PATHOLOGY

Basic Scientific Research

2018-2019

MASSACHUSETTS GENERAL HOSPITAL

COVER IMAGE:

Confocal image of a 3-dimensional culture model of biliary cells organizing into tubular structures. Cell fate and tubular organization are coordinated in normal liver development and this coordination is aberrantly recapitulated in biliary cancers. Red: E-cadherin. Green: F-actin. Blue: nuclei stained with DAPI.

Courtesy of Evan O'Loughlin (Andrea McClatchey laboratory); see also Benhamouche-Trouillet, O'Loughlin, et. al., *Development*, 2018.

CONTENTS

Pathology plays a key role in academic medicine, as a natural bridge between the study of human disease and experimental biological investigation. Major advances in molecular pathology and informatics are accelerating the pace of this translational research. In turn, the rapidity and frequency of interactions between the clinical and scientific areas makes this an exciting time in the field of pathology.

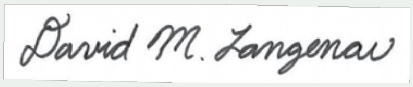
Laboratory-based scientific research is a major component of MGH Pathology, and is complemented by productive clinical research activities. As a result, MGH Pathology provides an exciting stage for basic and translational research. The present brochure highlights the basic scientific research activities in MGH Pathology.

Basic research at MGH Pathology, which is organized under the Division of Research, is divided among a variety of laboratories, both within the Pathology Service and other MGH departments. Peer-review funded investigators in MGH Pathology are centered in the Molecular Pathology Unit, with additional departmental laboratories in the Howard Hughes Medical Institute, the Center for Integrated Diagnostics, the Experimental Pathology Unit, and the Immunopathology Research Unit. In addition, many of our affiliated members have laboratories in other MGH departments, including the Cancer Center, the Infectious Disease Unit, Neurology, Urology and Wellman Photomedicine.

Basic research activities have been expanded greatly over the past ten years, including: creation of the Division of Research; recruitment of more than ten basic scientists at the Assistant Professor level (with nearly all as full members of the Center for Cancer Research, five as members of the Harvard-MIT Broad Institute and many as members of the Harvard Medical School Biological and Biomedical Sciences program); addition of five molecular diagnostic pathologists; acquisition of considerable additional Pathology research space; expansion in the number of Harvard and MIT Ph.D. candidates training in MGH Pathology laboratories; and provision of competitive pilot grants to junior clinical faculty. As a result, the group has seen an extraordinary increase in the amount of NIH funding over the past decade.

In coordination with the growth of molecular pathology research, molecular diagnostic activities have also expanded greatly, including: development of the MGH Center for Integrated Diagnostics; organization of the MGH Pathology component of the Harvard-wide Molecular Genetic Pathology fellowship; extension and further development of the molecular pathology rotation for Pathology residents; and extensive expansion of the CLIA-approved molecular diagnostics laboratory, with implementation of a novel, high-throughput clinical mutation screening program through the Translational Research Laboratory.

With support and resources from the department and the hospital, we have expanded our computational biology and bioinformatics resources for pathology, increasing collaborations and interactions with the Center for Integrated Diagnostics, and building additional links between basic and clinical/translational researchers within MGH Pathology. We also plan to continue to recruit additional basic science principal investigators and to develop new research space. These efforts will ensure that MGH Pathology faculty remain at the forefronts of their fields, enabling them to continue advancing our understanding and diagnosis of human diseases.



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MGH Training Grant Laboratories

MGH Pathology directs an NIH Training Grant that provides post-doctoral fellowship support for residents wishing to pursue scientific training following their clinical years. MGH Pathology trainees have had considerable success garnering individual grants for research fellowships. Our trainees have been authors on well over 100 publications, in journals that include Cell, Science, Nature, Cancer Cell, Cell Stem Cell, Developmental Cell, Molecular Cell, Nature Genetics, Nature Biotechnology, Nature Methods, Cancer Discovery, Current Biology and PNAS. Many trainees undertake post-doctoral fellowships in MGH Pathology laboratories. MGH Pathology trainees have also done fellowships with other investigators, including the following over the past 20 years:

Nancy Andrews, MD, PhD
Children's Hospital

Spyros Artavanis-Tsakonas, PhD
Harvard Medical School

David Bartel, PhD
Whitehead Institute

Alan Beggs, PhD
Children's Hospital

Brett Bouma, PhD
MGH Wellman Center for Photomedicine

Connie Cepko, PhD
Harvard Medical School

George Church, PhD
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Michael Detmar, MD
MGH Cutaneous Biology Research Center

Iain Drummond, PhD
MGH Renal Unit

Benjamin Ebert, MD, PhD
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Kevin Eggan, PhD
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Stephen Elledge, PhD
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James Fox, PhD
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Frank Gertler, PhD
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MGH Center for Regenerative Medicine

Bradley Hyman, MD, PhD
MGH Neurology

Frank Haluska, MD, PhD
MGH Hematology-Oncology

Donald Ingber, MD, PhD
Wyss Institute at Harvard

Ralph Isberg, PhD
Tufts University

Rudolf Jaenisch, MD
Massachusetts Institute of Technology

Rakesh Jain, PhD
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Jeannie Lee, MD, PhD
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Susan Lindquist, PhD
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Andrea McClatchey, PhD
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Matthew Meyerson, MD, PhD
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Carl Pabo, PhD
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Gary Yellen, PhD
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"Computational methods for genomics and epigenomics..."

We develop statistical methods to improve our understanding of tumor cell-to-cell variability and its relationship to cancer progression. Much of this work relates to the computational and statistical challenges posed by single-cell transcriptome and epigenome data.

Different tumors, even of the same type, can harbor extremely heterogeneous genetic and 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. The stability of these individualized DNA methylation profiles has 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.

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. 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:

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

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

Nordor AV, Nehar-Belaid D, Richon S, Klatzmann D, Bellet D, Dangles-Marie V, Fournier T, Aryee MJ. The early pregnancy placenta foreshadows DNA methylation alterations of solid tumors. *Epigenetics*. 2017; 12(9):793-803

Ziller MJ, Hansen KD, Meissner A, Aryee MJ. Coverage recommendations for methylation analysis by whole-genome bisulfite sequencing. *Nat Methods.* 2015; 12(3): 230-2.

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

Aryee MJ, Liu W, Engelmann JC, Nuhn P, Gurel M, Haffner MC, Esopi D, Irizarry RA, Getzenberg RH, Nelson WG, Luo J, Xu J, Isaacs WB, Bova GS, Yegnasubramanian S. DNA methylation alterations exhibit intraindividual stability and interindividual heterogeneity in prostate cancer metastases. *Sci Transl Med.* 2013; 5(169):169ra10.

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"Researching mucosal immunology and inflammatory bowel disease..."

In the two major forms of IBD, Crohn's disease and ulcerative colitis, the underlying etiological factors and the pathogenesis remain poorly defined. It is generally believed that exaggerated immune responses to luminal normal enteric flora are involved in the initiation and perpetuation of the disease process.

The availability of a wide variety of experimental models of intestinal inflammation has helped provide important clues about the pathogenesis of IBD. The commonly used models include chemically induced mucosal injury and colitis induced by the transfer of selected populations of T cells into immunodeficient mice. The spontaneous development of colitis in genetically engineered animal models has provided excellent experimental models to study the pathogenesis of IBD. One important lesson learned from IBD models is that many different immunologic and mucosal defects can lead to similar pathologic findings.

For the last several years, our laboratory has focused on defining the pathogenesis of chronic intestinal inflammation using TCR alpha KO mice as a model of human IBD. TCR alpha KO mice develop spontaneously chronic colitis with many features of ulcerative colitis. We have identified a regulatory B cell subset, which appears under chronic intestinal inflammatory conditions and suppresses the progression of intestinal inflammation by secreting IL-10. TCR alpha KO mice deficient in both IL-4 and B cells, but not in IL-4 alone, develop granulomatous colitis with features of Crohn's disease. This suggests that differences in the two major forms of IBD may reflect different immunological responses to similar initiating events.

The laboratory is closely associated with the Center for the Study of Inflammatory Bowel Disease at MGH and collaborates with the other members of the Center; Dr. Bhan is an Associate Director of the Center. In collaboration with Dr. Terhorst and Dr. Xavier we have studied the role of Th-1 and Th-17 pathways, innate immune system and autophagy in the development of intestinal inflammation. Collaborative studies with Dr. Scott Snapper's laboratory have shown that interleukin-10 receptor signaling in innate immune cells regulates mucosal immune tolerance and anti-inflammatory macrophage function. The studies with Dr. Richard Hodin's laboratory indicate that administration of intestinal alkaline phosphatase may have a beneficial effect in intestinal inflammatory conditions and metabolic syndromes. Dr. Bhan's consultant role in the newly established Harvard Institute of Translational Immunology-Helmsley Pilot Program in Crohn's Disease has led to his collaboration with Dr. Vijay Yajnik at MGH and Dr. Matthew Myerson at DFCI to identify microorganisms in Crohn's disease lesions.

