2016-2017 | Pathology Basic Scientific Research

MASSACHUSETTS GENERAL HOSPITAL

MASSACHUSETTS General Hospital
Molecular Pathology Unit
149 13th Street, 6th Floor
Charlestown, MA 02129
Phone: 617-726-3690
Fax: 617-726-5684

HTTP://WWW.MASSGENERAL.ORG/PATHOLOGY/RESEARCHPATH/
Cover Image:
“Constellation”
by ML Ginez and AJ Infrate

Multiplex FISH of breast cancer derived circulating tumor cells. Cells were hybridized with sequence-specific PCR-based FISH probe mix recognizing 16 genes. Nuclei were not counterstained.
INTRODUCTION..............................................................................................................................ii

TRAINING GRANT LABORATORIES..............................................................................................iii

FACULTY

Molecular Pathology Unit
J. Keith Joung .................................................................................................................................1
David M. Langenau ..........................................................................................................................2
Martin Aryee ..................................................................................................................................3
Atul K. Bhan ..................................................................................................................................4
A. John Iafrate ................................................................................................................................5
David N. Louis ................................................................................................................................6
Luca Pinello ....................................................................................................................................7
Miguel N. Rivera ...............................................................................................................................8
Dennis C. Sgroi ................................................................................................................................9
Anat Stemmer-Rachamimov .........................................................................................................10
Mario L. Suvà ................................................................................................................................11

Experimental Pathology Unit
Bradley Bernstein ...........................................................................................................................12
Frederic I. Preffer ..........................................................................................................................13
James R. Stone ................................................................................................................................14

Translational Oncology Laboratory
Dora Dias-Santagata .......................................................................................................................15
Long Phi Le ....................................................................................................................................16

Immunopathology Research Unit
Robert B. Colvin ...........................................................................................................................17
Rex Neal Smith ................................................................................................................................18

Howard Hughes Medical Institute
Jeannie T. Lee (Molecular Biology) ................................................................................................19

Pathology Imaging
Guillermo J. Tearney (Wellman Center for Photomedicine) ..........................................................20

Pathology Faculty Affiliated With Other Departments
Matthew P. Frosch: MassGeneral Institute for Neurodegenerative Diseases ..................................21
Gad A. Getz: Center for Cancer Research .........................................................................................22
John M. Higgins: Center for Systems Biology ..................................................................................23
Michael S. Lawrence: Center for Cancer Research .........................................................................24
Andrea I. McClatchey: Center for Cancer Research ........................................................................25
Eric S. Rosenberg: Infectious Diseases ............................................................................................26
Chin Lee Wu: Urology-Pathology Research Laboratory ..................................................................27
Ömer H. Yilmaz: Koch Institute for Integrative Cancer Research at MIT ........................................28
Lee Zou: Center for Cancer Research ..................................................................................................29
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 in pathology 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 a very 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, the Pathology Imaging Laboratory, and the Immunopathology Research Unit. In addition, many peer-review funded pathologists and members of the Harvard Medical School Department of Pathology 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; 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 that has been extensively renovated; expansion in the number of Harvard and MIT Ph.D.-candidate graduate students 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 (led by A. John Iafrate); 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.

We are currently implementing initiatives identified from our recent departmental strategic planning process. With support and resources from the department and the hospital, we are expanding computational biology and bioinformatics resources for pathology, expanding 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.

J. Keith Joung, MD, PhD
Associate Chief of Pathology (Research) and Pathologist, Massachusetts General Hospital
Professor of Pathology, Harvard Medical School
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, Developmental Cell, Molecular Cell, Nature Genetics, Nature Biotechnology, Nature Methods, 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:

