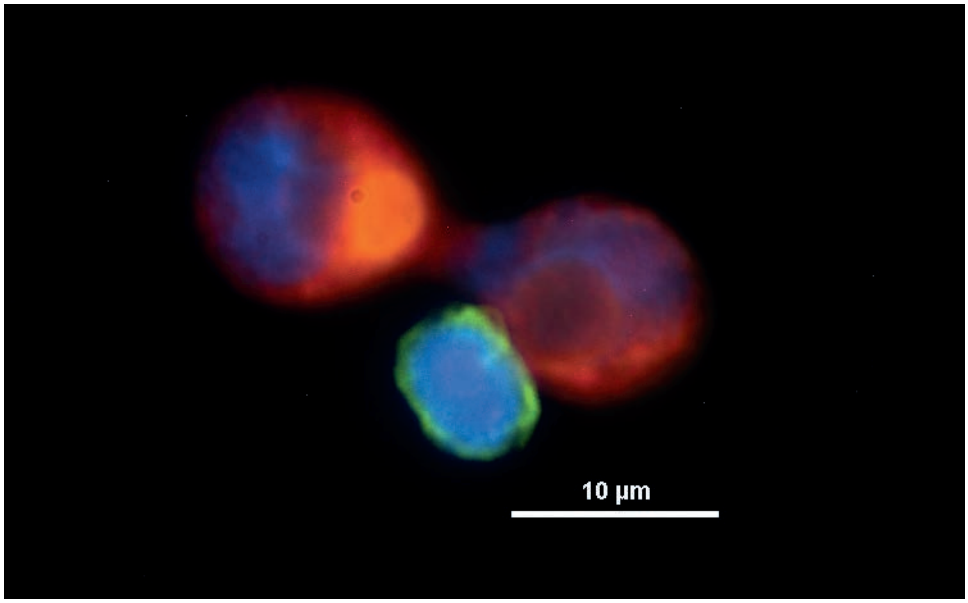
The mission of our translational research laboratory is to discover and develop molecular biomarkers that inform clinical decisions in the management of patients with genitourinary malignancies. We aim to develop circulating and tissue-based biomarkers in a variety of clinical contexts in order to actualize the concept "real-time precision medicine", integrating genomic analyses of liquid and tissue biopsies to guide the personalized care of patients with genitourinary malignancies.

Prostate cancer is the most common cancer in men and the second leading cause of cancer-related death in men. There is a critical unmet need for predictive biomarkers to guide prostate cancer therapy in settings ranging from localized to metastatic disease. In localized prostate cancer, reliable biomarkers are sorely needed to guide the rational selection of appropriate management options tailored to each patient's tumor, including active surveillance, radical prostatectomy, or radiation therapy. In metastatic prostate cancer, multiple FDA-approved therapeutic options that increase survival are now available, including androgen receptor (AR) targeted therapies, cytotoxic chemotherapy, and PARP inhibitors. However, we lack non-invasive biomarkers that can reliably predict treatment responses and precisely guide selection of the most appropriate therapy for each individual patient. A major focus of our laboratory is the investigation of circulating tumors cells (CTCs), which are rare cancer cells shed from primary and metastatic tumors into the peripheral blood. CTCs represent a type of "liquid biopsy" that may be performed repeatedly and non-invasively to monitor treatment efficacy and study tumor evolution during therapy. In collaboration with a multidisciplinary team at MGH, we have developed novel molecular assays using microfluidic technologies to isolate and analyze CTCs from the blood of cancer patients. Our recent studies include the interrogation of androgen receptor (AR) signaling status to predict therapeutic response in patients receiving AR-targeted therapies, and the use of single cell RNA-seq to nominate noncanonical Wnt signaling as a contributor to enzalutamide resistance. Most recently, we derived CTC RNA signatures that predict resistance to AR-targeted therapy in metastatic cancer



A dividing circulating tumor cell isolated from a prostate cancer patient, immunostained for PSA (red), PSMA (orange), and DNA (blue), adjacent to a leukocyte immunostained for CD45 (green) and DNA (blue).

and early dissemination in localized cancer. Ongoing projects include the development of CTC molecular signatures for the prediction of clinical outcomes after radiation therapy, and for the early detection of clinically significant prostate cancer. Another focus of the laboratory is the development of novel tissue-based biomarkers. We utilize technologies including microfluidic real-time PCR, next-generation sequencing, and RNA in situ hybridization (RNA-ISH) to evaluate molecular signatures in limited quantities of tumor biopsy tissues. Our past and ongoing efforts are directed at correlating molecular findings with clinical outcomes in order to identify novel biomarkers predictive of treatment response.

Bladder cancer is the fifth most common cancer in the US, causing 18,000 deaths per year. Muscle-invasive bladder cancer is aggressive and has a high propensity for metastasis, but can often be treated effectively with either radical cystectomy or bladder-sparing trimodality therapy (transurethral tumor resection followed by chemoradiation). However, the decision regarding which treatment to pursue is often made based on arbitrary factors including patient or physician preference. There is an urgent unmet need for molecular biomarkers to guide patients towards the most appropriate therapy based on the biology of their tumor. We recently performed gene expression profiling of bladder tumors from patients treated with bladder preservation therapy, and identified immune and stromal molecular signatures predictive of outcomes after chemoradiation therapy. We are currently evaluating these and other candidate biomarkers as predictors of treatment response in prospective clinical trials and carefully defined retrospective clinical cohorts.

Selected Publications:

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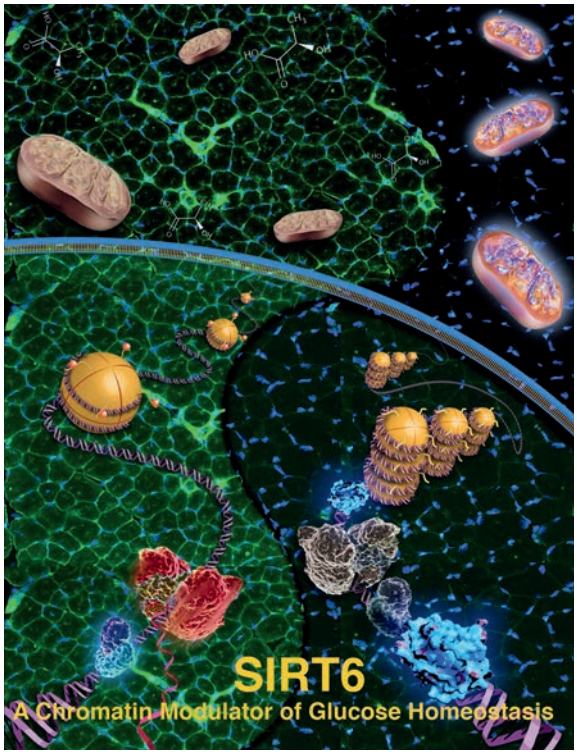
Research in **the Mostoslavsky laboratory** focuses on the crosstalk between chromatin dynamics and cellular metabolism. In particular, we have focused on sirtuins, a family of proteins first discovered in yeast that plays a critical role in many human diseases, including cancer. The yeast protein Sir2 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 indicates that SIRT6 modulates glucose metabolism and DNA repair and functions as a strong tumor suppressor gene. Using transgenic mouse models and other experimental systems, we are exploring the role of SIRT6 and metabolism in tumorigenesis and other disease processes, as well as trying to understand the crosstalk between metabolism and epigenetics.

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, they need a finely tuned system to modulate chromatin dynamics in order to respond to metabolic cues. Reciprocally, chromatin changes necessary for cellular functions need as well to be coupled to metabolic adaptations.

Our lab is interested in understanding the influence of chromatin on nuclear processes (gene transcription, DNA recombination and DNA repair) and the relationship between chromatin dynamics and the metabolic adaptation of cells. One of our interests includes 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. In the past few years, we have been exploring the crosstalk between epigenetics and metabolism. In particular, our work has focused on the mammalian Sir2 homologue, SIRT6. In recent years, we have identified SIRT6 as a key modulator of metabolism. 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 the TCA cycle to reduce intracellular ROS levels. This function appears critical for glucose homeostasis, as SIRT6 deficient animals die early in life from hypoglycemia. Remarkably,



SIRT6: A Chromatin Modulator of Glucose Homeostasis.

SIRT6 acts as a tumor suppressor in colon cancer, regulating 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. We have also uncovered key roles for SIRT6 in DNA repair (anchoring the chromatin remodeler SNF2H to DNA breaks) and early development (acting as a repressor of pluripotent genes), indicating broad biological functions for this chromatin deacetylase. More recently, we identified SIRT6 as a robust tumor suppressor in pancreatic cancer, where it silences the oncofetal protein Lin28b, protecting against aggressive tumor phenotypes. As such, SIRT6 represents an example of a chromatin factor modulated by cancer cells to acquire "epigenetic plasticity".

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

Projects:

1. Deciphering how SIRT6 regulates chromatin structure
2. Determining the role of SIRT6 in tumorigenesis using mouse models
3. Elucidating the role of histone modifications and chromatin dynamics in DNA repair
4. Determining molecular crosstalk between epigenetics and metabolism
5. Assessing metabolic liabilities in cancer and metastases

Selected Publications:

Etchegaray J-P, Zhong L, Li C, Henriques T, Ablondi E, Nakadai T, Van Rechem C, Ferrer, C, Ross KN, Choi J-E, Samarakkody A, Ji F, Chang A, Sadreyev RI, Ramaswamy S, Nechaev S, Whetstine JR, Roeder RG, Adelman K, Goren A, and **Mostoslavsky R**. (2019). The histone deacetylase SIRT6 restrains transcription elongation via promoter-proximal pausing. *Molecular Cell.* 2019 Jul 20. pii: S1097-2765(19)30491-5.

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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.* 2013 Aug 22;51(4):454-68.

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.* 2012 Dec 7;151(6):1185-99.

Mo Motamedi, PhD

Motamedi Laboratory

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Research in **the Motamedi Laboratory** focuses on a molecular memory system, called epigenetics, which allows cells to develop distinct identities during development. Cells develop identities when groups of genes are turned on and off at a given time in a given cell. A focus of the lab is studying the molecular machinery that transmits this gene regulatory information to progeny cells upon division. Another focus for the lab is cellular dormancy. Recently, scientists have discovered that a major reason for cancer resistance and recurrence is that a small number of dormant cancer cells originating from the primary tumor disperse throughout the body. These cancer cells are long-lived and can exit dormancy forming tumors years after remission. None of the existing therapies target dormant cancer cells. By studying dormancy, we have identified a pathway that specifically neutralizes these cells. We believe this discovery will help in addressing this unmet need in cancer therapy.

Epigenetic changes are heritable, phenotypic alterations which occur without mutations to the underlying genes. Once triggered, these phenotypic changes persist through numerous cell divisions independently of the original inducing signal. Epigenetic changes are critical for the stable formation of cellular identities, upon which all developmental processes depend. Disruption to epigenetic regulation underlies a variety of human maladies, including cancers. In fact, epigenetic pathways can contribute to all stages of cancer progression, including initiation, metastasis, resistance and recurrence. Therefore, understanding the molecular mechanisms that establish epigenetic states is fundamental to the development of therapies that target the epigenetic components of cancers.

Often, but not always, epigenetic changes are concomitant with alterations to the chromatin state of underlying genes. Most of what is known about how chromatin states are altered in response to epigenetic triggers comes from decades of research in model organisms. These studies have revealed highly conserved protein families, which are now used for therapeutic or diagnostic purposes in cancers. The Motamedi lab uses the fission yeast as a model to understand how changes to eukaryotic chromatin are made, maintained and propagated, and how these changes establish alternative transcriptional programs particularly in response to persistent stress.

Noncoding RNAs and chromatin – partners in epigenetic regulation

One of the first models for how long and small noncoding RNAs regulate chromatin states was proposed in the fission yeast. It posits that noncoding RNAs, tethered to chromatin, provide a platform for the assembly of RNA-processing and chromatin-modifying proteins (Motamedi et al 2004), leading to transcriptional regulation of the underlying genes. In addition to acting as platforms, RNA molecules also can function as *trans*-acting factors, targeting chromatin regulatory proteins to specific chromosomal regions. These principles now have emerged as conserved mechanisms by which noncoding RNAs partake in chromatin regulation in eukaryotes including in humans.

The image depicts cells as they enter quiescence (moon), and load Ago1 (ships) with euchromatic small RNAs to mediate Quiescent-induced Transcriptional Repression (Q) of a set of euchromatic genes. Exosome activity separates heterochromatic (dark blue) from euchromatic (yellow) regions. When entering quiescence, the exosome barrier opens, permitting euchromatic transcripts (differently colored dots) to become substrates for RNAi degradation. Ago1, acquiring new color (sRNAs) as it crosses the exosome barrier, targets Q to the corresponding color in euchromatin.

