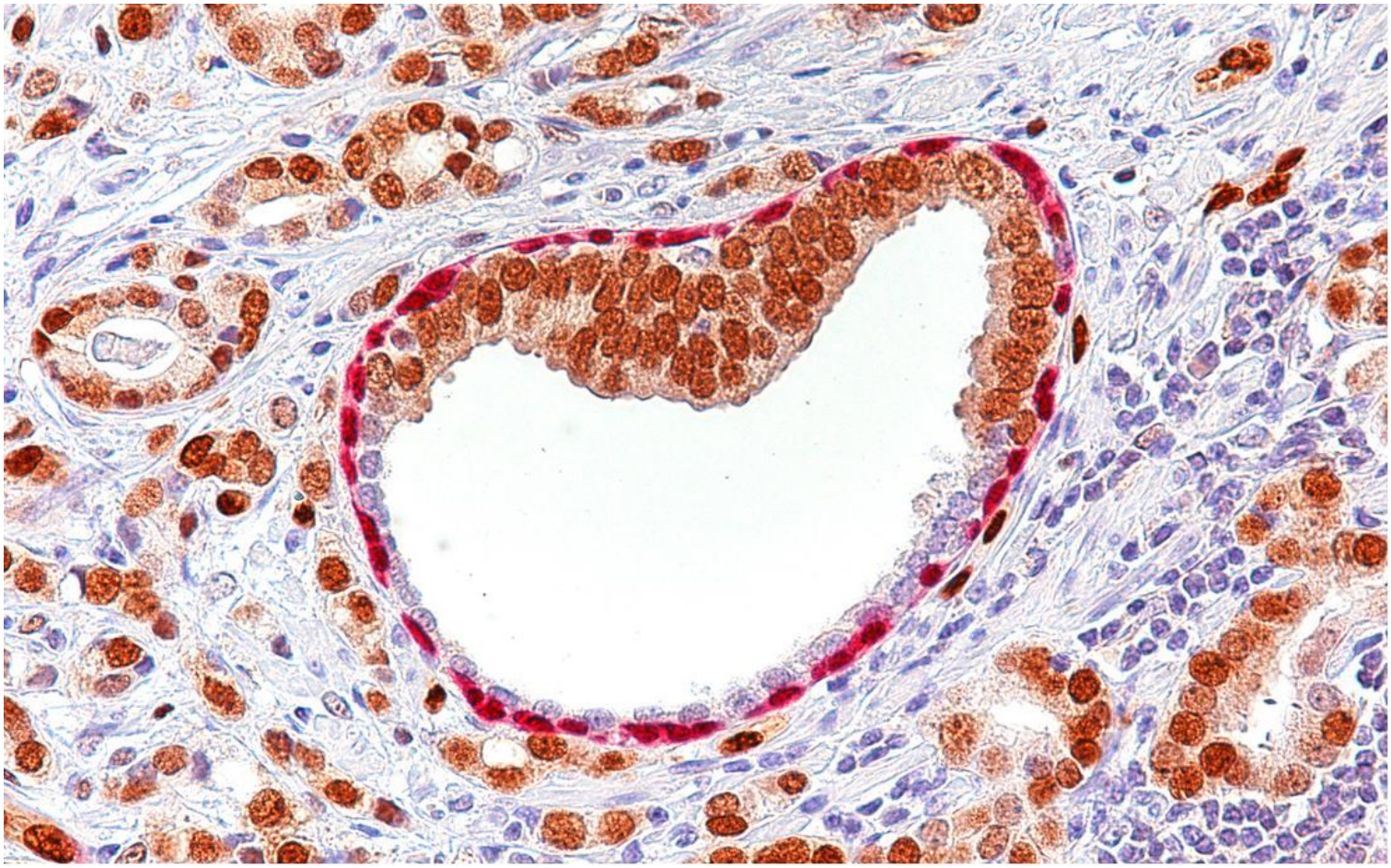
HUMAN BIOLOGY 2019-2020



About the cover: Provided by Assistant Member, Michael Haffner, MD, PhD

Prostate cancer cells (characterized by ERG overexpression, shown in brown) invade and colonize a benign prostate gland (highlighted by intact basal cells, shown in red). Such retrograde colonization of preexisting benign glands by cancer cells is increasingly recognized as a distinct pattern of cancer cell spread and is seen in many solid tumors, including prostate and pancreatic adenocarcinomas.