<table>
<thead>
<tr>
<th>Nancy Andrews, MD, PhD</th>
<th>Daniel Haber, MD, PhD</th>
<th>Sridhar Ramaswamy, MD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children's Hospital</td>
<td>MGH Center for Cancer Research</td>
<td>MGH Center for Cancer Research</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Spyros Artavanis-Tsakonas, PhD</th>
<th>Konrad Hochedlinger, PhD</th>
<th>David Sabatini, MD, PhD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harvard Medical School</td>
<td>MGH Center for Regenerative Medicine</td>
<td>Massachusetts Institute of Technology</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>David Bartel, PhD</th>
<th>Bradley Hyman, MD, PhD</th>
<th>David Scadden, MD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whitehead Institute</td>
<td>MGH Neurology</td>
<td>MGH Hematology-Oncology</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Alan Beggs, PhD</th>
<th>Frank Haluska, MD, PhD</th>
<th>Jeffrey Settleman, PhD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children's Hospital</td>
<td>MGH Hematology-Oncology</td>
<td>MGH Center for Cancer Research</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Brett Bouma, PhD</th>
<th>Donald Ingber, MD, PhD</th>
<th>Phillip Sharp, PhD</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGH Wellman Center for Photomedicine</td>
<td>Wyss Institute at Harvard</td>
<td>Massachusetts Institute of Technology</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Connie Cepko, PhD</th>
<th>Ralph Isberg, PhD</th>
<th>Carla Shatz, PhD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harvard Medical School</td>
<td>Tufts University</td>
<td>Harvard Medical School</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>George Church, PhD</th>
<th>Rudolf Jaenisch, MD</th>
<th>Melissa Suter, PhD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harvard Medical School</td>
<td>Massachusetts Institute of Technology</td>
<td>MGH Wellman Center for Photomedicine</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Michael Detmar, MD</th>
<th>Rakesh Jain, PhD</th>
<th>Jay Vacanti, MD, PhD</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGH Cutaneous Biology Research Center</td>
<td>MGH Radiation Oncology</td>
<td>MGH Pediatric Surgery</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Iain Drummond, PhD</th>
<th>Jeannie Lee, MD, PhD</th>
<th>Amy Wagers, PhD</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGH Renal Unit</td>
<td>MGH Molecular Biology</td>
<td>Harvard University</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Benjamin Ebert, MD, PhD</th>
<th>Susan Lindquist, PhD</th>
<th>Robert Weinberg, PhD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brigham &amp; Women’s Hospital</td>
<td>Massachusetts Institute of Technology</td>
<td>Massachusetts Institute of Technology</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Kevin Eggan, PhD</th>
<th>Andrea McClatchey, PhD</th>
<th>Ramnik Xavier, MD, PhD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harvard University</td>
<td>MGH Center for Cancer Research</td>
<td>MGH Gastrointestinal Unit</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stephen Elledge, PhD</th>
<th>Matthew Meyerson, MD, PhD</th>
<th>Gary Yellen, PhD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harvard Medical School</td>
<td>Broad Institute</td>
<td>Harvard Medical School</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>James Fox, PhD</th>
<th>Carl Pabo, PhD</th>
<th>Lee Zou, PhD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Massachusetts Institute of Technology</td>
<td>Massachusetts Institute of Technology</td>
<td>MGH Center for Cancer Research</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Frank Gertler, PhD</th>
<th>Shiv Pillai, MD, PhD</th>
<th>MGH Center for Cancer Research</th>
</tr>
</thead>
</table>
Molecular Pathology Unit
Massachusetts General Hospital
149 13th Street, 6th Floor
Charlestown, MA 02129
Phone: 617-726-9462 • Email: jjoung@mgh.harvard.edu

Selected Publications

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.

Tsai SQ, Joung JK. Defining and improving the genome-wide specificities of CRISPR-Cas9 nucleases.

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.

Kleinstiver BP, Prew MS, Tsai SQ, Topkar VV, Nguyen NT, Zheng Z, Gonzalez AP, Li Z, Peterson RT, Yeh JR, Aryee MJ, Joung JK.