A focus of the lab is cellular quiescence (or G0). G0 is a ubiquitous cellular state in which cells exit proliferation and enter a state of reversible dormancy. Developmental programs, such as wound healing, or exposure to a variety of stress, such as starvation, can trigger entry into or exit from G0. G0 cells have distinct transcriptional programs through which they acquire new properties compared to their proliferative selves, including long life, thrifty metabolism and resistance to stress. Loss of G0 regulation results in defects in developmental and adaptive programs. How cells enter, survive and exit G0 is a critical question in basic biology, which is largely unexplored. To address this knowledge gap, we modeled G0 in fission yeast and showed that when cells transition to G0, new ncRNAs emerge which coopt and deploy constitutive heterochromatin proteins (histone H3 lysine 9 methyltransferase, Clr4/SUV39H) to several euchromatic gene clusters to regulate the expression of a set of developmental, metabolic and cell cycle genes. We show that this pathway is critical for survival and the establishment of the global G0 transcriptional program. This work revealed a new function of heterochromatin proteins and noncoding RNAs, which orchestrate the genome-wide deployment of heterochromatin factors in response to long-term stress. It also led to the proposal of several hypotheses that we are currently testing. Moreover, in collaboration with several groups, we have begun to test whether this pathway also plays an important role in cancer dormancy and treatment resistance.

Selected Publications:

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Khanduja JS, Calvo IA, Joh RI, Hill IT, **Motamedi M**. Nuclear noncoding RNAs and genome stability. *Mol Cell* 2016; 63: 7-20.

Li H*, **Motamedi M***, Yip C, Wang Z, Walz T, Patel DJ, Moazed D. An alpha motif at Tas3 C terminus mediates RITS cis-spreading and promotes heterochromatic gene silencing. ††*Mol Cell*. 2009; 34: 155-167.

Motamedi M, Hong EE, Li X, Gerber S, Denison C, Gygi S, Moazed D. HP1 proteins from distinct complexes and mediate heterochromatic gene silencing by non-overlapping mechanisms. *Mol Cell.* 2008; 32: 778-790.

Motamedi M*, Verdel A*, Colmenares S*, Gerber S, Gygi S, Moazed D. Two RNAi complexes, RDRC and RITS, physically interact and localize to non-coding centromeric RNAs. †††*Cell*. 2004; 119: 789-802.

*Co-authors

†This paper was the cover story in *Molecular Cell* and featured in *Boston Magazine* (*http://www.bostonmagazine.com/sponsor-content/mgh-study-potentially-finds-the-achilles-heel-for-dormant-cancer-cells/*)

††This article was previewed in *Dev Cell*. 16: 630-632, 2009

†††This article was the cover story in *Cell*

Christopher J. Ott, PhD

Ott Laboratory

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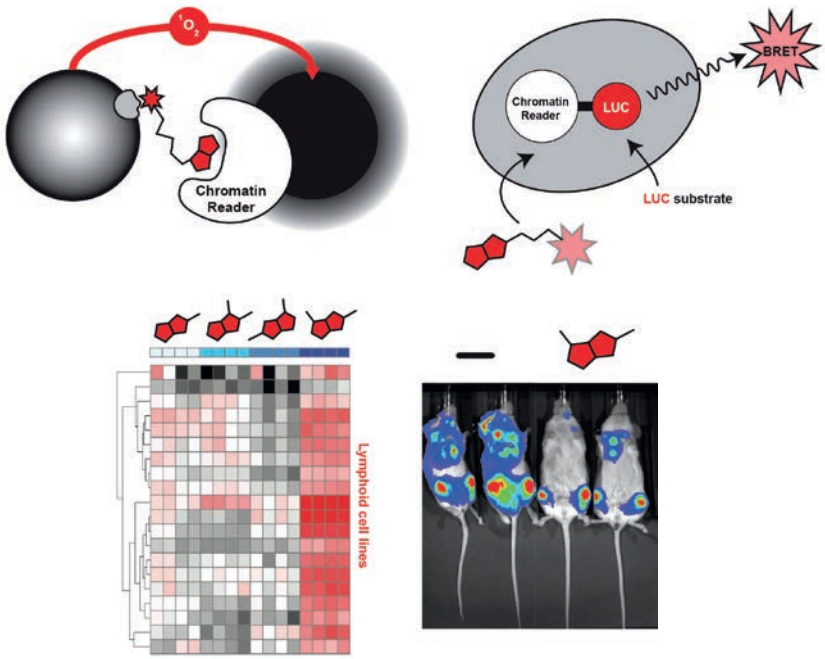
Mutations in cancer cells lead to malfunctioning control of gene expression. **The Ott laboratory** is dedicated to discovering the gene expression control factors that are essential for cancer cell survival. Discovery of these factors prompts further efforts in our group to design chemical strategies for the synthesis and deployment of prototype drugs targeting the aberrant mechanisms of gene control. Biologically, gene control factors represent compelling therapeutic targets for these cancers, as they are master regulators of cell identity. Yet despite this clear rationale, most are perceived as intractable drug targets owing to their large size, disordered shapes, and involvement in complex cellular circuits. Recent advances in gene editing technologies and discovery chemistry have advanced our capability to rapidly identify targetable aspects of gene control and methods to disrupt their function. We use these genetic and chemical tools to probe cancer cell circuitry and inform therapeutic hypotheses.

Chemical modulation of bromodomains

Gene control factors bind to regions of transcriptionally active chromatin called enhancers. Enhancers are critical for driving cell-type specific gene expression, and their chromatin structures are typically marked with specific histone modifications. Among the most distinctive is lysine side-chain acetylation, recognized (or 'read') by histone modules called bromodomains. Recently, novel chemical compounds have been advanced that selectively target the bromodomains of the bromodomain and extra terminal domain (BET) family. These compounds efficiently displace BET proteins from active enhancer chromatin, and we and others have found them to be active agents in models of acute leukemia, lymphoma, and several solid tumor types. Using a suite of genome-wide chromatin and transcriptomic assays, we aim to understand principles of bromodomain dependency in cancer. Efforts are ongoing to establish biomarkers for response and resistance, and realize promising rationales for combination therapies with other targeted agents.

Essential enhancers

Classic studies have described oncogenic enhancers in leukemia and lymphoma cells. This aberrant enhancer activity can occur by chromosomal translocation of proto-oncogenes such as *MYC* and *BCL2*. In addition to chromosomal translocations, cancer-specific enhancers have been described at proto-oncogene loci like *TAL1* and *MYC*, which are aberrantly bound by transcription factors through direct somatic mutation of enhancer DNA elements or focal amplification. We have generated high-resolution enhancer landscapes derived from primary patient samples, including a large cohort of chronic lymphocytic leukemia samples (Ott et al, *Cancer Cell* 2018). Current projects include construction of core regulatory transcription factor circuitries, and the discovery of inherited and somatic



Expanding the chromatin chemical probe toolbox with high throughput bead-based proximity assays, cellular target engagement assessment, cell line viability profiling, and in vivo pharmacology.

variants leading to aberrant gene expression. Using genetic and epigenetic genome editing techniques, we are functionally dissecting malfunctioning enhancers and their cognate bound factors to derive mechanistic understanding of the essential enhancers principally responsible for maintaining leukemia and lymphoma cell states.

Expanding the chromatin chemical probe toolbox

The successful discovery of chemistry efforts that yielded efficient BET bromodomain inhibitors has revealed chromatin reader domains broadly, and bromodomains specifically, as protein modules amenable for small molecule ligand development. Used experimentally, enhancer-targeting compounds enable precise disruption of chromatin features and can be used to identify and validate discrete biophysical and biochemical functions of target proteins. Paired with an understanding of integrated epigenomics, these probes enable the elucidation of fundamental insights into genome structure and function. We use high-throughput protein-protein interaction assays and cellular assays of chromatin reader activity to identify reader domain inhibitors. Lead compounds are iteratively optimized for potency and selectivity, followed by functional assessments on epigenome structure. Leukemia and lymphoma cell viability profiling and in vivo pharmacokinetic and pharmacodynamic studies enable the nomination of next-generation inhibitors of essential chromatin readers. Ongoing projects seek to expand our current toolbox of bromodomain inhibitors, with a particular focus on 'orphan' factors for which selective compounds have yet to be developed.

Selected Publications:

Ott CJ*^, Federation AJ*, Schwartz LS, Kasar S, Klitgaard JL, Lenci R, Li Q, Lawlor M, Fernandes SM, Souza A, Polaski D, Gadi D, Freedman ML, Brown JR^, Bradner JE^. Enhancer architecture and essential core regulatory circuitry of chronic lymphocytic leukemia. *Cancer Cell*. 2018; 34: 982-995.

Gechijian LN, Buckley DL, Lawlor MA, Reyes JM, Paulk J, **Ott CJ**, Winter GE, Erb MA, Scott TG, Xu M, Seo HS, Dhe-Paganon S, Kwiatkowski NP, Perry JA, Qi J, Gray NS, Bradner JE. Functional TRIM24 degrader via conjugation of ineffectual bromodomain and VHL ligands. *Nat Chem Biol*. 2018; 14: 405-412.

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^ Co-corresponding authors

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Grace Yuen, PhD

* PhD Candidate

The Pillai laboratory asks questions about the biology of the immune system and susceptibility to disease. Some of these questions are 1) can we manipulate the immune system to treat autoimmunity and 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 systemic sclerosis and IgG4-related 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 are also exploring novel ways to strengthen immune responses and enhance helper T cell memory that provide hope for developing more effective personalized immune-system based treatments for cancer.

Pathogenesis of fibrosis (NIAID Autoimmune Center of Excellence at MGH)

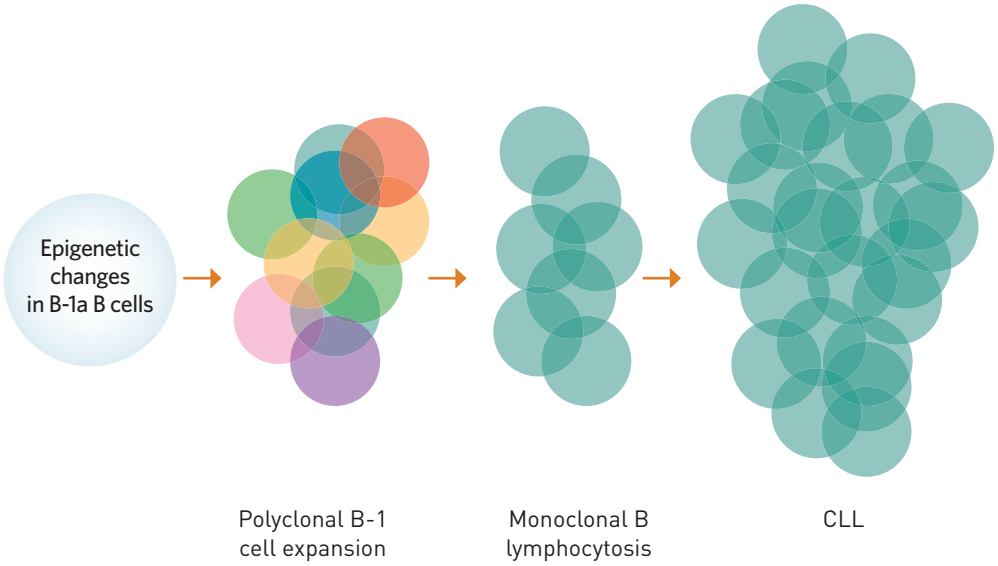
In studies on the immunology of IgG4 related disease and scleroderma, performed in collaboration with John Stone (MGH Rheumatology) and Dinesh Khanna, (U. of Michigan, Rheumatology), we have identified an unusual, clonally expanded and potentially "fibrogenic" human CD4+ effector T cell subset in affected tissues. The differentiation and protective role of these CD4+ CTLs in cancer and chronic viral infections are currently being investigated using chromatin accessibility mapping, DNA methylation studies and single cell RNA-seq approaches. We have also discovered unusual B cell populations that are potentially fibrogenic both in the context of autoimmune fibrotic diseases and pancreatic cancer.

Studies on murine and human B cell development and activation

We are using a number of single cell transcriptomic, epigenetic and genetic approaches to examine the heterogeneity and development of murine and human B cells, as well as the molecular bases of the processes of T-B collaboration and germinal center formation.

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. The contributions of DNA methylation and demethylation to the biology of CD4+ CTL and T_{FH} cells are also being investigated.



A model for the evolution of CLL.

Studies on Human *CTLA4* mutations and early B cell development

The underlying mechanism for the human B cell developmental defect in individuals with *CTLA4* mutations has been studied helping us to better understand how regulatory T cells can influence early B cell development and humoral autoimmunity.