FRED HUTCH
CURES START HERE™

Welcome to the Human Biology Annual Retreat!

This past year The Human Biology Division has continued its tradition of serving as the home base for a remarkable group of researchers with diverse interests in cancer research and other complex human diseases. Together we are a complimentary blend of fundamental, applied, and translational researchers.

We have accomplished a lot since our last retreat. We welcomed new primary and secondary members, promoted members, were awarded many new grants and initiated new collaborations. We hosted six speakers through our two programs **Human Biology Distinguished Speakers** and **Trends in Human Biology**. Our first round of professional development funds to HB Staff Scientists, Post Docs and techs were awarded. We also enjoyed celebrating together at our annual pumpkin carving, holiday parties, ice cream social and summer picnic.

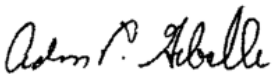
Our recruitment efforts have been successful in finding talented new faculty. Last spring, we welcomed **Susan Bullman, Ph.D.** as an Assistant Member. Susan studies the species of bacteria that is implicated in colorectal cancer. This summer, **Michael Haffner M.D. Ph.D.** joined us as an Assistant Member in the Prostate Cancer Program.

Our faculty have been recognized with many notable honors. This is a wonderful testament to our research excellence and the outstanding caliber of our faculty. To name just a few, **Denise Galloway, Ph.D.** was elected to the American Academy of Arts & Sciences, **Daphne Avgousti, Ph.D.** received an NIGMS MIRA and **Sita Kugel, Ph.D.** received an NCI MERIT Award.

The Human Biology division aims to continue the tradition of scientific excellence at Fred Hutch through active mentoring of our students and fellows. This brochure provides a snapshot of each lab with highlights from this past year.

Sincerely,

Eric Holland, MD, PhD
Sr. VP, Division Director



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**** very interested in taking a graduate student 2019-2020***

VIRAL MANIPULATION OF CHROMATIN

Our laboratory is focused on the mechanisms by which viruses hijack chromatin. Due to the major advancement in sequencing technologies and the expansion of the field of epigenetics, exploiting viruses to investigate chromatin biology has enormous potential. Our goal is to advance basic understanding of viral manipulation of chromatin and uncover new aspects of chromatin biology.

Much like the cellular genome, viral genomes must be compacted in virus particles with small basic molecules to maximize space and be poised for gene expression. Some DNA viruses use cellular histone proteins to compact their genomes whereas others use small basic molecules. Adenoviruses encode their own histone-like protein, called protein VII, that forms a 'beads on a string' assembly with the viral genome. By examining protein VII in host chromatin, we discovered that protein VII sequesters the immune danger signal, HMGB1, in chromatin thereby dampening downstream inflammation (Avgousti et al, Nature 2016). This discovery sets the framework for deciphering how adenovirus manipulates host chromatin and more broadly how DNA viruses use histones or histone-like proteins for dual function: to compact their genomes and control host genomes.

Research efforts in the lab use a multidisciplinary approach to address the following questions:

1. How does adenovirus protein VII impact nuclear architecture? The expression of protein VII in cells is sufficient to increase nuclear size and markedly disrupt the appearance of cellular chromatin. To investigate this phenotype, we carried out immunoprecipitation of protein VII followed by mass

spectrometry (IP-MS) and identified interacting proteins. In conjunction with chromatin fractionation proteomics, we have identified chromatin factor HMGB1 as well as linker histone H1 as potential key players in the chromatin distortion caused by protein VII. Projects in the lab will use cell culture, microscopy, biochemistry and genomic techniques to asses this phenotype and define the role of host chromatin factors in viral pathogenesis.

a. To which genomic sites does protein VII bind? What is the effect on transcription?
b. How do host chromatin factors, such as HMGB1, contribute to protein VII's impact on host chromatin?

2. How does herpes simplex virus (HSV-1) affect host chromatin? HSV-1 is an enveloped DNA virus with well characterized lytic and latent stages. HSV-1 first replicates in epithelial cells then enters peripheral neurons where it establishes latency. We developed a novel proteomic approach for temporal analysis of the host and viral proteome, phosphoproteome and chromatin-associated proteins during HSV-1 lytic infection (Kulej and Avgousti et al, MCP 2017). These data reveal that hundreds of host proteins are regulated during lytic HSV-1 infection, including many chromatin factors. We also uncovered dynamic changes in histone post-translational modifications throughout the course of HSV-1 infection. In addition to mining this vast dataset, projects in the lab will use molecular biology and genome editing techniques to investigate chromatin-related proteins and their role during HSV-1 infection.
    a. MacroH2A1 is upregulated during HSV-1 infection and localizes to viral genomes. What is the role of macroH2A during infection?
    b. Chromatin remodeling complexes such as SWI/SNF are also upregulated late during infection. What is the function of these complexes during infection?