Genome Editing Using Targeted Nucleases

Genome editing technology using CRISPR-Cas9 nucleases was recently named “Breakthrough of the Year” for 2015 by Science magazine. Much of our recent work with genome-editing nucleases has focused on CRISPR-Cas9. We and our collaborators were the first to demonstrate that these nucleases can function in vivo (Hwang & Fu et al., Nat Biotechnol. 2013), modifying endogenous genes in zebrafish embryos and the first to show that they can induce significant off-target mutations in human cells (Fu et al., Nat Biotechnol. 2013). We recently developed GUIDE-seq, an unbiased, genome-wide method for sensitive detection of CRISPR-Cas9-induced off-target mutations in human cells (Tsai et al., Nat Biotechnol. 2015). Using structure-guided design, we have engineered “high-fidelity” Cas9 variants that robustly fail to show detectable genome-wide off-targets as judged by GUIDE-seq (Kleinstiver & Pattanayak et al., Nature 2016). Finally, we used a combination of structure-guided design and molecular evolution to engineer Cas9 variants with novel DNA binding specificities, thereby broadening the targeting range and applications of this platform (Kleinstiver et al., Nature 2015).

Epigenome Editing Using Targeted Transcription Factors

We have also demonstrated that the TALE and CRISPR 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). In addition, we have collaborated with Brad Bernstein’s group to develop fusions of the histone demethylase LSD1 with TALE domains that can induce targeted histone alterations at endogenous human enhancers (Mendenhall et al., Nat Biotechnol. 2013). Finally, we have also developed fusions of engineered TALE domains with the catalytic domain of the TET1 enzyme, enabling the targeted demethylation of CpGs in human cells (Maeder et al., Nat Biotechnol. 2013).
The Langenau laboratory research focus is to uncover relapse mechanisms in pediatric cancer. Utilizing zebrafish models of T-cell acute lymphoblastic leukemia (T-ALL) and embryonal rhabdomyosarcoma (ERMS), we have undertaken chemical and genetic approaches to identify novel modulators of progression, therapy-resistance, and relapse.

Uncovering progression-associated driver mutations in T-cell acute lymphoblastic leukemia T-ALL is an aggressive malignancy of thymocytes that affects thousands of children and adults in the United States each year. Recent advancements in conventional chemotherapies have improved the five-year survival rate of patients with T-ALL. However, patients with relapse disease are largely unresponsive to additional therapy and have a very poor prognosis. Ultimately, 70% of children and 92% of adults will die of relapse T-ALL, underscores 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 trangenergisis platforms, and unbiased bioinformatic approaches, we have uncovered new oncogenic drivers associated with aggression, therapy resistance and relapse. A large subset of these genes exert important roles in regulating human T-ALL proliferation, apoptosis and response to therapy. Discovering novel relapse-driving oncogenic pathways will likely identify new drug targets for the treatment of T-ALL.

Visualizing and killing cancer stem cells in embryonal rhabdomyosarcoma ERMS is a common soft-tissue sarcoma of childhood and phenotypically recapitulates fetal muscle development arrested at early stages of differentiation. Microarray and cross-species comparisons of zebrafish, mouse and human ERMS uncovered the finding that the RAS pathway is activated in a majority of ERMS. Building on this discovery, our laboratory has developed a transgenic zebrafish model of KRASG12D-induced ERMS that mimics the molecular underpinnings of human ERMS. We used fluorescent transgenic zebrafish that label ERMS cell subpopulations based on myogenic factor expression to identify functionally distinct classes of tumor cells contained within the ERMS mass. Specifically, the myf5-GFP+ ERMS cell subpopulation contains self-renewing cancer stem cells that drive continued tumor growth at relapse and is molecularly similar to a non-transformed, activated muscle satellite cell. Building on the dynamic live cell imaging approaches available in the zebrafish ERMS model, our laboratory has undertaken chemical genetic approaches to identify drugs that kill relapse-associated, self-renewing myf5-GFP+ ERMS cells. We are currently assessing a subset of drugs for their ability to regulate growth of human ERMS cells and mouse xenografts.
My research involves computational methods that enable us to elucidate the genetic and epigenetic basis of cancer and other diseases from large genomic datasets.