Selected Publications:

Lassen KG, McKenzie CI, Mari M, et al. Genetic coding variant in GPR65 alters lysosomal pH and links lysosomal dysfunction with colitis risk. *Immunity*. 2016; 44:1392-405.

Lassen KG, Kuballa P, Conway KL, et al. Atg16L1 T300A variant decreases selective autophagy resulting in altered cytokine signaling and decreased antibacterial defense. *Proc Natl Acad Sci USA*. 2014; 111:7741-6.

Shouval DS, Biswas A, Goettel JA, et al. Interleukin-10 receptor signaling in innate immune cells regulates mucosal immune tolerance and anti-inflammatory macrophage function. *Immunity*. 2014; 40:706-19.

Kaliannan K, Hamarneh SR, Economopoulos KP, et al. Intestinal alkaline phosphatase prevents metabolic syndrome in mice. *Proc Natl Acad Sci USA*. 2013; 110:7003-8.

Chang SY, Song JH, Guleng B, et al. Circulatory antigen processing by mucosal dendritic cells controls CD8+ T cell. *Immunity*. 2013; 38:153-165.

Yilmaz OH, Katajisto P, Lamming DW, et al. mTORC1 in the Paneth cell niche couples intestinal stem-cell function to calorie intake. *Nature*. 2012; 486:490-5.

Sugimoto K, Ogawa A, Shimomura Y, et al. Inducible IL-12-producing B cells regulate Th2-mediated intestinal inflammation. *Gastroenterology*. 2007; 133:124-36.

Mizoguchi A, Ogawa A, Takedatsu H, et al. Dependence of intestinal granuloma formation on unique myeloid DC-like cells. *J Clin Invest*. 2007; 117:605-615.

Selected Publications:

Matissek KJ et al. Expressed Gene Fusions as Frequent Drivers of Poor Outcomes in Hormone Receptor-Positive Breast Cancer. *Cancer Discov.* 2018; 8(3):336-353.

Heist RS et al. MET Exon 14 Skipping in Non-Small Cell Lung Cancer. *Oncologist.* 2016; 21(4):481-486.

Zheng Z et al. Anchored multiplex PCR for targeted next-generation sequencing. *Nat Medicine.* 2014; 20(12):1479-84.

Bergethon K et al. ROS1 rearrangements define a unique molecular class of lung cancers. 2012; 10;30(8):863-70.

Snuderl M et al. Mosaic amplification of multiple receptor tyrosine kinase genes in glioblastoma. *Cancer Cell.* 2011; 20:810-7.

Kwak EL et al. Response of non-small cell lung cancers with Anaplastic Lymphoma Kinase (ALK) gene rearrangements to a targeted ALK inhibitor. *N Engl J Med*. 2010; 363(18):1693-703.

Iafrate AJ et al. Detection of large-scale variation in the human genome. *Nat. Genet.* 2004; 36(9):949-51.

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"To identify actionable genetic alterations in cancer"

Our lab has focused efforts on translating highly complex molecular analyses of tumor genetics using novel technologies into clinical use. We have previously developed the SNaPshot genotyping assay, which has enabled Mass General to make personalized cancer medicine a priority. We have a strong interest in the clinical implementation of genetic screening technologies that can help direct targeted therapies, focusing on lung, pancreatic and brain tumors. Our recent contributions in the treatment of a subset of lung tumors with rearrangements of the ALK, ROS1 and MET tyrosine kinases with a small molecule kinase inhibitors underscore the promise of personalized cancer care. Our long term goal is to develop high-throughput genetic screening approaches for all cancer patients. To address this need, we have developed a novel next generation sequencing technique termed "anchored multiplex PCR (AMP)" that is especially powerful at detecting gene fusion events from clinical specimens. We have shown that AMP is a sensitive as FISH in diagnosing ALK, ROS1 and RET fusions in lung cancer, and does not require knowing both fusion partners. We have recently used AMP to uncover novel gene fusions important for the development and progression of hormone receptor-positive breast cancer. In addition, AMP can be used for genomic DNA target enrichment, and is scalable and cost effective. Current work focuses on ultrasensitive detection of mutations in blood and urine.

We have also continued prior studies of tumor heterogeneity, by studying gene amplification of receptor tyrosine kinases in glioblastoma. This work has revealed a new subclass of brain tumors with mosaic gene amplification of up to 3 kinases in distinct but intermingled cell populations within the same tumor. We are exploring the therapeutic implications of such driver gene heterogeneity in model systems of glioblastoma using CRISPR-based screens of patient derived cell lines. A major effort here has been the development of multiplexed in situ genetic analysis using FISH. These techniques will allow us to analyze many more genes, and map copy number heterogeneity onto histology sections.

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

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"Genome-editing nuclease technologies have important applications in biological research and gene therapy..."

The Joung laboratory is developing strategies to reprogram the genome and epigenome of living cells to better understand biology and treat disease. We have created and optimized molecular tools for customized genome editing that enable scientists to alter the DNA sequence of a living cell — from fruit flies to humans — with great precision. We also use these targeting methodologies to enable activation, repression, or alteration of histone modifications of specific genes. These tools have many potential research uses and have promise for developing therapeutics.

Genome editing using targeted nucleases and base editors

Genome editing technology using CRISPR-Cas9 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*, in press). In addition, we have engineered "high-fidelity" Cas9 variants (Kleinstiver & Pattanayak et al., *Nature* 2016) and variants with novel DNA binding specificities, (Kleinstiver et al., Nature 2015; Kleinstiver et al., *Nat Biotechnol*. 2015). More recently, we have developed a novel base editor architecture that shows improved precision and reduced off-target effects (Gehrke et al., *Nat Biotechnol*. 2018).

Epigenetic 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 factors that can robustly alter expression of endogenous human 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:

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, in press.

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 Jul 30. doi: 10.1038/nbt.4199.

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.

Tsai SQ, Nguyen NT, Malagon-Lopez J, Topkar VV, Aryee MJ, Joung JK. CIRCLE-seq: a highly sensitive in vitro screen for genome-wide CRISPR-Cas9 nuclease off-targets. *Nat Methods*. 2017; 14(6):607-614.

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Kleinstiver BP, Pattanayak V, Prew MS, Tsai SQ, Nguyen NT, Zheng Z, Joung JK. High-fidelity CRISPR-Cas9 nucleases with no detectable genome-wide off-target effects. *Nature.* 2016; 529(7587): 490-5.

Kleinstiver BP, Prew MS, Tsai SQ, Nguyen NT, Topkar VV, Zheng Z, Joung JK. Broadening the targeting range of Staphylococcus aureus CRISPR-Cas9 by modifying PAM recognition. *Nat Biotechnol.* 2015; 33(12): 1293-1298.

Selected Publications:

Hayes MN, McCarthy K, Jin A, Oliveira ML, Iyer S, Garcia SP, Sindiri S, Gryder B, Motala Z, Nielsen GP, Borg JP, van de Rijn M, Malkin D, Khan J, Ignatius MS, Langenau DM. Vangl2/RhoA Signaling Pathway Regulates Stem Cell Self-Renewal Programs and Growth in Rhabdomyosarcoma. *Cell Stem Cell.* 2018; 22(3):414-427.

Lobbardi R, Pinder J, Martinez-Pastor B, Theodorou M, Blackburn JS, Abraham BJ, Namiki Y, Mansour M, Abdelfattah NS, Molodtsov A, Alexe G, Toiber D, de Waard M, Jain E, Boukhali M, Lion M, Bhere D, Shah K, Gutierrez A, Stegmaier K, Silverman LB, Sadreyev RI, Asara JM, Oettinger MA, Haas W, Look AT, Young RA, Mostoslavsky R, Dellaire G, Langenau DM. TOX Regulates Growth, DNA Repair, and Genomic Instability in T-cell Acute Lymphoblastic Leukemia. *Cancer Discov*. 2017; 7(11):1336-1353.

Tang Q, Moore JC, Ignatius MS, Tenente IM, Hayes MN, Garcia EG, Torres Yordán N, Bourque C, He S, Blackburn JS, Look AT, Houvras Y, Langenau DM. Imaging tumour cell heterogeneity following cell transplantation into optically clear immune-deficient zebrafish. *Nat Commun.* 2016; 7:10358.

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

Ignatius MS, Chen E, Elpek NE, Fuller A, Tenente IM, Clagg R, Liu S, Blackburn JS, Linardic CM, Rosenberg A, Nielsen PG, Mempel TR, Langenau DM. In vivo imaging of tumor-propagating cells, regional tumor heterogeneity and dynamic cell movements in embryonal rhabdomyosarcoma. *Cancer Cell.* 2012; 21(5):680-93.

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"Identifying molecular pathways that drive progression and relapse in pediatric cancer..."