Selected Publications:

Della-Torre E, Rigamonti E, Perugino C, Sain SB, Sun N, Maehara T, Kaneko N, Rovati L, Lanzillota M, Mahajan V, Mattoo H, Molineris I, Deshpande V, Stone JH, Falconi F, Manfredi AA and **Pillai S**. B lymphocytes directly contribute to fibrosis in IgG4-related disease. *Journal of Allergy and Clinical Immunology*. 2019 Jul 15. pii: S0091-6749(19)30936-4.

Perugino C, AlSalem SB, Mattoo H, Della-Torre E, Mahajan VS, Ganesh G, Allard-Chamard H, Wallace Z, Montesi MB, Kreuzer J, Haas W, Stone JH, **Pillai S**. Identification of Galectin-3 as an auto-antigen in IgG4 related-disease. *Journal of Allergy and Clinical Immunology* 2019 143, 736-745.

Maehara T, Mattoo H, Mahajan VS, Murphy SJH, Yuen GJ, Ishiguro N, Ohta M, Moriyama M, Saeki T, Yamamoto H, Yamauchi M, Daccache J, Kiyoshima T, Nakamura S, Stone JH and **Pillai S**. The expansion in lymphoid organs of IL-4+ BATF+ T follicular helper cells is linked to IgG4 class switching in vivo. *Life Science Alliance* 2019 Epub April 13.

Fraschilla I, **Pillai S**. Viewing Siglecs through the lens of tumor immunology. *Immunol Rev*. 2017 Mar; 276(1):178-191.

Luca Pinello, PhD

The focus of **the Pinello laboratory** is to use innovative computational approaches and cutting-edge experimental assays, such as genome editing and single cell sequencing, to systematically analyze sources of genetic and epigenetic variation and gene expression variability that underlie human traits and diseases. The lab uses machine learning, data mining and high performance computing technologies, for instance parallel computing and cloud-oriented architectures, to solve computationally challenging and Big Data problems associated with next generation sequencing data analysis. Our mission is to use computational strategies to further our understanding of disease etiology and to provide a foundation for the development of new drugs and novel targeted treatments.

Pinello Laboratory

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shared with Daniel Bauer lab

* Visiting PhD student

Epigenetic variability in cellular identity and gene regulation

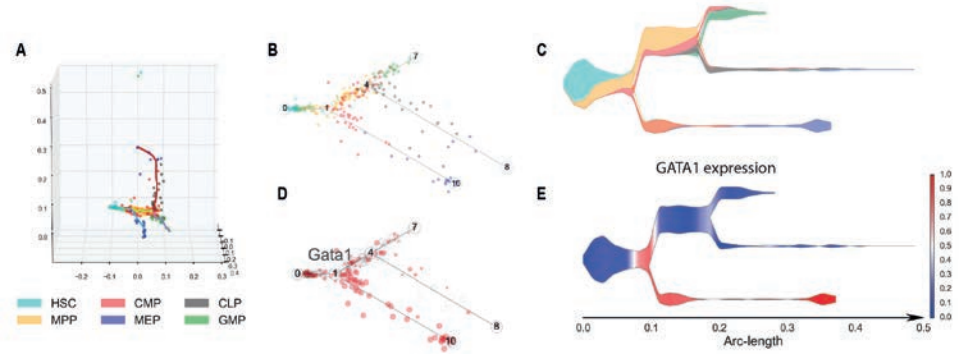
We are studying the relationship between epigenetic regulators, chromatin structure and DNA sequence and how these factors influence gene expression patterns. We recently proposed an integrative computational pipeline called HAYSTACK (*https://github.com/lucapinello/Haystack*). HAYSTACK is a software tool to study epigenetic variability, cross-cell-type plasticity of chromatin states and transcription factor motifs and provides mechanistic insights into chromatin structure, cellular identity and gene regulation. By integrating sequence information, histone modification and gene expression data measured across multiple cell-lines, it is possible to identify the most epigenetically variable regions of the genome, to find cell-type specific regulators, and to predict cell-type specific chromatin patterns that are important in normal development and differentiation or potentially involved in diseases such as cancer.

Computational methods for genome editing

Recent genome editing technologies such as CRISPR/Cas9 are revolutionizing functional genomics. However computational methods to analyze and extract biological insights from data generated with these powerful assays are still in an early stage and without standards. We have embraced this revolution by developing cutting-edge computational tools to quantify and visualize the outcome of CRISPR/Cas9 experiments. We created a novel computational tool called CRISPResso2 (*http://github.com/pinellolab/CRISPResso2*), an integrated software pipeline for the analysis and visualization of CRISPR-Cas9 and base editor outcomes from deep sequencing experiments, as well as a user-friendly web application that can be used by non-bioinformaticians (*http:// crispresso.rocks*). In collaboration with Daniel Bauer's and Stuart Orkin's groups, we recently applied CRISPResso and other computational strategies to aid the development of an *in situ* saturation mutagenesis approach for dissecting enhancer functionality in the blood system with the aim of developing potential therapeutic genome editing applications for hemoglobin disorders.

Exploring single cell gene expression variation in development and cancer

Cancer often starts from mutations occurring in a single cell that results in a



STREAM on transcriptomic data from the mouse hematopoietic system. A) Dimensionality reduction, reconstructed hierarchical structure composed of curves approximating the inferred trajectories. Single cells are represented as circles and colored according to the FACS sorting labels. B) Flat tree representation at single cell resolution; branches are represented as straight lines, (cells are represented as in A). The length of the branches and the distances between cells and assigned branches are proportional to the original representation in the 3D space. C) Rainbow plot: intuitive visualization to show cell type distribution and density along different branches. D) Single cell resolution expression pattern of GATA1, each circle is red filled proportionally to the relative expression of GATA1 in the whole population. E) Relative expression of GATA1 in each branch using the representation in C.

heterogeneous cell population. Although traditional gene expression assays have provided important insights into the transcriptional programs of cancer cells, they often measure a combined signal from a mixed population of cells and hence do not provide adequate information regarding subpopulations of malignant cells. Emerging single cell assays now offer exciting opportunities to isolate and study individual cells in heterogeneous cancer tissues, allowing us to investigate how genes transform one subpopulation into another. Characterizing stochastic variation at the single cell level is crucial to understanding how healthy cells use variation to modulate their gene expression programs, and how these patterns of variation are disrupted in cancer cells. We have developed a method called STREAM to model the variability of gene expression at single cell resolution, and to reconstruct developmental trajectories (see illustrative image) using data from single cell assays such as scRNA-seq, multiplexed qPCR or sc-ATAC-seq. This method can be used for disentangling complex cellular types and states in development, cancer, differentiation or in perturbation studies.

Selected Publications:

Chen H, Albergante L, Hsu JY, Lareau CA, Lo Bosco G, Guan J, Zhou S, Gorban AN, Bauer DE, Aryee MJ, Langenau DM, Zinovyev A, Buenrostro JD, Yuan GC†, **Pinello L**.† Single-cell trajectories reconstruction, exploration and mapping of omics data with STREAM. *Nat Commun*. 2019 Apr 23;10(1):1903.

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Canver MC*, Smith EC*, Sher F*, **Pinello L***, Sanjana NE*, Shalem O, Chen DD, Schupp PG, Vinjamur DS, Garcia SP, Luc S, Kurita R, Nakamura Y, Fujiwara Y, Maeda T, Yuan G-C, Zhang F, Orkin SH & Bauer DE. BCL11A enhancer dissection by Cas9-mediated in situ saturating mutagenesis. *Nature*. 2015 Nov 12;527(7577):192-72015 Sep.

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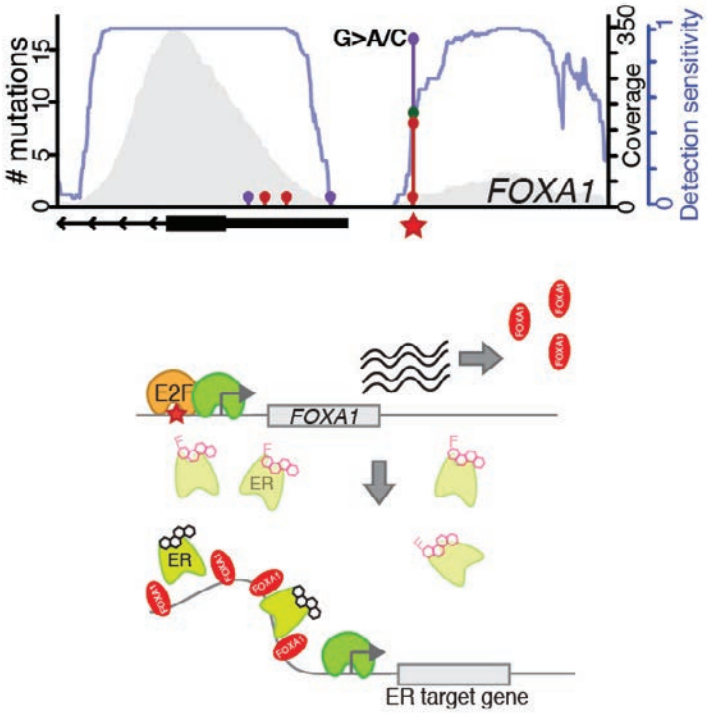
Esther Rheinbay, PhD

Most known genomic drivers of cancer are in coding genes, affecting the encoded protein's interaction with other proteins, DNA or biological compounds. Recent advances in DNA sequencing technology have made it possible to study non-coding regions that regulate these protein-coding genes. Several cancer drivers have been identified and characterized in these regulatory regions, however, this genomic territory remains relatively unexplored in human tumors. **The Rheinbay laboratory** concentrates on identifying and functionally characterizing these non-coding drivers in the sequences of tumor whole genomes through development of novel analysis strategies and collaborations with experimental investigators.

We are also interested in tumors, especially breast cancers, for which no known protein-coding driver alterations have been found. In the age of targeted therapy, these tumors pose a special challenge in that they leave few treatment options for patients beyond conventional chemotherapy. We believe that finding novel genomic and epigenomic, protein-coding and regulatory therapeutic targets in these tumors will have significant clinical implications.

Regulatory driver mutations in cancer genomes

Genomic cancer driver discovery has traditionally focused on protein-coding genes (the human exome), and large-scale sequencing of these genes in thousands of tumors has led to the discovery of novel frequently altered genes. However, exome sequencing focused only on coding genes does not allow analysis of non-coding regions in the human genome. Protein-coding genes are regulated by several types of genomic elements that control their expression (promoters, distal enhancers and boundary elements), translation (5'UTRs) and mRNA stability (3'UTRs). Alterations in the DNA sequence of these elements thus directly affect the expression and regulation of the target gene. Several such non-coding elements have been identified as recurrently altered in human cancer, and functionally characterized, although these non-coding drivers appear infrequent compared to protein-coding oncogenes and tumor suppressors. One reason might be that gene regulation is highly tissue-specific, and therefore driver alterations in non-coding regions might create a fitness advantage in only a single tumor type. Finding such a specific driver requires a sufficient number of whole genomes from this tumor type. With recent advances in DNA sequencing technology and an increasing number of whole cancer genomes available for analysis, we are just starting to map out and characterize regulatory driver alterations. The Rheinbay laboratory works on the development of novel methods to identify non-coding driver candidates using genomic and epigenomic sources of information, and to understand their impact on tumor initiation, progression and treatment resistance through collaborations with experimental colleagues. We have



Hotspot mutation in the FOXA1 promoter in breast cancer and proposed mechanism of action.

recently identified a recurrent mutation in the promoter of the breast cancer oncogene FOXA1. This mutation increases expression through augmenting a binding site for E2F, leading to E2F protein recruitment. In addition, *FOXA1* overexpression leads to resistance to the breast cancer drug, fulvestrant. We are now investigating the implications and mechanism of action of this mutation in breast cancer progression and treatment resistance.

Finding targetable vulnerabilities in cancers without known drivers

From recent large genome and exome sequencing studies of different cancer types, it has become apparent that there are almost always patients whose tumors harbor no common driver alteration such as *BRAF* mutation in melanoma, *HER2* amplification, or hormone receptor expression in breast and prostate cancer. In an era of treatments targeting such alterations specific to a patient's cancer cells, a lack of potentially druggable cancer drivers severely limits the repertoire of available therapy options. Rather than being truly without any drivers, these tumors are likely driven by yet uncharacterized protein-coding or regulatory genomic alterations, or an oncogenic state induced and maintained by epigenetic changes. Our research is focused on finding the drivers and vulnerabilities of these particular tumors by integrating genomics and epigenomics data, with the ultimate goal of connecting patients to effective targeted treatments.

Selected Publications:

Rheinbay E, Parasuraman P, Grimsby J, et al. Recurrent and functional regulatory mutations in breast cancer. *Nature*. 2017;547:55-60.

Suva ML*, **Rheinbay E***, Gillespie SM, et al. Reconstructing and reprogramming the tumor-propagating potential of glioblastoma stem-like cells. *Cell*. 2014;157:580-94.