**Tumor Heterogeneity**
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 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.
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


“...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 tyrosine kinase and with rearrangements of the ROS1 tyrosine kinase with a small molecule kinase inhibitor 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 detection 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. 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 patient derived cell lines and xenografts. 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 genomic DNA target enrichment, which 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.
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 oligodendrogial 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 diagnostic 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. 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.

“Elucidating the molecular basis of glioma formation impacts both diagnostic and therapeutic aspects of clinical neuro-oncology...”
Luca Pinello, PhD
Assistant Professor of Pathology, Harvard Medical School
Assistant Pathologist, Massachusetts General Hospital

Molecular Pathology Unit
Massachusetts General Hospital
149 13th Street, 6th Floor
Charlestown, MA 02129
Phone: 617-643-6522
Email: lpinello@mgh.harvard.edu

Selected Publications


“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 proposed an integrative computational pipeline called HAYSTACK (https://github.com/lucapinello/Haystack). HAYSTACK is a software tool to study epigenetic variability, cross-cell-type plasticity of chromatin states and transcription factor motifs and provides mechanistic insights into chromatin structure, cellular identity and gene regulation.

Computational methods for genome editing

We embraced the revolution in functional genomics made possible by the novel genome editing approaches such as CRISPR/Cas9 and TALENs by developing computational tools to quantify and visualize the outcome of sequencing data originating from these powerful assays. We created a novel 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.rc.fas.harvard.edu). 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.

Single cell analysis

We are developing tools to model the variability of gene expression at single cell resolution by using data from single cell assays such as single cell RNA-seq and multiplexed qPCR. By profiling the transcriptome of single cells, we are inferring cell states, detecting rare cell types, and we are able to track their state transitions during development.
Selected Publications


Molecular Pathology Unit
Massachusetts General Hospital
149 13th Street, 6th Floor
Charlestown, MA 02129
Phone: 617-726-6257
Email: mnrivera@partners.org

“Using genomics to identify critical pathways in pediatric tumors…”

Our research focuses on using genomic tools to identify and characterize critical pathways in pediatric tumors. An important feature shared by these tumors is their strong association with developmental processes and, in particular, with the gene regulation mechanisms that control stem cell populations during organ formation. Our work combines the use of genomic technologies for the direct identification of gene regulation abnormalities in tumors with functional analysis of critical pathways in several model systems. Given that the mechanisms that drive pediatric tumors are poorly understood at present, we anticipate that our work will point to new therapies for these diseases.

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 other tumor types. 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.

Epigenomic approaches to identify novel pathways in cancer

Given that alterations in transcriptional programs play critical roles in transformation, we are using genomic technologies to identify abnormal patterns of gene regulation in pediatric cancer. In particular, genome-wide chromatin profiling, which combines chromatin immunoprecipitation and high-throughput sequencing, is a powerful technology that can identify activation and repression states based on patterns of histone modification. Our initial 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 function of aberrant transcriptional regulators such as the EWS-FLI1 fusion protein in Ewing sarcoma. We are now extending our epigenomic analysis to other tumor types and other key transcriptional pathways in pediatric 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 achieve 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/IL7BR (H/I). MGI is a molecular surrogate for histological grade and a highly precise biomarker for risk of breast cancer recurrence. The HOXB13/IL7BR 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 assessing its possible role as a surrogate marker for a nonclassical estrogen receptor signaling pathway.
Selected Publications


“Investigating hereditary brain tumor syndromes...”

Our lab’s research focuses on identifying the underlying molecular changes in lesions and afformations associated with hereditary brain tumor syndromes (neurofibromatosis 1, neurofibromatosis 2, schwannomatosis and tuberous sclerosis, von Hipple Lindau), and the identification of activated pathways or events that lead to tumor progression. Although hereditary brain tumor syndromes are relatively uncommon, the same molecular events and pathways are often involved in tumorigenesis and progression of similar sporadic tumors that are much more frequent in the general population.