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

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 that the RAS pathway is activated in a majority of ERMS. Building on this discovery, our laboratory has developed a transgenic zebrafish model of RAS-induced ERMS that mimics the molecular underpinnings of human ERMS. We used fluorescent transgenic zebrafish to label functionally distinct tumor cells. Specifically, the myf5-GFP+ self-renewing cancer stem cells drive continued tumor growth at relapse and are molecularly similar to non-transformed, activated muscle satellite cells. Building on the dynamic live cell imaging approaches available in the zebrafish ERMS model, our laboratory has uncovered a number of molecular pathways that drive continued tumor growth and progression by regulating cancer stem cell function. Finally, using chemical genetic approaches, we have identified drugs that kill relapse-associated, self-renewing ERMS cells. We are currently assessing the genetic pathways uncovered by our work and a subset of drugs for their ability to regulate growth of patient-derived xenografts.

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 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 an important role in regulating human T-ALL proliferation, apoptosis and response to therapy. Discovering novel relapse-driving oncogenic pathways will likely identify new drug targets for the treatment of T-ALL.

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"Elucidating the molecular basis of glioma formation impacts both diagnostic and therapeutic aspects of clinical neuro-oncology…"

Over the past 25 years, we have demonstrated alterations characteristic of specific glioma subtypes and grades. We originally demonstrated that molecular genetic analysis could be used to define clinicopathologically relevant subsets of glioblastomas, and then showed that molecular genetic alterations are powerful predictors of therapeutic response and survival in patients with anaplastic oligodendrogliomas and in other oligodendroglial tumors. These findings have already led to incorporation of molecular diagnostic testing around the world for these parameters. Work over the past few years has been directed toward incorporating molecular testing into the World Health Organization Classification of Central Nervous System Tumors, a process directed by Dr. Louis, and toward making molecular diagnostics a practical and routine part of brain tumor diagnosis through international efforts led by Dr. Louis (the Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy [cIMPACT] and the CNS dataset of the International Collaboration on Cancer Reporting [ICCR]). The lab has also demonstrated that glioblastomas treated with the alkylating agent temozolomide (which is now the standard of care for such cases) frequently inactivate mismatched repair genes, leading to more rapid growth during therapy and to therapeutic resistance, and has worked collaboratively on epigenetic and single-cell studies of high-grade gliomas.

Selected Publications:

Louis DN, Wesseling P, Paulus W, Giannini C, Batchelor TT, Cairncross JG, Capper D, Figarella-Branger D, Lopes MB, Wick W, van den Bent M. cIMPACT-NOW update 1: Not Otherwise Specified (NOS) and Not Elsewhere Classified (NEC). *Acta Neuropathol*. 2018; 135(3):481-484.

Louis DN, Aldape K, Brat DJ, Capper D, Ellison DW, Hawkins C, Paulus W, Perry A, Reifenberger G, Figarella-Branger D, Wesseling P, Batchelor TT, Cairncross JG, Pfister SM, Rutkowski, Weller M, Wick W, von Deimling A. Announcing cIMPACT-NOW: The Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy. *Acta Neuropathol*. 2017; 133:1-3.

Louis DN, Ohgaki H, Wiestler OD, Cavenee WK (eds.). *World Health Organization Histological Classification of Tumours of the Central Nervous System*. Lyon: International Agency for Research on Cancer, 2016.

Louis DN, Perry A, Reifenberger G, von Deimling A, Figarella-Branger D, Cavenee WK, Ohgaki H, Wiestler OD, Kleihues P, Ellison DW. The 2016 WHO classification of tumours of the central nervous system: a summary. *Acta Neuropathol*. 2016; 131(6):803-820.

Louis DN. Perry A, Burger P, et al. International Society of Neuropathology-Haarlem consensus guidelines for nervous system tumor classification and grading. *Brain Pathol*. 2014; 24:429-435.

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"Understanding gene regulation using computational methods for epigenomics, genome editing and single cell analysis"

The focus of the Pinello laboratory is to use innovative computational approaches and cutting-edge experimental assays to systematically analyze sources of genetic and epigenetic variation and (single-cell) 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 more targeted treatments.

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 developed 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.

Computational methods for genome editing

We embraced the revolution in functional genomics made possible by the novel genome editing approaches such as CRISPR/Cas9 by developing computational tools to quantify and visualize the outcome of sequencing data originating from these powerful assays. We created a computational tool called CRISPResso (*http://github.com/lucapinello/CRISPResso*), an integrated software pipeline for the analysis and visualization of CRISPR-Cas9 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 the groups of Daniel Bauer and Stuart Orkin, 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. We also recently released an end-to-end protocol for the design, execution, and analysis of arrayed and pooled CRISPR genome-editing experiments.

Single cell analysis

We are developing tools to characterize cellular types and states at single cell resolution by using data from single cell transciptomic or epigenomics data. For example, we recently released STREAM (Single-cell Trajectories Reconstruction, Exploration And Mapping), an interactive computational pipeline for reconstructing complex celluar developmental trajectories from sc-qPCR, scRNA-seq or scATAC-seq data available at *http://stream.pinellolab.org*.

Selected Publications:

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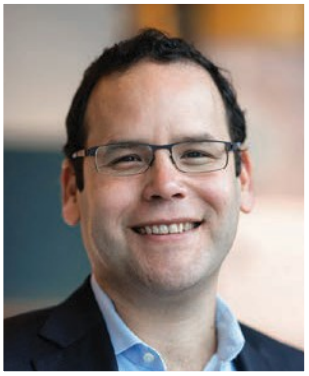

"Using genomics to identify critical pathways in pediatric tumors..."

Pediatric tumors are often driven by mutations in genes that directly control gene expression programs such as transcription factors and chromatin regulators. Our laboratory uses genomic technologies to identify abnormal gene regulation patterns in tumors and to analyze critical oncogenic pathways in several systems. Given that the mechanisms that drive pediatric tumors are poorly understood at present, we anticipate that our work will point to new therapies for these diseases.

Epigenomic approaches to identify critical pathways in cancer

We are using genomic technologies to identify abnormal gene regulation patterns in pediatric cancer. In particular, genome-wide chromatin profiling, which combines chromatin immunoprecipitation and high-throughput sequencing, is a powerful technology that can identify active and repressed states in the genome based on patterns of histone modifications. Our work using this technology has shown that Wilms tumors exhibit chromatin features typical of stem cells and that patterns of chromatin remodeling can reveal the mechanisms of action of aberrant transcriptional regulators such as the EWS-FLI1 fusion protein in Ewing sarcoma and the transcription factor OTX2 in medulloblastoma. We are now using a combination of biochemical and genomic tools to continue our analysis of these and other key transcriptional pathways in pediatric cancer.

Role of the WTX gene family in cancer and development

Wilms tumor, the most common pediatric kidney cancer, arises from kidney-specific stem cells and is a prime example of the connection between cancer and development. Through mapping genomic deletions in Wilms tumor we identified WTX, an X-linked tumor suppressor gene commonly inactivated in this disease and recently implicated in colon cancer. WTX is the founding member of a new protein family (FAM123) and our work using a conditional knockout mouse model has shown that it regulates mesenchymal stem cells in several organs, including kidneys, bones and fat. We are now studying the function of WTX and related proteins using several in vitro and in vivo model systems.

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*Co-first authorship

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"Identifying treatment predictive biomarkers to optimize personalize medicine for women with breast cancer..."

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 advanced molecular 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 and from novel targeted therapeutics.

My 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 arrays and real-time quantitative PCR and advanced tandem mass spectrometry technologies to identify novel gene and protein expression patterns in human breast cancer. 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 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 DNA microarray and proteomic technologies as a means to predict the clinical behavior of human breast cancer in the setting of 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 is a biomarker of endocrine responsiveness in ER+ breast cancer, as it has been shown to predict for benefit from adjuvant and extended anti-hormonal 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. Lastly, we are currently investigating the functional activity of HOXB13 and IL17BR and assessing their possible role as a surrogate marker for a nonclassical estrogen receptor signaling pathway.

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"Investigating hereditary brain tumor syndromes..."

Our lab's research focuses on identifying the molecular changes that lead to formation and progression of tumor in patients with hereditary brain tumor syndromes (neurofibromatosis 1, neurofibromatosis 2, schwannomatosis, tuberous sclerosis and von Hipple Lindau). In collaboration with both clinical groups and basic science/mouse modelling groups, our research focuses on translating novel findings to clinical pathology; identifying biomarkers that aid in diagnosis and classification of NF associated tumors; analyzing human tumors to validate novel findings in animal models; and identifying activated pathways that may be targetable by therapy and may help in the development of new treatment modalities by analyzing the effects on treated tumors (in animals and humans). For example, Schwannomas are benign nerve sheath tumors that may arise in people with no underlying genetic syndrome (solitary, sporadic schwannomas) or in the context of two hereditary tumor syndromes: neurofibromatosis 2 and schwannomatosis. Although all schwannomas share the loss of function of the NF2 gene, our hypothesis is that additional microenvironmental factors or epigenetic events are responsible for the clinical manifestations associated with these tumors, such as pain, hearing loss or rapid tumor growth. The identification of these events and of the pathways involved may aid in the diagnosis of the different subclinical types of schwannomas as well as in the development of targeted therapies. Our recent work in collaboration with researchers and clinicians at MGH has unraveled molecular pathways of angiogenesis in schwannomas, leading to targeted antiangiogenesis therapy with clinical improvement in a small series of patients with NF2-associated schwannomas.