Rheinbay E*, Suva ML*, Gillespie SM, et al. An aberrant transcription factor network essential for Wnt signaling and stem cell maintenance in glioblastoma. *Cell Reports*. 2013;3:1567-79.

*Equal contribution

Miguel N. Rivera, MD

Research in **the Rivera laboratory** focuses on using genomic tools to identify and characterize gene regulation pathways that are altered in cancer. An important feature shared by most tumors is the dysregulation of complex gene expression programs that control cell proliferation and differentiation. Our work combines the use of genomic technologies for the direct identification of gene regulation abnormalities in tumors with functional analysis of critical pathways in several model systems. Given that the mechanisms that drive changes in gene expression programs are poorly understood, we anticipate that our work will point to new therapeutic approaches.

Rivera Laboratory

Alexandra Cauderay
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Epigenomic approaches for the identification of novel pathways in cancer

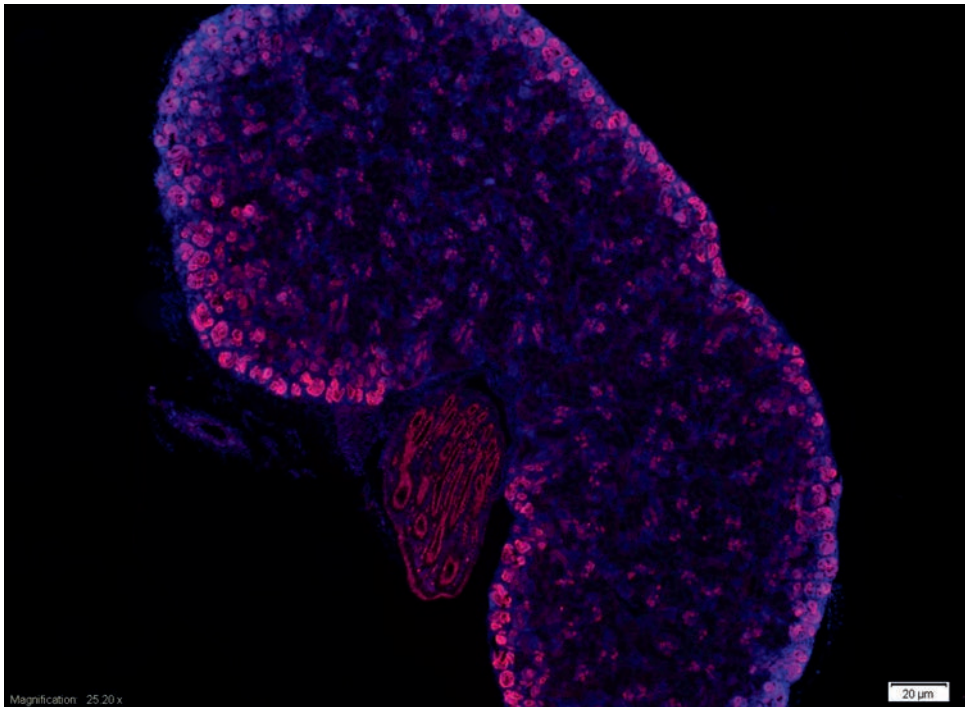
While genetic studies have led to the development of important cancer therapies, most genetic alterations in cancer do not point to specific therapeutic targets. In the case of pediatric cancers, which are often driven by low numbers of recurrent mutations, the identification of therapeutic targets through genetic studies has been particularly challenging. In order to discover new pathways involved in these tumors, we are using new genomic technologies to identify abnormalities in the mechanisms that regulate gene expression programs controlling cell proliferation and differentiation.

One of these technologies is genome-wide chromatin profiling, which combines chromatin immunoprecipitation and high-throughput sequencing. This approach has been used to study how genes are activated or repressed by regulatory elements in the genome such as promoters and enhancers. As a complement to gene expression studies, chromatin profiling provides a unique view of gene regulation programs by allowing the identification of both active and repressed genomic domains based on patterns of histone modification. Several studies have shown that prominent active histone marks are associated with genes that play key roles in cell identity and proliferation, including oncogenes that promote the growth of tumor cells. In contrast, repressive marks are found at loci that are maintained in an inactive state to prevent cellular differentiation.

In recent studies we have applied chromatin profiling to Wilms tumor, Ewing sarcoma and medulloblastoma, three pediatric tumors that are thought to arise from stem cell precursors and that have been linked to abnormalities in transcriptional regulation. Our work has uncovered novel genes and pathways involved in these diseases by comparing chromatin patterns in primary tumor samples and normal tissue specific stem cells. In addition, we have identified gene regulation mechanisms that play critical roles in tumor formation through functional studies of transcription factors and chromatin regulators. We are now characterizing these pathways in detail and extending our epigenomic analysis to other tumor types where oncogenic pathways are poorly defined.

Role of the WTX gene family in cancer and development

Wilms tumor, the most common pediatric kidney cancer, is a prime example of



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.

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 formation. We identified WTX, an 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). More recently, large tumor sequencing studies have shown that WTX is also inactivated in several other tumor types. WTX is the founding member of a new protein family (FAM123/AMER) and is expressed in the stem cells 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 profound alterations in the development of several organs including kidneys, bones and fat by causing changes in the differentiation programs of mesenchymal stem cells. In particular, we observed an expansion of mesenchymal kidney stem cells, suggesting that WTX regulates the balance between proliferation and differentiation in these cells. We are now using a combination of in vitro and in vivo approaches to elucidate the molecular mechanisms by which WTX and related proteins regulate stem cells. Given that the same mechanisms are likely to be operative in tumors where WTX is inactivated, we expect that our studies may reveal new therapeutic opportunities for a variety of tumor types.

Selected Publications:

Boulay G, Sandoval GJ, Riggi N, Iyer S, Buisson R, Naigles B, Awad ME, Rengarajan S, Volorio A, McBride MJ, Broye LC, Zou L, Stamenkovic I, Kadoch C, **Rivera MN**. Cancer-specific retargeting of BAF complexes by a prion-like domain. *Cell*. 171(1-16), 2017 Sept 21.

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*Co-authors

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Sgroi Laboratory

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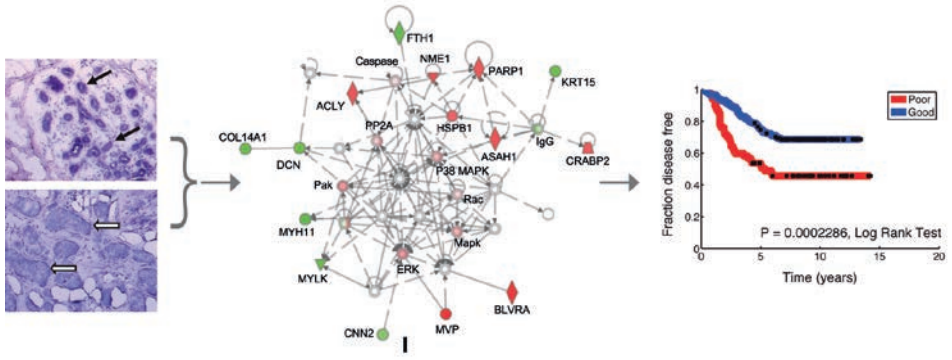
The overarching goals of research in **the Sgroi laboratory** are to develop better ways to identify patients who are at risk for the development of breast cancer and to identify those breast cancer patients who are likely to benefit from targeted drug therapies. We are taking several different approaches 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.

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.

Presently, my laboratory is focused on applying high-throughput molecular technologies to identify biomarkers that will 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



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).

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.

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Sgroi DC, Sestak I, Cuzick J, Zhang Y, Schnabel CA, Schroeder B, Erlander MG, Dunbier A, Sidhu K, Lopez-Knowles E, Goss PE, and Dowsett M. Prediction of late distant recurrence in patients with oestrogen-receptor-positive breast cancer: a prospective comparison of the Breast Cancer Index (BCI) assay, 21-gene recurrence score, and IHC4 in TransATAC study population. *Lancet Oncol*. 2013 Oct;14(11):1067-76.

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Toshihiro Shioda, MD, PhD

Shioda Laboratory

Molecular Profiling Laboratory

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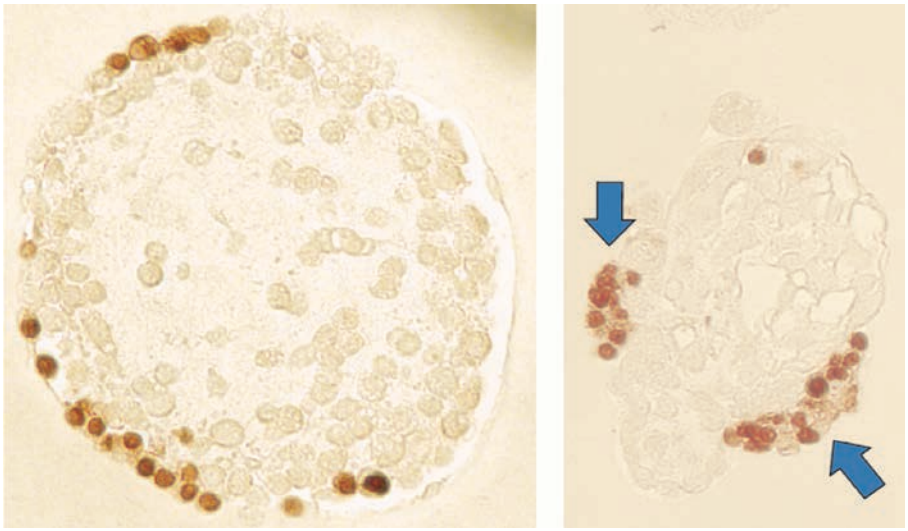
The Shioda laboratory is interested in the biology and diseases of human germline cells, which are committed to producing gametes (sperm or eggs). Primordial germ cells (PGCs) are the first germline cells emerging in human embryos during the third week of gestation. Malignant transformation of male PGCs results in testicular cancers, the most common cancers in young US men between the ages of 15 and 35. Whereas the DNA of PGCs loses most of its gene-silencing machinery to reset its gene expression program, DNA regions encoding the Human Endogenous Retroviruses (HERVs), which are remnants of ancient retroviral infection, selectively remain silent. Activation of HERVs may cause various disorders such as autoimmune diseases and cancers. Although mechanisms of HERV activation in diseases are largely unknown, we hypothesize that relaxed HERV silencing in PGCs under stresses may predispose HERVs to be activated beyond fertilization. Because access to human embryonic PGCs is extremely challenging, our laboratory takes advantage of human iPSC-derived PGC-like cell culture models to study normal biology and mechanisms of diseases involving PGCs.

Epigenetically Provoked Multi-generational Disease Predispositions Involving Aberrant Germline Epigenetic Reprogramming in Primordial Germ Cells

The germline is a series of specialized cell population destined for gametogenesis – i.e., production of sperm and eggs. Thus, germline cells are solely responsible for conveying genetic and epigenetic information to the subsequent generation. All heritable genetic aberrations, including mutations causing familial cancer predispositions, occur exclusively in the germline. Recent studies, including ours, showed that *in utero* exposure of mammalian germline cells to various types of stresses such as therapeutic drugs, toxic environmental chemicals, or malnutrition may create trans-generationally heritable epigenetic aberrations that could cause adult-onset diseases such as cancers or metabolic disorders. In the third week of gestation, human primordial germ cells (PGCs), the earliest-stage germline cells, are observed in the embryonic yolk sac as a cluster of only 40 cells. While rapidly proliferating, PGCs migrate towards genital ridges, where they differentiate into sex-specific germline stem cells. Genomic DNA of PGCs lose cytosine methylation globally and almost completely except for a few specific elements such as regions encoding the Human Endogenous Retroviruses (HERVs). We presume that this robust epigenetic reprogramming occurring uniquely in PGCs may make PGCs especially vulnerable to epigenetic aberrations that cause disease predispositions. Since activation of HERVs are linked to various human diseases such as cancers, autoimmune diseases, and resistance to cancer immunotherapy, our current hypothesis is that stress-induced relaxation of epigenetic machineries silencing HERVs in PGCs may predispose a subset of HERVs to accidental activation and thus increase risks of diseases observed in the



Emergence of human PGC-LCs on the surface of embryoid bodies. Human PGC-LCs are visualized by anti-OCT4 immunohistochemistry of FFPE slides. Most PGC-LCs are localized in the outermost surface layer of embryoid bodies (left). PGC-LCs often form dense clusters (arrows; right), which may mimic the embryonic niche involved in germline commitment of precursor cells.

subsequent generations.