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 in 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.

Finally, in collaboration with multiple groups, we perform extensive pathological analyses of new mouse models of neurofibromatoses and new diagnostic and therapeutic modalities.
MOLECULAR PATHOLOGY UNIT

Mario L. Suvà, MD, PhD
Assistant Professor of Pathology, Harvard Medical School
Assistant Molecular Pathologist, Massachusetts General Hospital

Massachusetts Pathology Unit
Massachusetts General Hospital
149 13th Street, 6th Floor
Charlestown, MA 02129
Phone: 617-726-6247
Email: Suva.Mario@mgh.harvard.edu

Selected Publications


“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 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 difficult-to-treat diseases.

**Gliomas heterogeneity 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 glioma specimens. Through our efforts, we are redefining our understanding of glioblastoma, oligodendroglioma, astrocytoma and diffuse intrinsic pontine glioma.

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

We have previously performed deep chromatin landscape profiling and analysis of primary glioblastoma models and have utilized this information to reconstruct functionally validated network models. We are applying similar approaches to genetically defined primary cultures of IDH1 mutant glioblastoma, H3F3A mutant pediatric glioblastoma and IDH1 mutant oligodendroglioma obtained through our collaborations with the MGH Brain Tumor Center.

**Targeting neurodevelopmental programs in primary human glioblastoma stem cells**

Our work has identified neurodevelopmental transcription factors as master regulators of a stem-like state in glioblastoma and reconstructed a transcriptional network model. Our lab is utilizing genome-editing technologies to generate functional knock-out of critical nodes in the network to identify novel dependencies in glioblastoma and assess novel therapeutic options.
Bradley Bernstein, MD, PhD

Professor of Pathology, Harvard Medical School; Pathologist, Massachusetts General Hospital; Institute Member, Broad Institute; Bernard and Mildred Kayden Endowed MGH Research Institute Chair; American Cancer Society Research Professor

Massachusetts General Hospital
185 Cambridge Street, Simches Research Building CPZN 8234
Boston, MA 02114
Phone: 617-726-6906 • Fax: 617-643-3566
Email: Bernstein.Bradley@mgh.harvard.edu

“Chromatin deregulation can result in inappropriate gene expression and contribute to the pathogenesis of cancer and other diseases...”

The Bernstein laboratory studies 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. His laboratory develops genomic technologies to study chromatin structure and epigenetic regulation. The 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.

Our long-term goal is to achieve a more complete understanding of how epigenetic alterations lead to cancer and other diseases, and how these may be corrected by ‘epigenetic’ or other targeted therapies.

Technologies for mapping histone modifications and chromatin proteins
We are combining tools in stem cell biology, biochemistry 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 are applying these approaches to characterize noncoding regulatory elements in the human genome and to understand how the resulting cell circuits control gene expression programs during development and in cancer. We also leverage emerging single-cell and single-molecule techniques to deconvolve heterogeneous cell populations and dynamic processes.

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 are now applying emerging chromatin and genome engineering approaches to study how bivalent domains and interacting regulatory elements program gene expression in development.

Chromatin regulation in cancer cells
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 and, in parallel, investigate representative tumor models in the laboratory. These synergistic approaches can inform therapeutic strategies for targeting epigenetic lesions or overcoming resistance mechanisms.