Selected Publications:

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"Genetic and non-genetic determinants of single-cell programs in human gliomas"

Our laboratory is focused on the biology of brain tumors, in particular diffuse gliomas in adults and children. We study primary human samples at single-cell resolution using transcriptomic and genomic approaches. We reconstruct the cellular composition of patient tumors and relate it to genetic mutations. We model how brain cancer cells exploit developmental programs to establish distinct cellular states. Additionally, the laboratory investigates how genetic events affecting genes involved in chromatin regulation rewire cancer cells' identities to contribute to cellular transformation. We seek to identify common programs that would offer novel therapeutic options in these dismal diseases.

Heterogeneity in brain tumors assessed at single-cell level

We are deploying cutting-edge single-cell genomic and transcriptional profiling to clinical samples. Our unique approach allows us for the first time to relate genotype to phenotype at single-cell resolution in clinical tumor specimens. Through our efforts, we are redefining our understanding of malignant cell subpopulations in glioblastoma, oligodendroglioma, astrocytoma, diffuse intrinsic pontine glioma, medulloblastoma and synovial sarcoma. In addition, our single-cell genomics efforts are providing invaluable insights into myeloid cells and T cell programs in clinical tumors.

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"A major focus of the lab is to understand how epigenetic lesions conspire with genetic events to drive tumorigenesis and drug resistance"

In the Bernstein laboratory we study epigenetics — changes in gene activity governed by influences outside the genes themselves — and specifically how modifications to the protein scaffold called chromatin contribute to mammalian development and human cancer. Our laboratory develops genomic technologies to study chromatin structure and epigenetic regulation. Our work is notable for the discovery of epigenetic mechanisms in stem cells, the annotation of thousands of enhancer "switches" in the human genome relevant to common disease, and the characterization of epigenetic lesions that drive brain tumors and other forms of cancer.

Cancer epigenetics

Genes encoding chromatin regulators are frequently mutated in human cancer. Moreover, cells in an individual tumor can vary markedly in their epigenetic states, transcriptional outputs, and functional phenotypes. We seek to understand how epigenetic lesions and epigenetic heterogeneity contribute to key cancer cell properties, such as tumor propagation, stemness, and drug resistance. We characterize the transcriptional and epigenetic landscapes of primary tumors at the single cell level. In parallel, we develop and perturb representative tumor models in the laboratory. These synergistic approaches can inform therapeutic strategies for targeting epigenetic lesions or overcoming resistance mechanisms.

Technologies for mapping histone modifications and chromatin proteins

We innovate and combine technologies in stem cell biology, biochemistry, imaging, and genome engineering with next-generation sequencing to achieve increasingly precise, genome-wide views of chromatin structure, chromatin regulator binding and genome organization. Genetic and chemical perturbations then allow us to test predicted regulatory interactions and functions. Ongoing projects apply these approaches to characterize noncoding regulatory elements and chromatin structure in the human genome and to understand how the resulting cell circuits control gene expression programs during development and in cancer. We also develop and leverage emerging single-cell and single-molecule techniques to deconvolve heterogeneous cell populations and dynamic processes in tumors.

Epigenetic regulation of stem cell differentiation

Chromatin regulators 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. In ES cells, bivalent domains appear to keep developmental genes poised for alternate fates. We now apply emerging chromatin and genome engineering approaches to study how bivalent domains and interacting regulatory elements program gene expression in development.

Selected Publications:

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"Elucidating the immunophenotype and functional capacity of stem cells capable of developing into various tissue lineages..."

The common lymphoid progenitor (CLP) responsible for the formation of T, B and NK cells is derived from a hematopoietic stem cell that is first identified in the embryonic aorto-gonad-mesonephros, a descendent of the mesoderm. The signals to initiate and regulate development are due to the control imposed by a variety of marrow stromal cells, transcription factors, and coordinated regulation by the nervous system, extracellular matrix, cytokines and adipocytes found in the bone marrow microenvironment. The general consensus of the ontological steps leading to production of naïve B-cells is summarized as follows; the earliest identifiable committed B-cells derived from the CLP are called progenitor (Pro) B-cells. Pro B-cells arise after obligate stimulation by the transcription factor PAX-5, which engenders CD19 production. These CD34+ CD19+ CD10+ CD38+ TdT+ expressing cells lack the pre-B-cell receptor or surface immunoglobulin (Ig) and initiate VDJ heavy chain rearrangements independent of any antigenic exposure. Pro B cells differentiate into CD34- CD19+ CD10+ CD38+ TdT- precursor (Pre) B-cells that acquire cytoplasmic and then surface mu heavy chain with a transient surrogate immunoglobulin light chain. Next, a CD19+ CD10-CD38- immature B-cell expresses surface IgM+ and physiologic light chain. Ultimately, CD19+ CD20+ B-cells co-expressing IgM and IgD heavy chains exit the bone marrow as transitional B-cells and home to secondary lymphoid organs as naive B-cells.

We are interested in the use of probability state modeling to quantify the locations of antigen modulations during the ontological development of human B-cells to determine the discrete progenitor and B-cell stages that occur during normal maturation. We will use this information to study and predict minimal residual disease in patients with B-lymphoblastic lymphoma.

The MGH Flow Cytometry research laboratories are located on the MGH campus in Simches 3.434 and CNY-5 [2015]. These hospital core resources will entertain research collaborations from throughout the pathology laboratories and greater hospital and university. The CNY flow laboratory, overseen by Dr. R. Mylvaganam, H. Ravichandran, R. Servis and J. Fung, contains a FACSAria II sorter, LSR-2, Fortessa, Cytoflex analyzer, Helios mass cytometer and FACSFusion sorter for BSL2+ operations. The Simches flow and imaging laboratory contains a DiVa cell sorter and LSR-2 operated by D. Dombkowski. A FACSFusion sorter permits BSL2+ sorting in that facility, as well. This laboratory also contains an Amnis ISX mkII imaging flow cytometer which permits bright-field and fluorescent visual analysis of immunophenotyped cells, run by S. Mordecai. The clinical flow cytometry laboratory is located on Warren 5 on the MGH campus in Boston, supervised by M. DeLelys with two FACSCanto-IIs available at that site.

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"Investigating the molecular mechanisms of vascular disease..."

The Stone Laboratory studies mechanisms underlying human vascular diseases, such as atherosclerosis and vasculitis. Atherosclerosis is the principal cause of heart disease and a leading cause of stroke, making it the most common cause of death in the U.S. The laboratory is seeking to understand the molecular processes resulting in atherosclerosis in order to combat this pervasive disease. Atherosclerosis is characterized by the development of necrotic/lipid cores within the intima of arteries at particular sites in the circulation. These necrotic/lipid cores form in the setting of a pre-existing intimal hyperplasia, characterized by the proliferation of smooth muscle-like cells in the intima. The laboratory is investigating both the signal transduction mechanisms responsible for the formation of the preatherosclerotic intimal hyperplasia as well as the factors stimulating the formation of intimal necrotic/lipid cores.

Essentially all risk factors for atherosclerosis result in the enhanced generation of hydrogen peroxide in the vessel wall by the activation of membrane-bound NADPH oxidases. These low physiologic levels of hydrogen peroxide are mitogenic, stimulating vascular cell growth and proliferation. The mechanisms by which low endogenous levels of hydrogen peroxide stimulate cellular proliferation are currently poorly understood. The laboratory is using molecular approaches with cultured vascular cells and cultured human arteries to identify signal transduction pathways activated by low physiologic levels of hydrogen peroxide. One such novel pathway identified in the laboratory is the CK1aLS/hnRNP-C signaling pathway, which has been shown to mediate hydrogen peroxide-stimulated mitogenic signaling in vascular cells and to promote intimal hyperplasia in cultured human arteries.

Intimal hyperplasia, the precursor lesion for atherosclerosis, forms both in vessels that are prone to develop atherosclerosis and in vessels remarkably resistant to atherosclerosis. Intimal hyperplasia can be formed in vitro with human artery segments in culture. The laboratory is using novel human artery culture models combined with molecular analyses of diseased human arteries to identify, characterize, and functionally assess the vascular wall factors that promote the transition from intimal hyperplasia to human atherosclerosis.

Selected Publications:

Siddiquee Z, Smith RN, Stone JR. An elevated IgG4 response in chronic infectious aortitis is associated with aortic atherosclerosis. *Mod Pathol*. 2015; 28:1428-1434.

Halushka MK, Eng G, Collins AB, Judge DP, Semigran MJ, Stone JR. Optimization of serum immunoglobulin free light chain analysis for subclassification of cardiac amyloidosis. *J. Cardiovasc Translat Res.* 2015; 8:264-268.

Wang H, Albadawi H, Siddiquee Z, Stone JM, Panchenko MP, Watkins MT, Stone JR. Altered vascular activation due to deficiency of the NADPH oxidase component p22phox. *Cardiovasc Pathol.* 2014; 23:35-42.