Experimental testing of the above hypothesis faces multiple hurdles. Access to human embryonic PGCs is extremely challenging due to technical and ethical reasons. Molecular mechanisms of PGC commitment and differentiation are significantly different between human and the conventional laboratory rodents. HERVs are unique to humans although the genome of mice harbors IAPs (Intra-cisternal A Particle), a rodent-specific group of endogenous retroviruses that are known to cause various epigenetically provoked diseases. To overcome these hurdles, my laboratory takes advantage of PGC-LCs (PGC-Like Cells), a cell culture model of PGCs generated from iPSCs. In contrast to other protocols that produce PGC-LCs inside iPSC aggregates, our protocol produces PGC-LCs exclusively on the surface of embryoid bodies. This is an important advantage to study effects of exposures to drugs or toxic chemicals on PGC-LCs. In our initial studies, we have shown robust and global DNA demethylation in the genome of mouse PGC-LCs whereas a few types of repetitive elements such as IAPs escaped the erasure, resembling late-stage mouse embryonic PGCs. We also demonstrated that aberrant DNA hypermethylation artificially introduced in mouse iPSCs was effectively repaired in PGC-LCs during the course of germline epigenetic reprogramming. Our recent studies produced PGC-LCs from human iPSCs using our own protocol for improved robustness and experimental reproducibility and showed that human PGC-LCs produced in our lab as well as other labs reflect an earlier stage of embryonic PGCs than mouse PGC-LCs. Thus, global DNA demethylation in the current version of human PGC-LCs was still in its early initiation state and weak. Nonetheless, we were able to detect activation of a specific subset of HERVs in human PGC-LCs that were strictly silenced in the precursor iPSCs, suggesting the existence of a group of HERVs that are especially prone to activation in human germline. Our current research focuses on the molecular mechanisms that silences HERVs in PGCs and their vulnerabilities to stresses. Attempts are also being made to determine whether germline activation of HERVs is involved in mechanisms of the epigenetically inherited disease predispositions to cancers and other human diseases.

Selected Publications:

Diaz-Castillo C, Chamarro-Garcia R, **Shioda T**, and Blumberg B. Transgenerational self-reconstruction of disrupted chromatin organization after exposure to an environmental stressor in mice. *Scientific Reports*. 2019, in press.

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David Spriggs, MD

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Oladapo Yeku, MD, PhD

The Spriggs laboratory has been focused on proteins present on the ovarian cancer cell surface and how those proteins regulate function in health and cancer. In particular, we are interested in MUC16 and Galectin 3. Our studies over the past several years have provided insights into the function of MUC16. It is now apparent that the MUC16 regulates functions like cancer growth and spreads through changes in the structure of sugars (glycosylation) on the surface of cancer cells. This regulation requires interaction with specialized sugar binding proteins called Galectins, which are key components of the tumor microenvironment. We are actively developing new antibodies against MUC16 and Galectin 3 for diagnosis, imaging and treatments. Our work has shown that antibodies which inhibit these cell – cell interactions can slow tumor growth and block the spread of cancer cells locally and inhibit the spread to new organs.

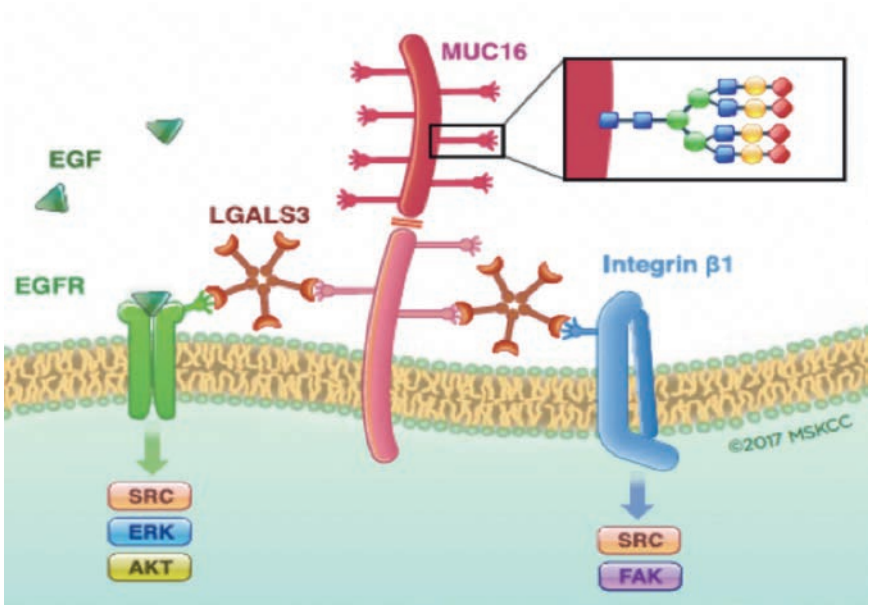
Our research group is actively examining the role of glycosylation, especially on mucins in tumor specific behaviors including uncontrolled growth, oncogene activation, invasion, immune system evasion angiogenesis, and metastatic spread. This work includes potential therapeutic antibodies against MUC16 and Galectin-3 in cancer.

Anti-MUC16 biology

Our current MUC16 work concentrates on development of our human MUC16 antibodies for targeting ovarian cancer. Our antibodies uniquely target the most proximal, retained portion of the MUC16 following cleavage and release of the CA125 antigen into the circulation. This retained ectodomain is a 58 amino acid peptide, linked to the membrane via a short transmembrane domain and a 31 amino acid cytoplasmic tail which is linked to the cellular cytoskeleton for mobility. We have shown that most of the adverse consequences relate to MUC16 expression. As little as 114 amino acids from the carboxyl terminal of the intact MUC16 sequence is sufficient to promote increased soft agar colony formation, Matrigel invasion with increased MMP2/MMP9 expression, activation of both AKT and ERK proto-oncogenes, and enhanced growth in nude mice. Deletion experiments demonstrate that the 58 amino acid MUC16 ectodomain is required for this effect. If one examines the ectodomain in greater detail, the portion of the sequence containing 2 N-glycosylation sites is the essential element. We (esp. Dr. Lee) are now actively examining the structure of the MUC16 – antibody interaction to improve the therapeutic efficacy of antibodies.

MUC16-directed Chimeric Antigen Receptor (CAR) T Cells

Chimeric Antigen Receptor (CAR) T cells have not been successful in the management of solid tumor malignancies. Reasons for this include: poor trafficking, the presence of an immunosuppressive tumor microenvironment, CAR T-cell dysfunction and immune escape via antigen-loss. In conjunction with Dr. Oladapo Yeku, from our junior faculty, we are using our antibodies as MUC16 targeted CAR T cells. We are developing strategies to further modify CAR



MUC16 is an example N-glycosylation - rich molecule which can regulate the cellular location and signal transduction mediated by TK receptors like EGFr or adhesion molecules (integrins) through Galectin 3 mediated interactions.

T cells to optimize their efficacy for ovarian cancer and gynecologic malignancies. Our approaches to further engineering these CAR T cells with Human Artificial Chromosomes (Dr. Kononenko) are informed by the ovarian cancer tumor microenvironment. Using syngeneic immune competent mouse models and subsequent validation in genetically engineered and xenograft models, we are able to effectively evaluate these rationally optimized CAR T cells as monotherapy or in combination with other immunomodulatory agents prior to initiation of clinical trials.

Glycosylation Dependence

Our work has been the first to show that the oncogenic effects of MUC16 require MGAT5 dependent tetra-antennary glycosylation of the MUC16 ectodomain and interaction with Galactin 3 (LGALS3). This complex then binds to glycosylation sites on growth factors including EGFr, Integrins, and immune receptors like CTLA4. This has provided us with new opportunities for MUC16+ cancer cell targeting.

Galectin 3 Targeting

LGALS3 regulates the interaction of surface proteins with the extracellular membrane domain and mediates a signal cascade leading to invasion, oncogene activation and growth. While anti-MUC16 glycosylation site antibodies inhibit oncogenic properties, LGALS3 represents a more general strategy for targeting glycosylation dependent oncogenesis. We have developed high-affinity antigalectin-3 antibodies directed at the carbohydrate recognition domain (CRD) of the galectin-3 carboxyl-terminus (to block sugar binding). These antibodies are able to block the oncogenic effects of MUC16 expression including invasion, oncogene activation (AKT, ERK, SRC) and reduced growth in nude mice. In addition, these antibodies appear able to decrease metastatic behaviors in lung metastasis models. Dr. Xu is focused on the functions of Galectin 3 in cancer while Dr. Lee has been producing a structural model of binding to the Galectin-3 surgery binding elements.

Selected Publications:

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The Stott laboratory is comprised of bioengineers, biologists and chemists focused on translating technological advances to relevant applications in clinical medicine. Specifically, we are interested in using microfluidics, imaging, and biopreservation technologies to create tools that increase our understanding of cancer biology and of the metastatic process. The Stott laboratory has co-developed innovative microfluidic devices that can isolate extraordinarily rare circulating tumor cells (CTCs) and extracellular vesicles (EVs) from the blood of cancer patients. New microfluidic tools are being developed to both manipulate and interrogate these cells and vesicles at a single particle level. We also look at tumor specimens using multispectral imaging, hoping that the exploration of the spatial relationships between immune cells and tumor tissue will help us better predict treatment response. Ultimately, we hope that by working in close partnership with the clinicians and cell biologists at the Mass General Cancer Center, we can create new tools that directly impact patient care.

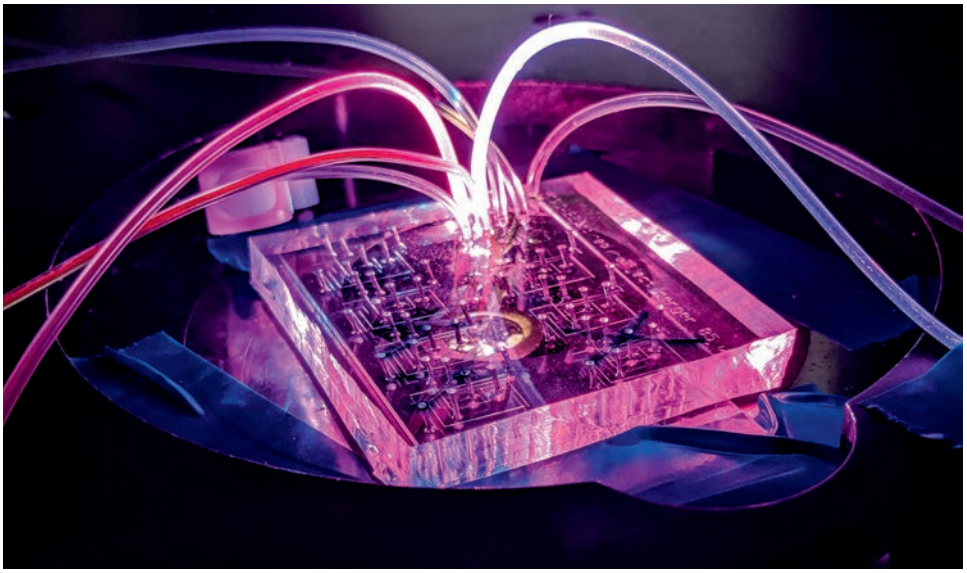
Rapid technological advances in microfluidics, imaging and digital gene-expression profiling are converging to present new capabilities for blood, tissue and single-cell analysis. Our laboratory is interested in taking these advances and creating new technologies to help build understanding of the metastatic process. Our research focus is on 1) the development and application of microfluidic devices and biomaterials for the isolation and characterization of extracellular vesicles, 2) the enrichment and analysis of CTCs at a single cell level, and 3) novel imaging strategies to characterize tumor tissue, cancer cells, and extracellular vesicles.

Extracellular Vesicle Isolation and Characterization

Extracellular vesicles (EVs), such as exosomes, microvesicles, and oncosomes, are small particles that bud off of cancer cells, with some cancer cells releasing up to thousands of EVs per day. Researchers have hypothesized that these EVs shed from tumors transport RNA, DNA and proteins that promote tumor growth, and studies have shown that EVs are present in the blood of most cancer patients. Ongoing work in my lab incorporates microfluidics and novel biomaterials to enrich cell-specific EVs from cancer patients, using as little as 1mL of plasma. Once isolated, we are exploring their protein and nucleic acid content to probe their potential as a less invasive biomarker. Droplet-based microfluidics are being developed to probe the EVs at a single vesicle level.

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, these cells provide a potentially accessible source for early detection, characterization and monitoring of cancers that would otherwise require invasive serial biopsies. Working in collaboration with Drs.



Droplet based microfluidics for the selective merging of encapsulated cells. Different cell populations can be sorted at a single cell level and then selectively placed into droplets, creating custom culture 'microdrops' for long term culture and monitoring.

Image courtesy of Rohan Thakur

Mehmet Toner, Shyamala Maheswaran and Daniel Haber, we have designed a high throughput microfluidic device, the CTC-Chip, which 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. Ongoing projects include translating the technology for early cancer detection, exploring the biophysics of the CTC clusters, and the design of biomaterials for the gentle release of the rare cells from the device surface. We are also developing new strategies for the long term preservation of whole blood such that samples can be shipped around the world for CTC analysis.