Selected Publications


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 ontogenetic 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 obligatory 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 naïve 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, R. Ravichandran and C. Luo, contain a FACsAria II sorter, LSR-2, Fortessa and FACSFusion sorter for BSL2+ operations. The laboratory is slated to have a Helios Mass Cytometer in 2017. 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. Two FACS Canto-IIs are available at that site.
Selected Publications


“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 CK1αLS/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.
**Dora Dias-Santagata, PhD, FACMG**

Assistant Professor of Pathology, Harvard Medical School  
Assistant Molecular Pathologist, Massachusetts General Hospital  
Co-Director, Translational Research Laboratory

Center for Integrated Diagnostics  
Molecular Pathology Unit  
Massachusetts General Hospital  
55 Fruit Street (GRJ-10)  
Boston, MA 02114  
Phone: 617-724-1261  
Email: ddiassantagata@mgh.harvard.edu

**Selected Publications**


"Molecular characterization of human tumors to identify markers of response to targeted therapeutics..."
Cancer genetics has expanded significantly in recent years due to various parallel efforts in cancer genotyping by next-generation sequencing. At the same time, targeted therapies have also advanced and require identification of molecular signatures to predict their response. Translating recent discoveries into clinical practice for patient management depends on two key challenges: implementing high-throughput cancer genotyping at the clinical level and establishing the informatics framework to support clinical implementation of these high-throughput technologies.

Our clinical molecular diagnostic laboratory has implemented a custom next-generation approach based on our own target enrichment method called anchored multiplex PCR (AMP). We have applied AMP to detect single nucleotide variants, indels, copy number changes, and fusion transcripts for solid tumors and hematopoietic malignancies. In addition, we are using hybridization-based capture technology to profile tumors across a broad range of genes.

To support our clinical next-generation sequencing operation, we have developed custom bioinformatics tools for indel, fusion, and copy number detection. Streamlining the clinical next-generation sequencing operation requires an end-to-end solution. We have employed modern web technologies to create a suite of laboratory tools: molecular laboratory information management system (LIMS), interface for dynamic variant review, variant curation knowledge base, and dynamic clinical reporting interface. We are currently developing an informatics environment to integrate clinical information with our genomics data to derive new genotype-phenotype associations and enable clinical decision support. Our efforts will drive not only the clinical operation but also research and discovery.
Robert B. Colvin, MD
Benjamin Castleman Distinguished Professor of Pathology,
Harvard Medical School (Formerly Chief of Pathology, 1991-2006)

Immunopathology Research Laboratory
Thier Building 8th Floor
Massachusetts General Hospital
Boston MA 02114
Phone: 617-724-3631 • Fax: 617-724-5833
Email: rbcolvin@partners.org

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.

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. Dr. Colvin leads the pathology core for several NIH and industry-sponsored clinical trials as well as an international NIH genomics project in renal allograft.

A major problem in long-term organ grafts is the development of a 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 is under investigation with proteomic techniques.
Rex Neal Smith, MD, PhD
Professor of Pathology, Harvard Medical School
Pathologist, Massachusetts General Hospital

Pathology Service
Massachusetts General Hospital
55 Fruit Street
Boston, MA 02140
Phone: 617-726-1835 • Fax: 617-726-2365
Email: rnsmith@partners.org

“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 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 have been able to establish 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. 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. With Dr. M. Soares investigations are ongoing into the mechanism of cerebral malaria. New work with Dr. E. Zorn and J Fraser seeks to identify new alloantibodies in graft rejection by novel proteomic approaches.
“The role of long noncoding RNA in epigenomic regulation…”

Our laboratory uses X-chromosome inactivation (XCI) as a model to study the structure and function of long noncoding RNAs (lncRNA) in epigenetic regulation. Nowhere in the mammalian genome are the abundance and roles of lncRNA more evident than at the X-inactivation center (Xic). This region harbors transcripts that serve as both repressors and activators in the regulation of genes on the X-chromosome. Interestingly, until 150 million years ago, the Xic genes were actually coding and functioned in pathways unrelated to XCI. The replacement of the Xic with noncoding transcripts suggests that lncRNAs may be uniquely suited to some types of epigenetic processes. Our research indicates that two such processes are allelic (cis-regulatory) and locus-specific targeting of chromatin factors (e.g., Polycomb complexes). In addition to pursuing mechanisms of action at the Xic, we are extending analysis to lncRNA occurring on a genome-wide scale and developing novel molecular techniques to do so. Our long-term goal is to understand how lncRNAs interact with chromatin complexes to achieve locus-specific and temporally specific gene expression patterns.
Guillermo J. Tearney, MD, PhD