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My research program focuses on phylodynamic analysis of pathogen sequence data with an intent of making inferences that are actionable to public health. This research program spans a number of viral systems including seasonal and avian influenza, Ebola, Zika, SIV, MERS-CoV, dengue and mumps. This requires development of mathematical and statistical methods to integrate infectious disease sequence data into evolutionary and epidemiological models.

I've co-developed the open-source Nextstrain platform (nextstrain.org) that aims to harness the scientific and public health potential of pathogen genome data by providing a continually-updated view of publicly available data alongside powerful analytic and visualization tools. This platform is used by the World Health Organization Global Influenza Surveillance and Response System (GISRS) to aid in vaccine strain selection for seasonal influenza virus. This platform was also highlighted during the Zika epidemic in the Americas and the Ebola epidemic in West Africa as a central source for data sharing and up-to-date insights.

I have published over 50 scientific journal articles and my awards include a MIRA R35 investigator award from NIGMS, a Pew Biomedical Scholar Award and the NIH / HHMI / Wellcome Trust Open Science Prize.

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Genome sequencing studies have demonstrated that cancer develops as an evolutionary process of somatic mutation and clonal selection. The underlying genetic fingerprint of each cancer is different and the presence of specific mutations, e.g. *EGFR* activating mutations in lung cancer, can dramatically influence the response of tumors to specific therapies.

The goal of my laboratory is to enable precision medicine by systematically uncovering the molecular alterations in cancer, determining the function of these variant alleles, and understanding how these alleles modulate response to cancer therapies. Although many of the *genes* involved in cancer have now been identified, a major challenge is discovering which specific *alleles* of these genes are involved and how these alleles modulate therapeutic response. My group uses functional genomics, computational biology, biochemistry, and genetics to understand the mechanism of somatic cancer variants. Our goal is to identify drug targets and biomarkers and to translate this knowledge into clinical benefit for patients.

A central theme in the laboratory is understanding the mechanism and therapeutic targeting of cancer oncogenes in the RAS/MAPK pathway. Both *KRAS* and *RIT1* encode small GTPase proteins and are mutated in lung adenocarcinoma and in the germline of “Ras-opathy” patients. Although *KRAS* has been extensively studied, *RIT1* mutations were discovered <5 years ago and the role of these mutations in cancer is poorly understood. We are using unbiased proteomic approaches and genome-wide CRISPR knockout screens to determine the functional similarities and differences between *KRAS* and *RIT1*, with the goal of identifying critical effectors of these proteins that might be targeted for therapeutic benefit. Candidate drugs will be tested in a genetically-engineered mouse model generated by our lab.

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REGULATION OF EPITHELIAL GROWTH IN DEVELOPMENT AND CANCER

My laboratory studies molecular and cellular mechanisms that regulate tissue growth in development and tumorigenesis. Our goal is to identify genes and gene pathways that can be used as novel targets in cancer therapy, with a particular focus on regulators of the balance between stem cell renewal and differentiation.

We use mouse skin epidermis and epidermal squamous cell carcinoma (SCC) as models of tissue growth in development and disease. The skin epidermis is particularly suited for our investigations for the following reasons:

A. It is a well-defined physiological system: The skin consists of an epithelial compartment, the epidermis, and mesenchymal compartment, the dermis, separated by a basement membrane. Epidermal growth during the embryonic development and its maintenance in the adult are achieved through continuous cycles of progenitor cell self-renewal and differentiation under the control of cell extrinsic signals from the surrounding mesenchyme.

B. It has implications to human health: SCC of the skin is the second most common cancer in people, with an estimated 700,000 new cases in the US each year. Fortunately, most lesions are detected early and surgically removed, accounting for the disease's high survival rate. Importantly, ontogeny of epidermal SCC parallels cancers with much higher mortality rates, including the SCC of the head and neck, and the lung SCC.