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Panchenko MP, Siddiquee Z, Dombkowski DM, Alekseyev YO, Lenburg ME, Walker JD, MacGillivray TE, Preffer FI, Stone JR. Protein kinase CK1aLS promotes vascular cell proliferation and intimal hyperplasia. *Am J Pathol*. 2010; 177:1562-1572.

Panchenko MP, Silva N, Stone JR. Upregulation of a hydrogen peroxide responsive pre-mRNA binding protein in atherosclerosis and intimal hyperplasia. *Cardiovasc Pathol*. 2009; 18: 167-172.

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"Molecular characterization of human tumors to identify markers of response to targeted therapeutics…"

Targeted cancer therapies require the rapid and accurate identification of genetic abnormalities predictive of therapeutic response. Our lab developed the first high-throughput clinical genotyping platform designed to detect specific mutations in a broad range of human malignancies and enable prospective patient selection to the most appropriate targeted treatments. To obtain a more complete tumor genetic fingerprint and expand the scope of therapeutic options available to each patient, we improved upon our original platform by the development of next generation sequencing technologies. Our clinical molecular panels identify somatic mutations, genetic rearrangements and copy number alterations in a wide range of solid tumors and hematopoietic malignancies.

Our research efforts have focused on the molecular characterization of rare tumor types and led to the identification of clinically relevant genetic alterations in Merkel cell carcinoma, pleomorphic xanthoastrocytoma and salivary duct carcinoma. We have developed research collaborations with the MGH Thoracic Oncology, the Endocrine Unit and the Gastrointestinal Cancer teams, to uncover novel genetic drivers of malignancy, monitor disease progression, and identify mechanism of acquired resistance to therapy.

Selected Publications:

Dias-Santagata D, Selim MA, Su Y, Peng Y, Vollmer R, Chłopik A, Tell-Marti G, Paral KM, Shalin SC, Shea CR, Puig S, Fernandez-Figueras MT, Biernat W, Ryś J, Marszalek A, Hoang MP. KIT mutations and CD117 overexpression are markers of better progression-free survival in vulvar melanomas. *Br J Dermatol*. 2017;177(5):1376-1384.

McFadden DG*, Dias-Santagata D*, Sadow PM, Lynch KD, Lubitz C, Donovan SE, Zheng Z, Le L, Iafrate AJ, Daniels GH. Identification of oncogenic mutations and gene fusions in the follicular variant of papillary thyroid carcinoma. *J Clin Endocrinol Metab*. 2014; 99(11):E2457-62.

Nardi V, Sadow PM, Juric D, Zhao D, Cosper AK, Bergethon K, Scialabba VL, Batten JM, Borger DR, Iafrate AJ, Heist RS, Lawrence DP, Flaherty KT, Bendell JC, Deschler D, Li Y, Wirth LJ, Dias-Santagata D. Detection of novel actionable genetic changes in salivary duct carcinoma helps direct patient treatment. *Clin Cancer Res.* 2013; 19(2):480-90.

Nardi V, Song YC, Santamaria-Barria JA, Cosper AK, Lam Q, Faber AC, Boland GM, Yeap BY, Bergethon K, Scialabba VL, Tsao H, Settleman J, Ryan DP, Borger DR, Bhan A, Hoang MP, Iafrate AJ, Cusack JC, Engelman JA, Dias-Santagata D. Activation of PI3K signaling in Merkel cell carcinoma. *Clin Cancer Res*. 2012; 18(5):1227-36.

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*Equal contribution

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"Enabling computational pathology…"

Computational Pathology is defined as "an approach to diagnosis that incorporates multiple sources of raw data; extracts biologically and clinically relevant information from those data; uses mathematical models . . . to generate diagnostic inferences and predictions; and presents that clinically actionable knowledge to customers" (*Arch Pathol Lab Med* 2014). Other industries such as finance, e-commerce, social media, and travel have benefited from access to and computation of structured, harmonized data to drive descriptive and predictive analytics. The same analytics and machine learning tools that have been developed for these industries could be leveraged to make our practice of pathology more effective, efficient, and economical.

In the Center for Integrated Diagnostics, we have developed the computational pathology infrastructure to generate, capture, and integrate genomics results with laboratory data. Having access to this integrated data store has greatly enhanced the practice of clinical genomics in the molecular diagnostics laboratory. By storing the data in a readily accessible database and combining it with a user interface for querying, pathologists, technicians, software engineers, bioinformaticians, data scientists, residents, and fellows have been able to generate queries to explore the data for both clinical and research purposes. Interfaces have been built to take advantage of historical data to present descriptive analytics about variant detection across all prior cases. In addition, data scientists in the team have used the data to generate several predictive models that are shown during clinical signout. These models include prediction of variant reporting, patient gender, sample swap, and microsatellite instability from the genomics data.

We have built a strong computational pathology team of software engineers, web developers, and data scientists who will integrate the data that we generate across our pathology laboratories with the electronic medical record. The integration of pathology data with clinical data will allow us to explore, gain insight, derive hypotheses, and generate models/tools to help with our day to day workflow. Our efforts will drive not only the clinical operation but also research and discovery.

Selected Publications:

Zomnir MG, Lipkin L, Pacula M, Dominguez Meneses E, MacLeay A, Duraisamy S, Nadhamuni N, Al Turki SH, Zheng Z, Rivera M, Nardi V, Dias-Santagata D, Iafrate AJ, Le LP, and Lennerz JK. Artificial Intelligence Approach for Variant Reporting. *JCO Clinical Cancer Informatics*. 2018; 2:1-13.

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"Pathogenesis of rejection and tolerance of organ transplants..."

The mechanisms of graft acceptance (tolerance) have been a major area of investigation in the transplant group at MGH, with mouse, pigs, non-human primates and most recently a clinical trial. Dr. Colvin is currently seeking the mechanisms of graft acceptance and the role of Foxp3+ Treg cells in mouse kidney allografts. These studies have revealed a novel Treg-rich organized lymphoid structure (TOLS) in accepted allografts that surround small arteries. Depletion of Treg causes dissolution of the TOLS and precipitates acute graft rejection. Further studies have revealed that mixed chimerism-induced tolerance leads to deletional tolerance of MHC antigens and regulatory tolerance of non-MHC antigens. Current studies are focused on the mechanism of kidney-induced tolerance using mRNA Nanostring analysis.

In studies in human kidney allografts, Dr. Colvin's group was the first to describe chronic antibody-mediated rejection, now recognized as the most common cause of late graft dysfunction. He has shown that deposition of the classical complement component, C4d, in peritubular capillaries is a useful marker of acute and chronic antibody-mediated rejection. C4d is the most specific marker of these conditions. Through the efforts of Dr. Colvin and others, new categories of acute and chronic antibody-mediated rejection have been incorporated into the Banff criteria and have become the standard of care. Protocol biopsies from non-human primate studies have demonstrated sequential stages of chronic humoral rejection and tolerance with distinctive mRNA signatures.

A major problem in long-term organ grafts is the development of chronic arteriopathy, which has an unknown pathogenesis. Dr. Colvin and Dr. Paul Russell developed and characterized a model of the disease, using heart grafts in mice. Coronary arteries develop florid lesions over 4-8 weeks, resembling closely the lesions in human organ grafts. The group showed that chronic allograft arteriopathy can be produced by three distinct immune pathways, humoral antibody (passive transfer of anti-donor antibodies into RAG-1 knockout mice), T cells (male to female grafts) or natural killer cells (parental graft to F1 recipients). Such antibodies can mediate chronic arteriopathy in the absence of complement, through an NK cell-dependent FcR mechanism.

The immunopathogenesis of renal diseases is Dr. Colvin's other long-term interest. He has recently identified a new disease due to anti-brush border antibodies (ABBA) that deposit in the proximal tubules. The publication led to the discovery of several other cases. The nature of the antigen was recently identified as megalin.

Selected Publications:

Smith RN, Adam BA, Rosales IA, Matsunami M, Oura T, Cosimi AB, Kawai T, Mengel M and Colvin RB. RNA expression profiling of renal allografts in a nonhuman primate identifies variation in NK and endothelial gene expression. *Am J Transplant*. 2018; In Press.

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Akiyoshi T, Hirohashi T, Alessandrini A, Chase CM, Farkash EA, Neal Smith R, Madsen JC, Miyajima M, Chase CM, Alessandrini A, Farkash EA, Della Pelle P, Benichou G, Graham JA, Madsen JC, Russell PS, Colvin RB. Early acceptance of renal allografts in mice is dependent on Foxp3+ cells. *Am J Pathol*. 2011; 178:1635-45.

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"Investigating the causes of acute and chronic rejection..."

Dr. Smith's research focuses primarily on the immunology of transplantation, with emphasis on the transplantation pathology of the heart, kidney, and pancreatic islets. He is particularly interested in how the acute and chronic rejection of allografts and xenografts come about. Studies involve patients and animal experimentation with heart, kidney and pancreatic islet grafts. With expertise in these areas, Dr. Smith is a consultant pathologist to investigators within the Harvard community, national consortia, and the Transplant Biology Research Program at MGH with clinical and preclinical transplant programs. Dr. Smith is also a consultant to revisions of the classification scheme for human heart allograft biopsies.