Professor of Pathology, Harvard Medical School
Pathologist, Massachusetts General Hospital
Physicist, Massachusetts General Hospital
Faculty, Wellman Center for Photomedicine
Mike and Sue Hazard Family MGH Research Scholar

Massachusetts General Hospital
55 Fruit Street, BHX604A • Boston, MA 02114
Phone: 617-724-2979 • Fax: 617-726-4103
Email: gtearney@partners.org • www.tearneylab.org

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

Selected Publications


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 subcellular 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 230 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.
Matthew P. Frosch, MD, PhD

Lawrence J. Henderson Associate Professor of Pathology and Health Sciences & Technology, Harvard Medical School
Director, C.S. Kubik Laboratory for Neuropathology, Massachusetts General Hospital
MassGeneral Institute for Neurodegenerative Diseases (MIND)

MassGeneral Institute for Neurodegenerative Diseases (MIND)
Massachusetts General Hospital
114 16th Street, Room 2700
Charlestown, MA 02129
Phone: 617-726-5156 • Fax: 617-724-1813
Email: mfrosch@partners.org

“Development and characterization of animal models of human neurodegenerative diseases...”

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 that have been shown to alter Aβ deposits in the brain (immunotherapy, gamma-secretase inhibitors). Current topics of particular interest involve the timing of oxidative stress induction by CAA, expression of matrix degrading enzymes such as MMP-9 and induction of apoptosis in vascular smooth muscle cells. We use serial in vivo 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 immunohistochemistry, image reconstruction and laser capture microdissection to define alterations in gene expression that occur in smooth muscle cells in the proximity of amyloid deposits of CAA. Finally, our observations in mouse models of CAA can be validated through the use of human autopsy tissue, collected through the Massachusetts Alzheimer Disease Research Center Neuropathology Core.

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

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 etc.). We are also developing benchmarking approaches to assess the accuracy of the tools to help guide and interpret the experiments.

Detecting Cancer-Associated Genes: Next, we analyze the detected events across a cohort of samples searching for genes/pathways 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 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). We recently reported the importance of modeling the heterogeneity of these models across patients, sequence contexts and the genome, when searching for cancer genes. We are continuously improving these methods and working towards generating a unified approach that integrates all types of alterations to better detect 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 are developing tools (ABSOLUTE) 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


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

I study the dynamics of human pathophysiologic processes by developing mathematical descriptions of complex human disease phenotypes and how they change over time. The research combines medical insight, dynamical systems theory, and experiments utilizing 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 both existing basic research and clinical experience, and once validated will enable the estimation of dynamic parameters. Personalized estimates of parameters often quantify unmeasurable aspects of biological 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 inflammatory states, blood flow in sickle cell disease, and immunologic response to transfusion.
Selected Publications


“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 1000. Mutations have many causes, and each mutation 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 C’s preceded by T. Loss of mismatch repair causes microsatellite instability (MSI), marked by expansion and contraction of simple-sequence repeats, as well as characteristic 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.

We have used our algorithm MutSig (Mutation Significance) to reveal novel cancer driver genes such as D1S3 and FAM46C in multiple myeloma and SF3B1 in chronic lymphocytic leukemia. An improved version of MutSig accounts for the variable mutational baseline in genomes, with lowest mutation rates seen in highly transcribed, early-replicating regions. It now handles very large, noisy mutation datasets, and has been used to identify dozens of new cancer driver genes, which we have begun to validate experimentally in collaboration with wet-lab colleagues.
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. Cancer cells exhibit defective membrane organization and therefore 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 such as cell:cell and cell:matrix adhesions, the intestinal brush border and immunological synapse. The assembly of such cortical domains involves the coordination of processes occurring at the plasma membrane with those in the underlying cytoskeleton. Cortical protein complexes position membrane receptors, control their abundance and activity and link them to the cortical cytoskeleton, thereby serving both regulatory and architectural functions. The overarching goal of my laboratory is to understand how the organization of protein complexes at the cell cortex contributes to morphogenesis and tumorigenesis. This 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.