High-Content and High-Throughput Imaging of Tumor Specimens

Tumors can be highly heterogeneous, and their surrounding stroma even more so. Traditionally, the tumor and surrounding cells are dissociated from the tissue matrix for high throughput analysis of each cell. While this allows for important information to be gained, the spatial architecture of the tissue and corresponding interplay between tumor and immune cells can be lost. The Stott lab is developing quantitative, robust analysis for individual cells within the tumor and neighboring tissue using multispectral imaging. We are using this technology alongside downstream imaging processing algorithms to interrogate signaling activity in cancer cells, immune cell infiltration into to the tumor and pEMT in cancer cells. 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.

Selected Publications:

Wong KHK, Edd JF, Tessier SN, Moyo WD, Mutlu BR, Bookstaver LD, Miller KL, Herrara S, **Stott SL**†, Toner M†, "Antithrombotic strategies for microfluidic blood processing", *Lab Chip*, 2018; 18(5).

Reátegui E*, van der Vos KE*, Lai CP*, Zeinali M, Atai NA, Floyd FP, Khankhel A, Thapar V, Toner M, Hochberg FH, Carter B, Balaj L, Ting DT, Breakefield XO, **Stott SL**. Engineered Nanointerfaces for Microfluidic Isolation and Molecular Profiling of Tumor-specific Extracellular Vesicles. *Nat. Comm*. 2018; 9(1).

Wong KHK*, Tessier SN*, Miyamoto D, Miller KL, Bookstaver LD, Carey TR, Stannard C, Tai EC, Vo KD, Sandlin RD, Thapar V, Sequist LV, Ting DT, Haber DA, Maheswaran S, **Stott SL**†, Toner M†. Whole blood stabilization for precision oncology: isolation and molecular characterization of circulating tumor cells. *Nat. Comm*., 2017 8(1).

Park MH*, Reátegui E*, Li W, Jensen AE, Toner M, **Stott SL**†, Hammond PT†. Enhanced Isolation and Release of Circulating Tumor Cells Using Nanoparticle Binding in a Microfluidic Chip Via Place-Exchangeable Ligands. *JACS*. 2017; 139(7).

Au SH, Storey BD, Moore JC, Tang Q, Chen Y-L, Sarioglu AF, Javaid S, Langenau DM, Haber DA, Maheswaran S, **Stott SL**†, Toner M†. Clusters of circulating tumor cells traverse capillary-sized vessels. *PNAS*. 2016; 113 (18).

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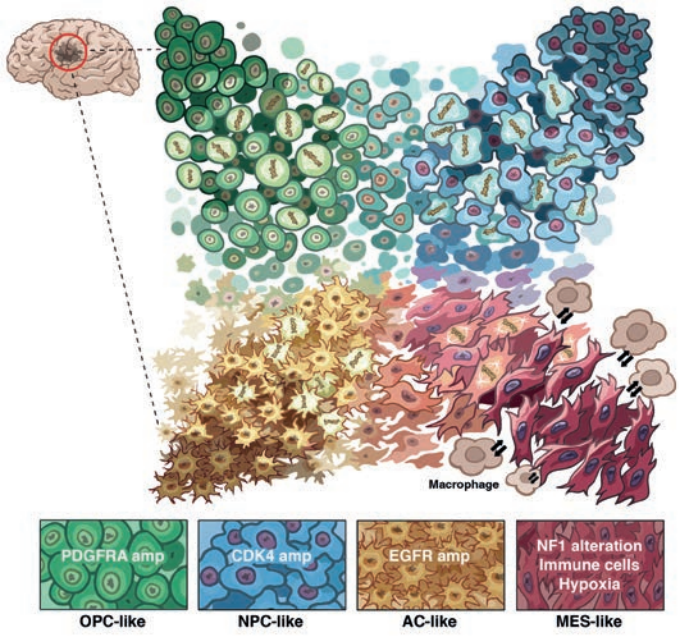
The Suvà laboratory is primarily focused on developing and applying single-cell genomic technologies to dissect the biology of brain tumors, in particular adult and pediatric high-grade gliomas and medulloblastoma. We study patient samples at single-cell resolution and establish genetically and epigenetically relevant cellular models directly from clinical 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. We seek to redefine tumor cell lineages and stem cell programs across all types of gliomas, and to leverage the information for renewed therapeutic attempts targeting cellular states. The laboratory is also invested in single-cell genomics efforts to dissect the immune system of gliomas, and in charting the cellular programs in sarcomas.

Gliomas are heterogeneous disease in which intra-tumoral heterogeneity contributes to disease progression and therapeutic failure. Glioma cells vary in stemness, proliferation, invasion, chemoresistance, apoptosis, and metabolism. Various factors contribute to this heterogeneity, on the one hand, branched genetic evolution of cancer cells generates distinct tumor sub-clones; on the other hand, it is also becoming increasingly clear that gliomas cells display functional properties related to developmental pathways and transcriptional programs, such as those associated with the self-renewal of tissue stem cells and their differentiation into specialized cell types. In order to dissect those influences and obtain a comprehensive view of gliomas biology, my laboratory is leveraging single-cell expression profiling across the spectrum of human gliomas, directly in patient samples. Analysis of transcriptomes of individual cells from human malignancies offers a compelling approach to dissect the cellular state and infer partial genetic information from cancer cells in an unbiased way. We seek to discover novel therapies for gliomas.

Assessing Malignant Cells Heterogeneity at the Single-Cell Level in Gliomas

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 intra-tumoral heterogeneity is increasingly appreciated as a determinant of treatment failure and disease recurrence. The Suvà Lab is performing large-scale single-cell RNA-seq analyses in IDH-mutant gliomas, histone H3-mutant midline gliomas, IDH-wildtype glioblastoma, and medulloblastoma to assess tumor cell lineages, stem cell programs and genetic heterogeneity at an unprecedented scale and depth. Our work in IDH-mutant gliomas highlighted a rare subpopulation of actively dividing stem/progenitor cells, solely responsible for fueling tumor growth in patients. Single cell profiling of H3K27-mutant pediatric gliomas highlighted specific vulnerabilities and revealed a differentiation block, maybe explaining the more aggressive



Model for the cellular states of glioblastoma and their genetic and micro-environmental determinants. Mitotic spindles indicate cycling cells. Lighter/darker tones indicate strength of each program. Intermediate states are shown in between the four states and indicate transitions.

nature of this cancer type. More recently, we provided a comprehensive model of glioblastoma biology that integrates single-cell expression programs, genetic composition and tumor subtypes (see figure). Our study of medulloblastoma single-cell programs provided clarifications on tumor histogenesis and classification. Overall, our goal is to identify both lineage-defined and somatically-altered therapeutic targets in brain cancer in both children and adults.

Dissecting the Ecosystem of Gliomas

The composition of the tumor micro-environment (TME) has an important impact on tumorigenesis and modulation of treatment responses. For example, gliomas contain substantial populations of microglia and macrophages, with putative immunosuppressive functions but whose precise programs remains uncharted at single-cell resolution. In addition, very little is known about the functional state of T cells in human gliomas. As is the case in diverse other conditions, the CNS may create a unique microenvironment that impacts T cell function by distinct mechanisms.

The laboratory leverages single-cell analyses in clinical samples to dissect the functional programs of immune cells in gliomas that can be used to elucidate mechanisms relevant to immuno-oncology. We profile both dysfunctional T cells that express multiple inhibitory receptors and T cells that are functional based on expression of multiple genes required for T cell cytotoxicity. We find these modules to be distinct from observations in other types of tumors (such as melanoma), underscoring the necessity to perform these analyses directly in gliomas. By analyzing modules of co-expressed genes in subsets of T cells in patients with glioma we seek to shed light on mechanism of activation and exhaustion in patient tumors and to highlight candidate novel regulatory programs that can be exploited for therapeutics.

Selected Publications:

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Filbin MG†, Tirosh I†, Hovestadt V†, Shaw ML, Escalante LE, ..., Getz G, Rozenblatt-Rosen O, Wucherpfennig KW, Louis DN, Monje M, Slavc I, Ligon KL, Golub TR, Regev A*, Bernstein BE*, **Suvà ML*** Developmental and oncogenic programs in H3K27M gliomas dissected by single-cell RNA-seq. *Science*, 2018 Apr 20;360(6386).

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Tirosh I†, Venteicher AS†, Hebert C, Escalante LE, Patel AP, Yizhak K, Fisher JM, ..., Rivera MN, Getz G, Rozenblatt-Rosen O, Cahill DP, Monje M, Bernstein BE, Louis DN, Regev A*, **Suvà ML***. Single-cell RNA-seq supports a developmental hierarchy in human oligodendroglioma. *Nature*. 2016 Nov 10;539(7628).

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David A. Sweetser, MD, PhD

Sweetser Laboratory

Carl Holland, PhD
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The Sweetser laboratory investigates how leukemia and other cancers develop with the goal of developing novel, safer, and more effective therapies. We have two major lines of research - the first investigating the function of a novel family of tumor suppressor genes and the second investigating the supportive role of the bone marrow niche in leukemia. Our lab has identified how the Groucho/TLE family of co-repressors function as potent tumor suppressors of acute myeloid leukemia, and has been defining 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. It is this ability to regulate inflammatory pathways that appears to underlie their tumor suppressor activity. We have defined critical inflammatory signaling pathways mediating cell proliferation and synergistic cross talk within the cancer niche that stimulated the proliferation and survival of leukemia. The laboratory is also involved in characterizing cancer predisposition genes and genes influencing therapy toxicity. As the MGH site director for the Undiagnosed Diseases Network and Chief of Medical Genetics and Metabolism at MGH, 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.

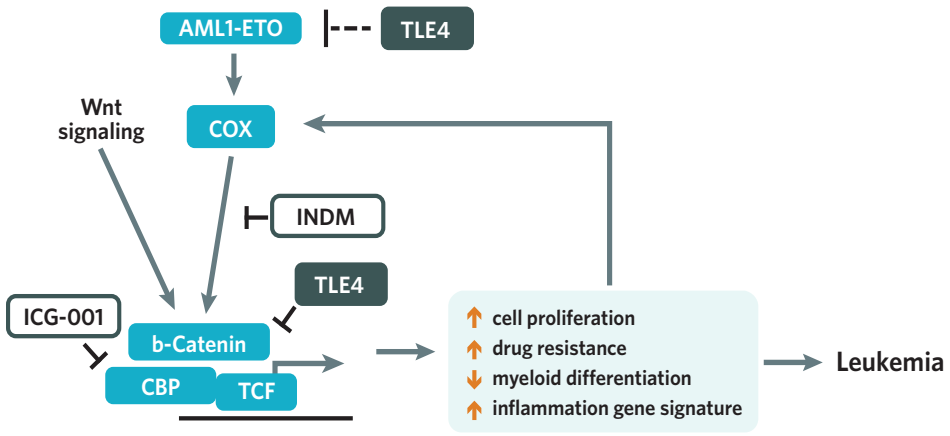
Genetics of Acute Myeloid Leukemia

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

Evaluation of the Role of the Groucho/TLE Family of Corepressors in Cancer and Development

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 and other cancers including melanoma.

The Groucho/TLE family of corepressor proteins can modulate many of the major pathways involved in development and oncogenesis, including Wnt/β-catenin, Notch, Myc, NFκB, and TGFβ. However, we 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. For example, in synovial cell sarcoma, TLE1 is over-expressed 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. TLE1 and TLE4 are potent inhibitors of the AML1-ETO oncogene in the most common subtype of AML. The mechanism of this inhibition appears to involve both regulation of gene transcription and chromatin structure. In



Schematic diagram summarizing proposed TLE4 regulation of AML1-ETO/COX/Wnt axis. The TLEs have potent anti-inflammatory effects and inhibitory effects on oncogene pathways involving AML1-ETO and B-catenin, which also underlie their tumor suppressor activity.

large part this cooperative effect appears to involve regulation of Wnt signaling and inflammatory gene pathways. This work has led to the demonstration that specific anti-inflammatory agents can have potent anti-leukemic effects. We have also been studying the role of TLE1 in melanomas. In this context TLE1 appears to have a critical role in inhibiting the oncogenicity of oncogenic BRAF. The mechanism of this inhibition is being investigated.

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 characterizing the 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 and T-cell differentiation, and is required for hematopoietic stem cell maintenance, as well as bone development.

The Role of the Bone Marrow Niche in Nurturing Leukemia

The bone marrow niche is remodeled in the process of leukemia development to provide a supportive environment that contributes to leukemic cell proliferation, survival, and resistance to chemotherapy. Our lab is working to define the critical cells and components of this niche with an eye towards designing targeted adjunctive therapies.