Current emphasis and ongoing work includes studies of cellular and humoral rejection in cardiac allografts of humans and mice (hearts) and in kidneys of monkeys and humans. Dr. Smith has been able to correlate by indirect immunofluorescence C4d staining and the presence of alloantibodies in cardiac allografts. With investigators at other institutions, using clinical data, criteria are being established for the diagnosis of acute antibody-mediated rejection in human cardiac transplants. Dr. Smith and Dr. Colvin are studying the progression of monkey kidney allograft rejection that comes about with development of alloantibodies, chronic antibody-mediated rejection. They established that alloantibodies are the causative of the glomerulopathy of chronic humoral rejection in allografted kidneys, and established that chronic antibody-mediated rejection develops through four stages. Using RNA gene expression and statistical modelling, Dr. Smith has identified biologically important subgroups in allografts, including tolerance, which is not readily classified by just pathological review. Dr. Smith, along with other investigators studying islet allograft survival, has established that portal vein-based islet allografts can undergo a non-immunological senescence. Dr. R Abdi and Dr. Smith are investigating why knockout of certain chemokine genes, dendritic cells, and stem cells affect graft rejection and donor dendritic cell migration. In some autologous stem cell transplants in mice, sarcomas developed. With AB Collins and Dr. JR Stone we have established the utility of immunofluorescence for the classification of amyloid deposits.

Selected Publications:

Hotta K, Oura T, Dehnadi A, Boskovic S, Matsunami M, Rosales I, Smith RN, Colvin RB, Cosimi AB, Kawai T. Long-term Nonhuman Primate Renal Allograft Survival Without Ongoing Immunosuppression in Recipients of Delayed Donor Bone Marrow Transplantation. *Transplantation.* 2018 ;102(4):e128-e136.

Smith RN, Matsunami M, Adam BA, Rosales IA, Oura T, Cosimi AB, Kawai T, Mengel M, Colvin RB. RNA expression profiling of nonhuman primate renal allograft rejection identifies tolerance. *Am J Transplant.* 2017;18(6):1328-1339.

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"The role of long noncoding RNA in epigenomic regulation..."

X-chromosome inactivation (XCI) is a biological process used by mammals like us to ensure that boys (XY) and girls (XX) have equal sex chromosome dosage despite having a different number of X-chromosomes. XCI happens very early during uterine development and leads to inactivation of one of the female embryo's 2 X-chromosomes. For XCI to occur, every embryo "counts" chromosomes. In embryos with 2 Xs, one of the Xs makes a "choice" to undergo inactivation. For this to happen properly, each X has to know what the other X intends to do. We believe that the Xs communicate this choice through a "pairing" process in which they make contact briefly at two noncoding loci — Tsix/Xite and the telomere. The chosen X chromosome then initiates "silencing" within a specific region — called the "X-inactivation center" — and silencing is "spread" throughout the large sex chromosome. During the silencing process, the X chromosome is folded like origami into a unique shape that helps to suppress gene activity. Thus, XCI involves a series of highly orchestrated steps. It is particularly interesting, because the major decision-makers (molecular regulators) appear to be non-coding in nature. Non-coding RNA plays a key role in every step of the XCI process, from counting to pairing to silencing and to 3D chromosome-folding. Our lab's goal is to understand how RNA interfaces with protein at each step of the XCI process.

We also aim to translate the knowledge gained from basic studies to treat human disorders — particularly neuro-developmental disorders and X-linked intellectual disabilities (XLID). Three disease areas of current interest are Rett Syndrome, the Fragile X Syndrome, and CDKL5 Syndrome. In each case, the girls and boys who suffer from the disorder harbor a perfectly good copy of the necessary gene, but the good copy is locked up by a silencing mechanism: MECP2 in the case of Rett Syndrome, FMR1 for Fragile X, and CDKL5 for CDKL5 Syndrome. Our goal is to use our understanding of XCI to unlock the silent copy of each gene for therapeutic benefit.

Selected Publications:

Wang CY, Jegu T, Chu HP, Oh HJ, Lee JT. SMCHD1 merges chromosome compartments and assists formation of Super-structures on the inactive X. *Cell*. 2018;174(2):406-421.

Carrette LG, Wang CY, Press W, Ma W, Kelleher RJ, Lee JT. Xi-reactivation: A mixed modality approach to MECP2 restoration for Rett Syndrome and other X-linked disorders. *Proc Natl Acad Sci*. USA 2017 ;115(4):E668-E675.

Del Rosario BC, Del Rosario AM, Anselmo A, Wang PI, Sadreyev RI, Lee JT. Genetic intersection of Tsix and Hedgehog signaling during the initiation of X chromosome inactivation. *Dev Cell*. 2017; 43, 359-371.

Rosenberg M, Blum R, Kesner B, Maier VK, Szanto A, Lee JT. Denaturing CLIP, dCLIP, pipeline identifies discrete RNA footprints on chromatin-associated proteins and reveals that CBX7 targets 3' UTRs to regulate mRNA expression. *Cell Syst.* 2017; 5, 368-385.

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Chu HP, Cifuentes-Rojas C, Kesner B, Aeby E, Lee HG, Wei C, Oh HJ, Boukhali M, Haas W, Lee JT. TERRA RNA antagonizes ATRX and protects telomeres. *Cell*. 2017; 170:86-101.

Zovoilis A, Cifuentes-Rojas C, Chu HP, Nernandez AJ, Lee JT. Destabilization of B2 RNA by EZH2 activates the stress response. *Cell*. 2017; 167, 1788-1802.

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"The development and validation of non-invasive, high-resolution optical imaging methods for disease diagnosis..."

Dr. Tearney's research interests are focused on the development and clinical validation of non-invasive, high-resolution optical imaging methods for disease diagnosis. Dr. Tearney's lab was the first to perform human imaging in the coronary arteries and gastrointestinal tract in vivo with Optical Coherence Tomography (OCT), which provides cross-sectional images of tissue architectural microstructure at a resolution of 10 μm. He has also conducted many of the seminal studies validating OCT and is considered an expert on OCT image interpretation. Recently, Dr. Tearney's lab has invented a next generation OCT technology, termed μOCT, which has a resolution of 1 μm and is capable of imaging cells and sub cellular structures in the coronary wall. Dr. Tearney has also developed several other technologies, including a confocal endomicroscope capable of imaging the entire esophagus, a capsule that once swallowed captures three-dimensional microscopic images of the GI tract, an ultraminiature three-dimensional endoscope, a highly efficient form of near field scanning optical microscopy (NSOM), and novel fluorescence spectroscopy and multimodality imaging techniques. He has an active program in Raman spectroscopy and has conducted the first intracoronary Raman in vivo. Dr. Tearney is co-editor of *The Handbook of Optical Coherence Tomography* and has written over 250 peer-reviewed publications.

Dr. Tearney's work extends beyond his laboratory at MGH. Many of his technologies are being produced commercially; he is the vice-chair of CAP's in vivo microscope committee and he has founded the International Working Group on Intravascular OCT Standardization and Validation, a group that is dedicated to establishing standards to ensure the widespread adoption of this imaging technology.

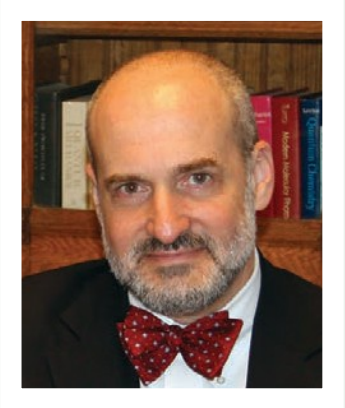
Selected Publications:

Yin B, Hyun C, Gardecki JA, Tearney GJ. Extended depth of focus for coherence-based cellular imaging. *Optica.* 2017; 4:959-965.

Ughi GJ, Wang H, Gerbaud E, Gardecki JA, Fard AM, Hamidi E, Vacas-Jacques P, Rosenberg M, Jaffer FA, Tearney GJ. Clinical characterization of coronary atherosclerosis with dual-modality OCT and near-infrared autofluorescence imaging. *JACC Cardiovasc Imaging*. 2016; 9(11):1304-14.

Gora MJ, Sauk JS, Carruth RW, Gallagher KA, Suter MJ, Nishioka NS, Kava LE, Rosenberg M, Bouma BE, Tearney GJ. Tethered capsule endomicroscopy enables less invasive imaging of gastrointestinal tract microstructure. *Nat Medicine*. 2013; 19(2):238-40.

Yoo H, Kim JW, Shishkov M, Namati E, Morse T, Shubochkin R, McCarthy J, Ntziachristos V, Bouma B, Jaffer F, Tearney GJ. Intra-arterial catheter for simultaneous microstructural and molecular imaging in vivo. *Nat Medicine*. 2012; 17:1680-4.



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"Understanding the pathogenesis of cerebral amyloid angiuopathy (CAA)..."