Through the generation of mouse models, we identified key roles for Merlin/ERMs in morphogenesis and tumorigenesis in many tissues. Molecular and cell-based studies suggest that these phenotypes are caused by defective distribution of membrane receptors such as EGFR/ErbBs, cell junctions and/or protein complexes that guide the orientation and function of the mitotic spindle. We also found that a key function of Merlin is to restrict the distribution of Ezrin. In the absence of Merlin, as in NF2-mutant cancers, unrestricted cortical Ezrin drives aberrant membrane receptor distribution and defective spindle orientation/integrity. These studies yield new insight into how the organization of the cell cortex drives normal cell behavior and how aberrant cortical organization contributes to unscheduled cell proliferation and tumorigenesis.

Ongoing studies extend basic and translational aspects of this work. A key goal is to define the molecular mechanism by which Merlin/ERMs organize the cell cortex and control receptor distribution and spindle orientation/integrity. We are also pursuing translational avenues for NF2-mutant tumors that stem directly from our basic studies such as targeting aberrant ErbB signaling or centrosome/spindle function. We believe that the continued partnering of basic and translational studies will lead to novel therapeutic options for NF2-mutant tumors and advance our understanding of how these basic cellular activities contribute to other human cancers.
In the vast majority of individuals infected with HIV-1, infection is characterized by the inability of the immune system to control viral replication. This failure of containment of HIV-1 replication inevitably results in disease progression. The one notable exception to this observation is in persons with long-term non-progressive infection who appear to contain viral replication in the absence of antiretroviral therapy. My laboratory investigates the mechanisms used by the cellular immune system in these individuals who are successful in mounting effective responses against HIV-1. Recent work has focused on characterizing CD4+ T helper cell function in individuals with long-term non-progressive infection and in a cohort of persons identified with acute HIV-1 infection prior to antibody seroconversion who when treated also generate functionally relevant immune responses. Currently, we are studying immunologic and virologic mechanisms employed by both host and virus that result in success or failure of the host immune response. In particular, we are studying how HIV-specific CD4+T helper cell responses are generated and subsequently disarmed during acute HIV-1 infection. In addition, we are studying the immunologic, virologic and clinical impact of antiretroviral therapy initiated during acute HIV-1 infection. Further investigation into the function of CD4+ T helper cells in these individuals will hopefully provide critical insight into the pathogenesis of HIV and support rationale for further immunotherapeutic interventions and vaccine development.
Chin-Lee Wu, MD, PhD
Associate Professor of Pathology, Harvard Medical School
Associate Pathologist, Massachusetts General Hospital
Director, Urology Research Laboratory
Massachusetts General Hospital
55 Fruit Street
Warren Building, Room 333A
Boston, MA 02114
Phone: 617-726-8454 • Fax: 617-724-7803
Email: cwu2@partners.org

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 methods and therapeutic regimens 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.
Ömer H. Yilmaz, MD PhD

Assistant Professor of Biology
Member, Koch Institute for Integrated Cancer Research
Massachusetts Institute of Technology
Assistant Pathologist, Massachusetts General Hospital

77 Massachusetts Avenue, 76-353D
Cambridge MA 02139 USA
Phone: (617) 324-7633
email: ohyilmaz@mit.edu • website: yilmaz-lab.mit.edu

“...on 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.
Lee Zou, PhD

Professor of Pathology, Harvard Medical School
Associate Scientific Director, Massachusetts General Hospital Cancer Center
James & Patricia Poitras Endowed Chair for Cancer Research

MGH Cancer Center
Massachusetts General Hospital
149 13th Street, 7th Floor
Charlestown, MA 02129
Phone: 617-724-9534 • Fax: 617-726-7808
Email: lzou1@partners.org

“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.