The Undiagnosed Diseases Network

The Harvard Medical School hospital consortium of MGH, Brigham and Women's Hospital and Children's Hospital together with 10 other clinical sites around the US comprise the NIH sponsored 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, using comprehensive clinical phenotyping, whole exome/whole genome sequencing, paired with RNASeq and metabolomics profiling, and in collaboration with zebrafish and Drosophila model organism cores to identify the underlying basis of a variety of challenging human diseases. Over a dozen new genetic disorders have been characterized with these efforts. The Sweetser lab also participates in the functional characterization of identified candidate genes.

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Gastrointestinal cancers are highly lethal 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. **The Ting laboratory** has been utilizing innovative technologies to characterize RNA expression patterns in cancer. Using single molecule sequencing, we have discovered a significant amount of "non-coding" repeat RNAs to be produced in high amounts at the earliest stages of cancer development, but not in normal tissues. These repeat RNAs can serve as a novel early detection cancer biomarker and they can be targeted as a new therapeutic avenue. In parallel, we have used microfluidic chip technologies to capture circulating tumor cells (CTCs), the cells that disseminate to distant organs. Using single cell RNA-seq we have gained unprecedented insight into the programs that drive metastatic spread. We are using these studies to develop blood based "liquid biopsy" biomarkers and generate new therapies to stop the spread of cancer.

The Ting laboratory has utilized RNA-sequencing and RNA in situ hybridization technology to understand the complex transcriptional landscape of cancers. We have used these technologies to characterize non-coding repeat RNA expression across cancer and normal tissues. This has provided novel insight into the role of the repeatome in cancer development and offers a method to identify novel biomarkers and therapeutic targets. In addition, we have been able to capture 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 and to develop novel early detection biomarkers.

Repeat Non-coding RNAs

RNA sequencing of a broad spectrum of carcinomas demonstrated a highly aberrant expression of non-coding repeat RNAs emanating from regions of the genome previously thought to be inactive due to epigenetic silencing. Analysis of all human repeats identified the HSATII satellite as being exquisitely specific for epithelial cancers, including carcinomas of the pancreas, colon, liver, breast, and lung. HSATII expression was confirmed by RNA in situ hybridization (RNA-ISH), and was present in preneoplastic lesions in mouse models and human specimens of the pancreas and colon suggesting satellite expression occurs early in tumorigenesis, which provides for a potential biomarker for early detection and a novel therapeutic avenue. Recently, we have discovered that HSATII is reverse transcribed in cancer cells and can integrate back into the genome and expand these pericentromeric regions. These expansions were found to be a poor prognostic marker in cancer. Moreover, work with others has found that these satellite repeats can affect the local tumor microenvironment with implications for immunotherapies. We are now trying to identify the HSATII reverse transcriptase and better understand the biological role of satellites in cancer progression.

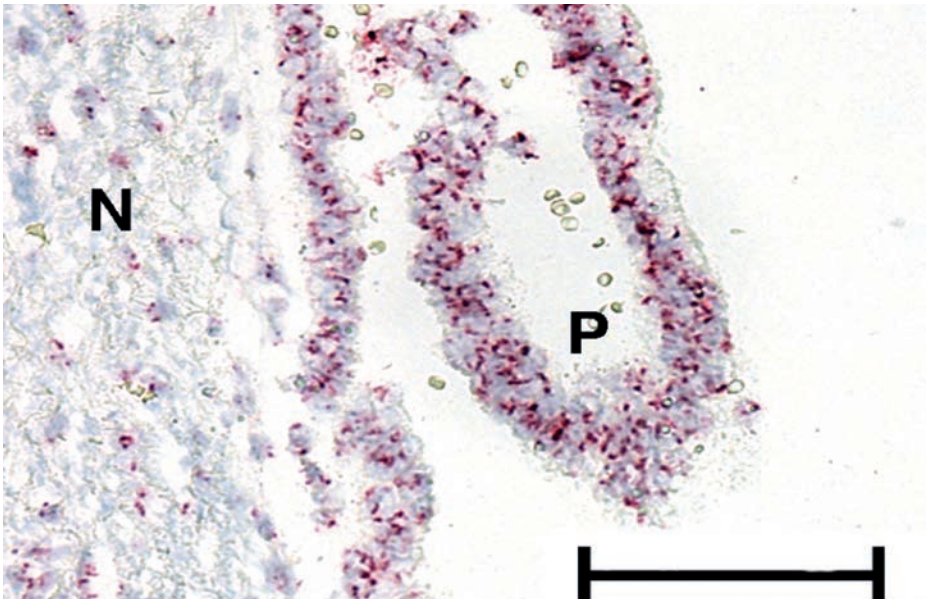

Image of a preneoplastic pancreatic intraepithelial neoplasm (P) positive for the HSATII ncRNA (Red dots).Normal adjacent reactive stroma (N) with minimal expression. Counterstain hematoxylin (blue). Scale bar = 100 μm.

Circulating Tumor Cells: The Liquid Biopsy

The temporal development of circulating tumor cells (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. Using a novel microfluidic device developed at MGH, we have isolated pancreatic and liver CTCs and perform RNA sequencing on these rare cells. This has revealed the opportunity to develop a novel early detection blood based biomarker and study the metastatic cascade. Using single cell RNA-sequencing, we have characterized the heterogeneity of pancreatic CTCs into three major subclasses, and note that over half of the CTCs are not viable. This illustrates that not all CTCs have the full capacity to metastasize, and that there are likely multiple paths for cancer cell dissemination. In addition, single cell RNA-seq has provided unprecedented transcriptional resolution of CTCs that has revealed significant enrichment for stem cell and epithelial mesenchymal transition markers of these metastatic precursors. Notably, we have also found that CTCs express a significant amount of extracellular matrix proteins normally found in the stroma of primary tumors. This suggests that the seeds of metastasis are in fact producing their own soil during the metastatic cascade. We have recently identified the stromal microenvironment is responsible for generating a significant amount of heterogeneity in pancreatic cancer and drive the development of these CTC phenotypes in both mouse models and patients. 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.

Selected Publications:

Ligorio M*, Sil S*, Malagnon-Lopez J, Nieman LT, Misale S, Di Pilato M, Ebright RY, Karabacak M, Kulkarni A, Liu A, Jordan NV, Franses JW, Philipp J, Kreuzer J, Desai N, Arora KS, Rajurkar M, Horwitz E, Neyaz A, Tai E, Magnus NKC, Vo KD, Yashaswini CN, Marangoni F, Boukhali M, Fatherree JP, Damon LJ, Xega K, Desai R, Choz M, Bersani F, Langenbucher A, Thapar V, Morris, R, Wellner UF, Schilling O, Lawrence MS, Liss AS, Rivera MN, Deshpande V, Benes CH, Maheswaran S, Haber DA, Fernandez-Del Castillo C, Ferrone CR, Haas W, Aryee M†, **Ting DT**†. Stromal Microenvironment Shapes the Intratumoral Architecture of Pancreatic Cancer. *Cell* (2019); 178(1):160-175.e27.

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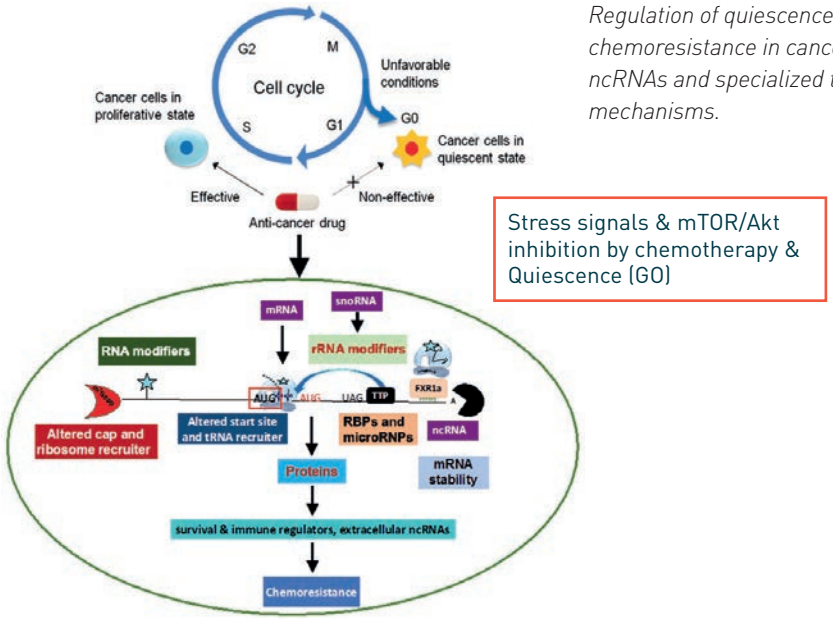
The Vasudevan laboratory focuses on the role of post-transcriptional mechanisms in clinically resistant quiescent cancer cells. Tumors demonstrate heterogeneity, harboring a small subpopulation that switch from rapid proliferation to a specialized, reversibly arrested state of quiescence that decreases their susceptibility to chemotherapy. Quiescent cancer cells resist conventional therapeutics and lead to tumor persistence, resuming cancerous growth upon chemotherapy removal. Our data revealed that post-transcriptional mechanisms are altered, with modification of noncoding RNAs, associated complexes and ribosomes—molecules that control vital genes in cancer—which are important for the persistence of quiescent cancer cells. The primary goal of our research is to characterize the specialized gene expression and their post-transcriptional regulators that underlie persistence of resistant cancer cells. A complementary focus is to investigate the modification of post-transcriptional regulators and their mechanisms in response to quiescent conditions and chemotherapy-induced signaling. Our goal is to develop a comprehensive understanding of the versatile roles of regulatory RNAs in cancer as a basis for early detection of refractory cancers and for designing new therapies.

Quiescent (G0) cells are observed as a clinically relevant population in leukemias and other tumors associated with poor survival. G0 is a unique, nonproliferative phase that provides an advantageous escape from harsh situations like chemotherapy, allowing cells to evade permanent outcomes of senescence, differentiation, and apoptosis in such tumor-negative environments. Instead, the cell is suspended reversibly in an assortment of transition phases that retain the ability to return to proliferation and contribute to tumor persistence. G0 demonstrates a switch to a distinct gene expression program, upregulating the expression of mRNAs and regulatory non-coding RNAs required for survival. Quiescence regulators that maintain the quiescent, chemoresistant state remain largely undiscovered.

Our studies revealed that specific post-transcriptional regulators, including AU-rich elements (AREs), microRNAs, RNA-protein complexes (RNPs), ribosome factors and RNA modifiers, are directed by G0- and chemotherapy-induced signaling to alter expression of clinically important genes. AU-rich elements (AREs) are conserved mRNA 3'-untranslated region (UTR) elements. MicroRNAs are small noncoding RNAs that target distinct 3'UTR sites. These associate with RNPs, ribosome associated factors and their modifiers to control post-transcriptional expression of cytokines and growth modulators. Their deregulation leads to a wide range of diseases, including tumor growth, immune and developmental disorders.

We identified post-transcriptional effectors associated with mRNAs and noncoding RNAs by developing in vivo crosslinking-coupled RNA affinity purification methods to purify endogenous RNPs. Our recent studies revealed mechanistic changes in



Regulation of quiescence & chemoresistance in cancer by ncRNAs and specialized translation mechanisms.

Stress signals & mTOR/Akt inhibition by chemotherapy & Quiescence (G0)

G0: uncovering inhibition of conventional translation and its replacement by non-canonical mechanisms that enable specific gene expression in G0 to elicit chemoresistance. These specialized mechanisms are driven by modifications of mRNAs, associated regulator RNAs and proteins, and ribosomes, which are induced in G0- and chemotherapy-induced signaling. These investigations reveal gene expression control by RNA regulators and non-canonical translation mechanisms that cause tumor persistence. Based on our data demonstrating altered RNPs, modifications, and specific translation in G0, we propose that transiently quiescent, chemoresistant subpopulations in cancers are maintained by specialized post-transcriptional mechanisms that permit selective gene expression, necessary for chemotherapy survival and tumor persistence.

The primary goal of our research is to characterize the specialized gene expression program in quiescent, chemoresistant cancers, and its underlying post-transcriptional and translational regulators that contribute to G0 and tumor persistence. A concurrent focus is to investigate RNA modifications and mechanisms of noncoding RNAs, RNPs, and ribosomes in G0 that contribute to chemoresistance, using cancer cell lines, in vivo models, patient samples, and stem cells. An important direction is to identify unique G0-specific RNA markers and develop novel therapeutic approaches to block selective translation in G0, of targets that encode for critical immune and tumor survival regulators—and thereby curtail chemoresistance.

The lab has four core directions:

1. To characterize microRNAs and noncoding RNAs, and their cofactors that control the expression of tumor survival regulators, using in vivo biochemical purification methods.
2. To investigate the mechanisms of post-transcriptional and translational regulation by noncoding RNAs, RNPs, and ribosome regulators.
3. To elucidate the modification and regulation of key mRNAs and ribosomes, by G0- and chemotherapy-induced signaling.
4. To develop therapeutic approaches that interfere with selective translation, and manipulate interactions of noncoding RNAs with targets that encode for critical tumor survival regulators. These studies should lead to a greater understanding of the versatile role of post-transcriptional mechanisms in cancer persistence and to novel approaches in RNA-based therapeutics.