My lab aims to understand cerebral amyloid angiopathy (CAA), using mouse models and human tissue. In this disease, the peptide Aβ deposits in the walls of blood vessels and is associated with risk of hemorrhage ("lobar hemorrhages"). This peptide is the same material that forms the plaques of Alzheimer disease, and nearly all patients with Alzheimer disease have pathologic evidence of CAA as well. CAA also occurs in the absence of histologic evidence of Alzheimer disease, and can present with hemorrhages or with cognitive changes. In clinicopathologic studies, we have found that this latter presentation is associated with the presence of an inflammatory response, often containing giant cells. This subset of patients can have dramatic recoveries of cognitive function after immunosuppressive therapy.

We are interested in the sequence of events by which Aβ is deposited in blood vessels, what factors determine the distribution of involvement, what the consequences are for the cells of the vessel and how this material can respond to therapeutic interventions focused on Aβ currently in clinical trials. For in vivo studies, we use serial multiphoton imaging with specific probes for these various processes and link the spatial and temporal distribution of the pathologic changes with the development of CAA. We complement these studies with work on human autopsy tissue, collected through the Massachusetts Alzheimer Disease Research Center Neuropathology Core. Those samples are examined through combinations of high field ex vivo MRI, optical clearing and volumetric imaging. We are particularly interested in the changes which result in bleeding in the setting of CAA (hemorrhagic strokes) as well as microinfarcts which can markedly impair cognition.

I also work with a range of collaborators to understand the relationship between neuropathologic findings in the setting of disease — including Alzheimer disease, Parkinson disease, Amyotrophic Lateral Sclerosis and others — and other biochemical or functional markers of disease. These studies include advancing imaging methods (DTI, OCT and others) as well as various genetic studies (deep sequencing as well as GWAS), cell biology and structural biology.

Selected Publications:

Marquié M, Siao Tick Chong M, Antón-Fernández A, Verwer EE, Sáez-Calveras N, Meltzer AC, Ramanan P, Amaral AC, Gonzalez J, Normandin MD, Frosch MP, Gómez-Isla T. [F-18]-AV-1451 binding correlates with postmortem neurofibrillary tangle Braak staging. *Acta Neuropathol*. 2017; 134(4):619-628.

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Marquié M, Normandin MD, Vanderburg CR, Costantino IM, Bien EA, Rycyna LG, Klunk WE, Mathis CA, Ikonomovic MD, Debnath ML, Vasdev N, Dickerson BC, Gomperts SN, Growdon JH, Johnson KA, Frosch MP, Hyman BT, Gómez-Isla T. Validating novel tau positron emission tomography tracer [F-18]-AV-1451 (T807) on postmortem brain tissue. *Ann Neurol.* 2015; 78(5):787-800.

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"Finding cancer genes and pathways is a crucial first step towards therapy..."

Characterizing the cancer genome

Cancer is a disease of the genome that is driven by a combination of possible germline risk-alleles together with a set of "driver" somatic mutations that are acquired during the clonal expansion of increasingly fitter clones. In order to generate a comprehensive list of all germline and somatic events that occurred during life and the development of the cancer, we are developing and applying highly sensitive and specific tools for detecting different types of mutations in massively-parallel sequencing data. The volume, noise and complexity of these data require developing computational tools using state-of-the-art statistical and machine learning approaches to extract the signal from the noise (e.g., MuTect, CapSeg, dRanger, BreakPointer, MSMuTect, etc.).

Detecting cancer-associated genes

Next, we analyze the detected events across a cohort of samples searching for genes/pathways, as well as non-coding variants, that show significant signals of positive selection. To that end, we construct a statistical model of the background mutational processes and then detect genes that deviate from it. As part of constructing the models, we study and infer the mutational processes (using SignatureAnalyzer) that affected the samples (carcinogens, defects in repair mechanisms, etc.) and their timing.

We have developed tools for detecting significantly gained or lost genes in cancer (GISTIC) and genes with increased density or irregular patterns of mutations (MutSig suite, CLUMPS/EMPRINT, MSMutSig, NetSig). Our work demonstrated the importance of modeling the heterogeneity of these models across patients, sequence contexts and the genome, when searching for cancer genes.

Heterogeneity and clonal evolution of cancer

Cancer samples are heterogeneous, containing a mixture of normal cells and cancer cells that often represents multiple subclones. We developed and continue to develop tools (ABSOLUTE, Phylogic, PhylogicNDT) for characterizing the heterogeneity of cancer samples using copy-number and mutation data measured on bulk samples and now also using single cells. Using these tools, we can infer which mutations are clonal or sub-clonal, as well as estimate the number of subclones and their distribution over space and time. We are now working to introduce these concepts to clinical trials and eventually clinical care.

Selected Publications:

Rheinbay E, Parasuraman P, et al, Ellisen LW, Iafrate AJ, Boehm JS, Gabriel SB, Meyerson M, Golub TR, Baselga J, Hidalgo-Miranda A, Shioda T, Bernards A, Lander ES, Getz G. Recurrent and functional regulatory mutations in breast cancer. *Nature.* 2017; 547(7661):55-60.

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Haradhvala NJ, Polak P, Stojanov P, Covington KR, Shinbrot E, Hess JM, Rheinbay E, Kim J, Maruvka YE, Braunstein LZ, Kamburov A, Hanawalt PC, Wheeler DA, Koren A, Lawrence MS, Getz G. Mutational strand asymmetries in cancer genomes reveal mechanisms of DNA damage and repair. *Cell.* 2016; 164(3):538-49.

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"Developing mathematical descriptions of complex human disease phenotypes and how they change over time..."

I study the dynamics of human pathophysiologic processes by developing mechanistic mathematical descriptions of complex human disease phenotypes and how they change over time. The research combines medical insight, dynamical systems theory, and experiments utilizing clinical specimens, microfluidics, video processing, flow cytometry, simulation, and large-scale analysis of medical databases in pursuit of two goals: (1) advancing fundamental understanding of human pathophysiologic process and their dynamics, and (2) improving patient diagnosis, monitoring, and treatment.

Pathophysiology may be described at the molecular, cellular, tissue and organismal levels and may show clinically significant variation over time scales ranging from less than a second to more than a decade. Using clinical laboratory data and experiments with clinical specimens, we can develop detailed descriptions of pathophysiologic states in terms of clinically relevant and measurable quantities. We can then propose mathematical models describing the interrelationships between these state variables and how those relationships change when perturbed by disease. Models must be consistent with mechanisms established by both existing basic research and clinical experience, and once validated will enable the estimation of dynamic parameters. Personalized estimates of parameters often quantify unmeasurable pathophysiologic processes, revealing new insight into pathophysiology and providing opportunities for novel approaches to diagnosis and patient monitoring. Recent work has focused on population dynamics of cell characteristics in anemia and inflammation due to ischemia, infection, autoimmune disease, and more.

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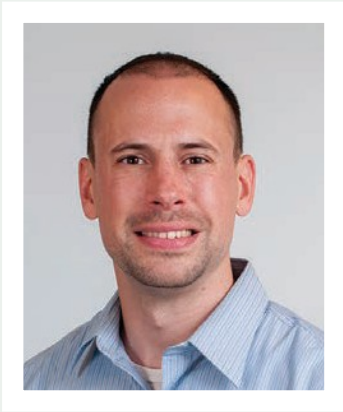

"Understanding mutagenic processes and identifying mutations that drive cancer..."

Cancer results from alterations to DNA that lead to the activation of oncogenes or the inactivation of tumor suppressors. We focus on understanding the many ways this can happen, using computation as a powerful microscope through to which 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 many causes. The vast majority 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 to 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.

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 1,000. 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 mutations. Ultraviolet radiation causes C-to-T. Activated APOBEC enzymes cause mutations at Cs preceded by T. Loss of mismatch repair causes microsatellite instability (MSI), marked by expansion and contraction of simple-sequence repeats, as well as characteristic 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 affected by MSI and/or POLE mutagenesis are known to experience better clinical outcomes, possibly thanks to their high neoantigen loads which attract a powerful immune response. Our most recent research has focused on 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.

A particular interest of the lab is the APOBEC mutational process, which results from aberrant activation of APOBEC cytidine deaminase enzymes. These enzymes normally function in innate immunity, attacking viral DNA inside cells. For reasons not fully understood, cancer cells upregulate APOBEC activity, leading to introduction of large numbers of mutations at TC sequences in the genome. We have observed that resistance mutations to targeted therapies often emerge during these APOBEC mutational flareups, suggesting they might be a vulnerability to target in order to delay or prevent the emergence of drug resistance.

Selected Publications:

Buisson R, Lawrence MS, Benes CH, Zou L. APOBEC3A and APOBEC3B activities render cancer cells susceptible to ATR inhibition. *Cancer Res*. 2017; 77(17) 4567-4578.

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"The role of the membrane: cytoskeleton interface in receptor tyrosine kinase signaling, tissue morphogenesis and tumorigenesis..."

The McClatchey laboratory seeks to understand how cells organize their outer membrane or cortex, which, in turn, determines their identity, behavior, and interface with the external environment. Defective cortical organization is one of the first signs of a developing tumor and causes tumor cells to interact inappropriately with other cells and with their environment. Our research stems from a longstanding dedication to understanding the molecular basis of neurofibromatosis type 2 (NF2), a familial tumor syndrome caused by mutation of the NF2 tumor suppressor gene. The NF2-encoded protein, Merlin, and closely related ERM proteins (Ezrin, Radixin, and Moesin) are key architects of the cell cortex.

Understanding morphogenesis and tumorigenesis

The vast array of forms and functions exhibited by different cell types is made possible by the organization of specialized domains within the cell cortex. Examples include cell:cell and cell:matrix adhesions, the T cell immunological synapse, the luminal surface of polarized epithelial cells, and the elaborately organized myelinating Schwann cell membrane. The assembly of such cortical domains involves the coordinated organization of membrane lipids and the underlying actomyosin cytoskeleton and thereby influences both the distribution and activity of membrane receptors and cellular architecture. These organizational principles are central to many developmental processes and often inappropriately re-enacted during tumor development. The overarching goal of my laboratory is to understand how this critical cellular compartment contributes to morphogenesis and tumorigenesis — a focus that stems from our dedication to defining the molecular function of the NF2 protein Merlin, which, like the ERMs, can link membrane proteins to the cytoskeleton.

We utilize in vivo mouse models and engineered three dimensional in vitro cellular models, to study key roles for Merlin/ERMs in morphogenesis and tumorigenesis in several tissues including the liver, kidney, mammary gland and nervous system. Complementary molecular and cellular studies taught us that a fundamental function of Merlin is to restrict the cortical distribution of Ezrin and associated actomyosin cytoskeleton — even in single cells. Among many ramifications of this are that Merlin/ERMs spatially control: 1) Membrane receptor trafficking and signaling; 2) The interphase centrosome and mitotic spindle and 3) Apical membrane (lumen) distribution. In the absence of Merlin unrestricted Ezrin drives spatially unregulated receptor trafficking, aberrant spindle orientation, centrosome unclustering/ multipolar spindles and ectopic apical lumens. These studies yield new insight into how the organization of the cell cortex drives morphogenesis and how aberrant cortical organization contributes to unscheduled cell proliferation, tumorigenesis and metastatic progression.

Selected Publications:

Benhamouche-Trouillet S, O'Loughlin E, Liu CH, Polachek W, Fitamant J, McKee M, El-Bardeesy N, Chen CS, McClatchey AI. Proliferation-independent role of NF2/Merlin in limiting biliary morphogenesis. *Development*, 2018 in press.

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

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" Novel diagnostics of infectious diseases..."

The Clinical Microbiology Laboratory at the Massachusetts General Hospital uses cutting edge methods to diagnosis infectious diseases of humans. Although classical microbiology has undergone transformative changes resulting in faster and more accurate diagnoses, there remains a critical need to further improve our diagnostic capability directly from primary specimens. To do this, my laboratory is involved in the development of novel methods to detect volatile organic compounds directly from primary specimens. If successful, this approach may speed up the time to diagnosis, which usually takes several days, to only taking several minutes.

In addition, there are several other longstanding projects that we are investigating including: 1) The diagnosis of acute HIV-1 infection and the immunologic and virologic correlates of immune protection in persons treated during primary HIV infection, and 2) The diagnosis of tick born illness and understanding the biology of human babesiosis and Lyme disease.

Selected Publications:

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"Studying the molecular basis of human urologic tumors, including cancers of the prostate, bladder and kidney..."

Our laboratory studies the molecular biomarkers of urologic tumors, including cancers of the prostate, bladder and kidney. The long-term goal of these studies is to develop new diagnostic method and therapeutic regimen for these cancers.

Prostate cancer is the most common cancer and the second leading cause of cancer death of men in the US. We are interested in identifying gene expression profiles associated with the development, diagnosis and prognosis of prostate cancer. We have used laser capture microdissection and DNA microarray techniques to identify a group of genes whose expression can be used to predict the prostate cancer outcome. We are in the process of developing a new gene-based diagnostic test to guide clinical management of prostate cancer. The genes identified by this approach may also be used as new therapeutic targets.

Currently, there is a clinical need to improve the method for imaging prostate cancer in vivo. Through collaboration with Dr. Leo Cheng, MGH Pathology and Radiology, we have identified a metabolomic signature of prostate cancer. We are applying this signature in the development of an in vivo imaging technique for prostate cancer. The new imaging method may help to detect, localize and quantify prostate cancer in vivo. Most prostate cancer death is due to the development of androgen independence. Androgen receptor is responsible for cell growth in both androgen dependent and independent prostate cancers. We identified two novel androgen receptor co-activators that may be involved in the development of androgen independence in prostate cancer. Characterizing these androgen receptor co-activators may lead to new drug targets for androgen independent prostate cancer.

Our laboratory is jointly supported by the MGH Urology and Pathology Departments and the MGH Cancer Center. In addition to our own investigations, we have established productive collaborations with investigators both locally and around the world. We provide clinical, research and technical expertise as well as pathology specimens to these collaborative studies.

Selected Publications:

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"The connection between diet, physiology, and stem cells in regeneration and cancer..."

The goal of the Yilmaz laboratory is to understand how diverse diets influence the regeneration and development of cancers in the intestine. Although diet is known to impact the regeneration of the intestine and the incidence of intestinal cancers, very little is understood about the cellular and molecular mechanisms that underlie these processes. The intestine is a rapidly proliferating organ that on average replaces its entire lining every 5 days, which in an average adult human equates to approximately 300 grams of new intestinal tissue being generated daily. Intestinal stem cells power this regeneration by undergoing either self-renewal divisions that generate more stem cells or a series of divisions that engender the various differentiated cell types of the adult intestine.

To function properly, intestinal stem cells also require support cells, or niche cells, consisting of Paneth cells that play a key role in modulating stem cell function in response to calorie intake. By integrating cues from their Paneth cell niche, intestinal stem cells remodel the composition and function of the intestine, allowing for the intestine to dynamically adapt to different diets. Since stem cells and their niche drive intestinal regeneration in response to diet and because most cancers are understood to arise from transformed or mutated stem cells, it is likely that intestinal stem cells, diet, and cancer are interconnected. The Yilmaz lab is working on elucidating the molecular mechanisms underpinning this connection between stem cells, diet, and cancer in conditions of low calorie diets as well as in high fat diet-induced obesity. By better understanding how intestinal stem cells adapt to diverse diets, his lab hopes to identify and develop new strategies that prevent and reduce the growth of cancers involving the intestinal tract that includes the small intestine, colon, and rectum.

Selected Publications:

Mihaylova M, Cheng CW, Cao AQ, Tripathi S, Mana MD, Bauer-Rowe KE, Abu-Remailleh M, Clavin L, Erdemir A, Lewis C, Freinkman E, Huang Y, Bell G, Deshpande V, Carmeliet P, Katajisto P, Sabatini DM, Yilmaz ÖH. Fasting-Activated Fatty Acid Oxidation Enhances Intestinal Stem Cell Function. *Cell Stem Cell*. 2018;22(5):769-778.

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"Understanding the underlying principles of cellular responses to chromosomal insults and their roles in the maintenance of genomic stability..."

Genomic instability is one of the hallmarks of cancer. On one hand, the genomic instability of cancer cells fuels tumorigenesis. On the other hand, the genomic instability of cancer cells offers a unique vulnerability that can be exploited therapeutically. While radiotherapy and chemotherapy have been successfully used to kill cancer cells with genomic instability, their cytotoxicity in normal cells presents a major challenge to cancer therapy today. The research of Dr. Zou's laboratory is focused on understanding how genomic instability arises in cancer cells, and how it can be targeted selectively and effectively in cancer therapy. In particular, Dr. Zou and colleagues have extensively characterized the DNA damage checkpoint, a pathway that detects and signals various types of problems in the genome. Dr. Zou's work has identified the critical sensors of DNA damage in human cells, and elucidated how these sensors activate the ATR kinase, a master regulator of the DNA damage response. The findings by Dr. Zou and colleagues have shed important light onto a fundamental cellular process that is critical for both tumor suppression and cancer therapy.

The recent and ongoing studies in Dr. Zou's laboratory have provided new opportunities for targeted cancer therapy. They find that activation of the alternative telomere-lengthening (ALT) pathway in a subset of cancers renders tumor cells hypersensitive to ATR inhibitors. Several cancer types, including various sarcomas, pediatric and high-grade glioblastomas, and neuroendocrine pancreatic tumors, are prevalent for ALT. In collaboration with Dr. Miguel Rivera, a pathologist, Dr. Zou's lab is developing a new assay for identifying ALT tumors. These studies may lead to new clinical trials for the treatment of ALT tumors with ATR inhibitors. Recent studies from the Zou lab also reveal that ATR inhibitors are able to selectively kill cancer cells under high levels of DNA replication stress. Importantly, their studies identified single-stranded DNA (ssDNA) as a general indicator of replication stress, which may provide a useful biomarker for the use of ATR inhibitors in targeted cancer therapy. These and other studies in the Zou lab have highlighted the value of the ATR checkpoint pathway as a therapeutic target, bringing about a new way to exploit the genomic instability and DNA repair dependency in cancer cells with increased selectivity and efficacy.

Selected Publications:

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