Selected Publications:

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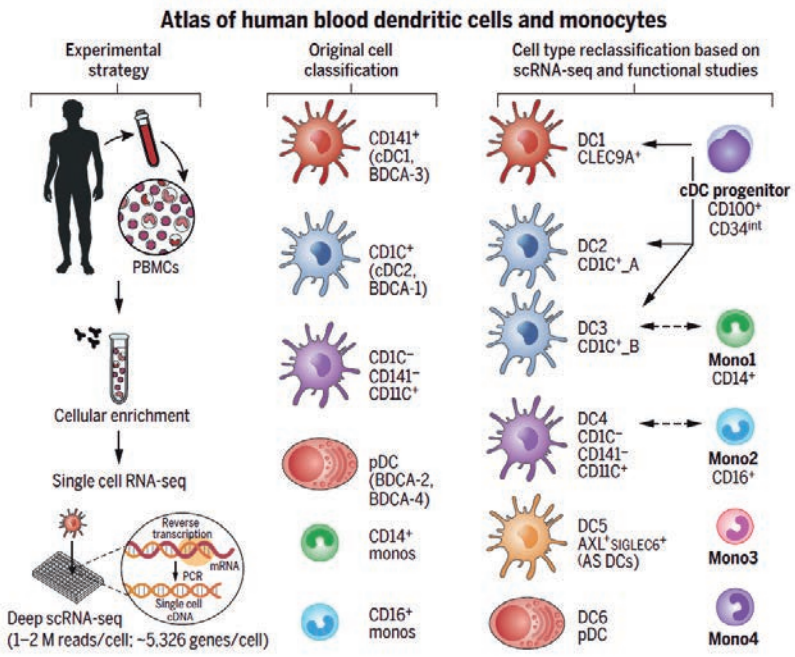
The Villani laboratory seeks to establish a comprehensive roadmap of the human immune system by achieving a higher resolution definition and functional characterization of cell subsets and rules governing immune response regulation, as a foundation to decipher how immunity is dysregulated in diseases. We use unbiased systems immunology approaches, cutting-edge immunogenomics, single-cell 'multi-omics' strategies, and integrative computational frameworks to empower the study and modeling of the immune system as a function of "healthy" and inflammatory states, disease progression, and response to treatment. Our multi-disciplinary team of immunologists, geneticist, computational biologists, and physicians work towards answering several key questions: Do we know all existing blood immune cell subsets? How do circulating immune cells mirror those in tissue microenvironment in the context of health and disease? Can we identify targets that would improve immunotherapy efficacy by increasing specificity? Collectively, our groundwork is paving the way for developing a human immune lexicon that is key to promoting effective bench-to-beside translation of findings.

Leveraging single-cell '*omics*' to unravel new insights into the human immune system

Achieving detailed understanding of the composition and function of the immune system at the fundamental unit of life — the cell — is essential to determining the prerequisites of health and disease. Historically, leukocyte populations have been defined by a combination of morphology, localization, functions, developmental origins, and the expression of a restricted set of markers. These strategies are inherently biased and recognized today as inadequate. Single-cell RNA sequencing (scRNAseq) analysis provides an unbiased, data-driven way of systematically detecting cellular states that can reveal diverse simultaneous facets of cellular identity, from discrete cell types to continuous dynamic transitions, which cannot be defined by a handful of pre-defined markers or for which markers are not yet known. We combine scRNAseq strategies together with in-depth follow-up profiling, phenotypic and functional characterization of prospectively isolated immune subsets defined by scRNAseq data to overcome such limitations. Our analyses of the human blood mononuclear phagocyte system resulted in the identification of six dendritic cell (DC), four monocyte, and one DC progenitor populations, thus revising the taxonomy of these cells (*Villani et al., Science 2017*). Noteworthy, five of these subsets had never been reported, illustrating the power of our integrative strategies to reopen the definition of these cell types. Our study highlighted the value of embarking on a comprehensive Human Cell Atlas initiative and offered a useful framework for conducting this kind of analysis on other cell types and tissues. We are currently contributing to the immune cell atlas effort by charting at high-resolution the human blood cellular landscape, and are studying paired human tissues with blood to better



Establishing a human blood dendritic cell and monocyte atlas. *We isolated ~2400 cells enriched from the healthy human blood lineage– HLA-DR+ compartment and subjected them to single-cell RNA sequencing. This strategy, together with follow-up profiling and functional and phenotypic characterization, led us to update the original cell classification to include six DCs, four monocyte subtypes, and one conventional DC progenitor.*

establish how circulating immune cells mirror those in tissue microenvironment in the context of health and disease.

We also continuously support development of in-depth expertise in single-cell 'omics' approaches, including single-cells strategies to map X-chromosome inactivation (Tukiainen, Villani, *Nature* 2017), new enrichment method targeting individual cell transcriptome in pooled library (Ranu, Villani, *Nucleic Acid Res* 2019), method's development to study single-T cells (Villani, *Methods Mol Biol* 2016) and application to study T cells infiltrates in tumor lesions (Izar *Science* 2016; Sade-Feldman,*Cell* 2019; Di Pilato, *Nature* 2019) and myeloid cell infiltrates (Olah M, *Nat Commun* 2018; Balan S, *Cell Rep* 2018; Chapuy L, *Mucosal Immunol* 2019).

Deciphering immune-related adverse events (irAEs) induced by immune-checkpoint inhibitor (ICI) therapy.

While ICI therapy is revolutionizing the treatment of solid cancers, its success is currently being limited by treatment-induced irAEs resembling autoimmune diseases that are affecting nearly every organ system. With ICI becoming first- and second-line of cancer treatments, it is expected that the number of irAEs will continue rising and limit immunotherapy efficacy unless we find solutions. Our multi-disciplinary translational group of scientists and clinicians are working towards developing a better understanding of the biological players and underlying molecular and cellular mechanisms involved in driving irAEs by directly studying patient blood and matched affected tissue samples using a range of systems immunology, immunogenomics and single-cell 'omics' strategies. Our translational research program may result in identifying putative cellular components and mechanisms that could be (i)targeted in a 'primary-prevention' approach to prevent irAE development, or (ii)targeted after onset of irAEs, without reducing the efficacy of the immunotherapy.

Selected Publications:

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Cancer is a complex disease driven by genetic and epigenetic alterations in the genome. To prevent these detrimental alterations, cells have evolved an intricate signaling network, called the DNA damage 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 increasingly dependent upon specific DNA repair and 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.

Sensing of DNA Damage, Replication Stress, and Transcription Problems

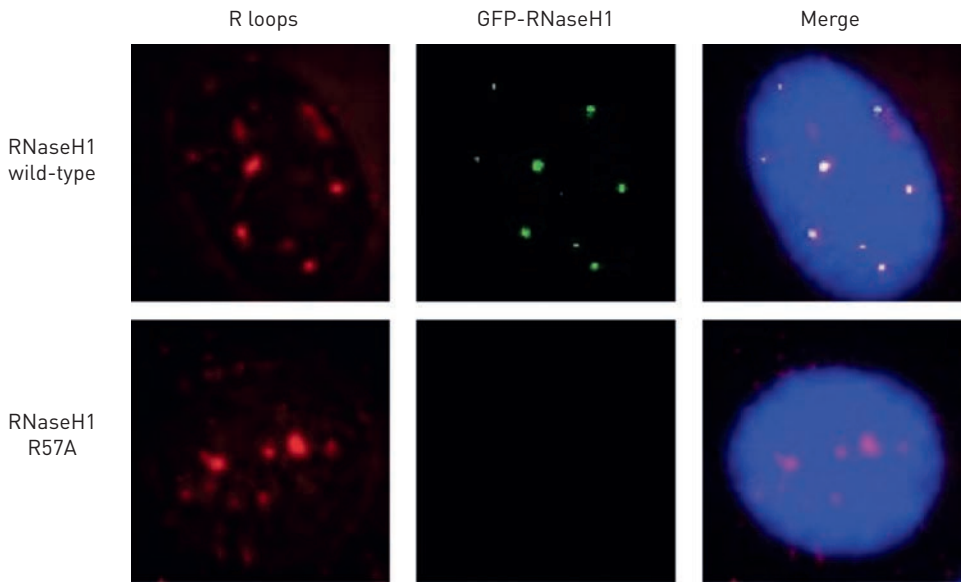
ATM and ATR are two master checkpoint kinases in human cells. In particular, ATR is the key responder to a broad spectrum of DNA damage and DNA replication problems. To understand how ATR is activated, we sought to identify the key DNA structural elements and sensor proteins that activate ATR. We have developed unique biochemical and cell biological assays to dissect the process of ATR activation. Our recent studies have revealed that ATR is important not only for sensing DNA damage and replication stress, but also for problems associated with transcription. R loops, which arise from stable DNA:RNA hybrids during transcription, are a major source of genomic instability. We found that ATR is activated by R loops and plays a key role in suppressing R loop-induced genomic instability, thus, uncovering a new function of ATR in safeguarding the genome.

Checkpoint, DNA Replication, DNA Repair, Telomeres, Centromeres and the Cell Cycle

The ATR checkpoint plays a key role in regulating and coordinating DNA replication, DNA repair, and cell cycle transitions. During the past few years, our studies have identified a number of novel roles that ATR plays in protecting the genome, such as: suppressing single-stranded DNA (ssDNA) accumulation during DNA replication, regulating homologous recombination (HR), and promoting alternative lengthening of telomeres (ALT). Recently, we have discovered a surprising function of ATR in mitosis. We have shown that ATR is localized to centromeres in mitosis, where it is activated by centromeric R loops. The activation of ATR at centromeres is critical for faithful chromosome segregation, thus revealing the unexpected importance of ATR in suppressing chromosomal instability (CIN).

RNA, DNA repair and Genomic Integrity

Non-coding RNAs are important components and regulators of chromatin. We are interested in understanding how non-coding RNAs affect DNA repair and genomic stability in specific chromosomal regions.



This image shows that GFP-tagged RNaseH1 (green) localizes to sites of R loops (red) through binding to RPA. R loops are transcription intermediates that contain RNA:DNA hybrids and single-stranded DNA (ssDNA). RPA is a protein complex that recognizes ssDNA. RNaseH1 is an enzyme that suppresses R loops by cleaving the RNA in RNA:DNA hybrids. Wild-type RNaseH1 recognizes R loops through binding to RPA, but the R57A mutant of RNaseH1, which is defective for RPA binding, fails to recognize R loops.

For example, the telomere non-coding RNA TERRA is upregulated in ALT-positive tumors, and may regulate the lengthening of telomeres through a unique DNA repair pathway. Moreover, centromeric RNAs form R loops in mitotic cells, promoting ATR activation and accurate chromosome segregation. In addition to non-coding RNAs, our recent studies also suggest that even coding RNA transcripts may directly participate in the repair of DNA breaks, revealing another function of RNA in the regulation of genomic integrity.

Cancer Genomics, Tumor evolution and Targeted Cancer Therapy

During the evolution of tumors, cancer cells acquire mutations through a variety of mechanisms. We recently discovered that APOBEC3A/B proteins, two cytidine deaminases that are aberrantly expressed in multiple types of cancers, induce DNA replication stress and render cancer cells susceptible to ATR inhibition. Working with the team of Dr. Michael Lawrence, we find that APOBEC3A prefers substrate sites in DNA hairpins, leading to the discovery of passenger hotspot mutations in cancer. Furthermore, in collaboration with Dr. Tim Graubert, we find that the splicing factor mutations associated with myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) induce R loops and trigger an ATR response. Cells that express these splicing factor mutants are sensitive to ATR inhibitors, providing a new strategy for the treatment of MDS and possibly other malignancies associated with RNA splicing defects.

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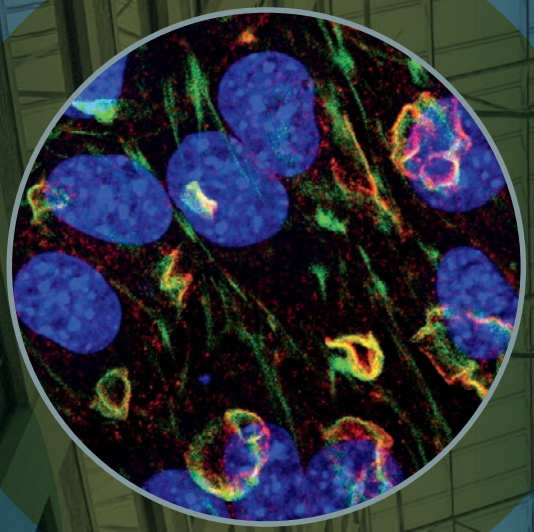

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