C. There are mature tools for analysis of gene function: In addition to established methods of creating transgenic animals, we have shown that mouse epidermis can be efficiently and stably targeted through *in*

utero injection of lentivirus. Using lentiviral vectors for RNAi-mediated gene knockdown or gene overexpression, we can rapidly assess gene function and complex genetic interactions *in vivo.*

Research efforts in the lab are divided between several approaches:

1. Candidate-based analysis of gene function in regulation of epidermal tissue growth. We have completed an RNAi screen of ~16,000 mouse genes and uncovered putative regulators of epidermal tissue growth during embryonic development and oncogenic hyperplasia. We are now testing the precise cellular and molecular mechanisms behind the observed growth effects, with a focus on genes that specifically operate within the physiological environment by altering the balance between stem cell renewal and differentiation.

2. Large–scale investigation of modifiers of epidermal tumor initiation. We have successfully combined pooled-format, lentiviral-mediated RNAi and quantitative Illumina sequencing in a rapid, comprehensive, and relatively low-cost approach to genome-wide gene function analysis during embryogenesis. We have now extended the use of this approach to identify *bona fide* enhancers of tumor initiation and progression in the oncogenic Ras animal model postnatally. The complexity of our lentiviral pools vary from patient-specific to genome-wide.

3. Development of a general model of epithelial growth and tumorigenesis. Our technique of injecting lentivirus *in utero* can be modified to produce efficient transduction of other tissues, including the oral, mammary and airway epithelium. These are distinct from the skin in their organization, physiological environment, and rate of developmental and regenerative growth, and carcinomas in these epithelia are the leading cause of tumor-associated deaths worldwide. Using RNAi-mediated gene knockdown, we test the general applicability of molecular mechanisms uncovered in our studies of epidermal growth and tumorigenesis.

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TRANSLATIONAL RESEARCH PROGRAM

Our mission is to prevent disease, advance treatment, and increase patient survival. To this end, we pursue a broad-based methodological approach to elucidate the fundamental and clinical implications of nuclear and mitochondrial DNA mutations in the pathogenesis of cancer and age-related disease.

The majority of our projects set out to address long-standing intractable questions in mutation research, which have remained unanswered, largely due to technical limitations. Thus, our first step toward their resolution typically involves the development of new methods and technologies (featured examples highlighted below) to sensitively measure biological information. The application of these tools to the question at hand, more often than not, reveals new insights into biology that exceed the scope of the hypothesis being tested, driving us down new and exciting pathways of discovery in pursuit of our overarching mission.

As such, while mutagenesis remains at the core of our research program, the focus of the laboratory continues to diversify and expand. Current and ongoing areas of interest include nuclear and mitochondrial genomics, DNA repair, transcriptomics, metabolomics, single cell biology, tumor immunology, cancer therapeutics and diagnostics.

SELECTED PUBLICATIONS

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The Tumor Associated Microbiota: A role in cancer progression and treatment

My lab focuses firstly on understanding the translational impact of the tumor microbiota in human cancers, and secondly on the delineation of specific mechanisms involved in the pathogenesis of microbe-associated human cancers. We combine molecular microbiology, computational biology, biochemistry, and genetics to understand host-microbial interactions within the tumor microenvironment. Through such efforts, we seek to make discoveries that have both a scientific and clinical impact in the emerging area of bacterial-associated malignancies. Bacterial agents that have a role in cancer initiation or progression provide a viable route for prevention and treatment of these cancers.

We have a particular interest in the colorectal cancer associated bacterium, *Fusobacterium nucleatum*. Utilizing state-of-the-art functional and computational approaches, we aim to improve the understanding of processes that contribute to *Fusobacterium*-associated cancer, its contribution to the tumor microenvironment and accelerate the development of new targeted therapeutics or personalized approaches that take in to account the tumor microbiota for cancer treatment. Our current projects touch on two broad areas, noted below.

Host-Microbe Interactions:

(i) Spatial analyses of the microbiota within the tumor microenvironment
(ii) Reductionist approach to functionally characterizing the capabilities of tumor-associated microbiota

Microbe-Drug Interactions:

(i) Assessing microbiota modulation by routine chemotherapeutics
(ii) Assessing chemotherapeutic modulation by tumor microbiota
(iii) Development of novel targeted antimicrobials against selected tumor enriched bacteria

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Recent studies by our group and others have uncovered that tumor cells invade and metastasize to distant organs as cell clusters. Clinically, the presence of circulating tumor cell clusters is associated with significantly worse patient outcomes in a variety of cancer types. Our laboratory's major focus is to decipher the fundamental mechanisms explaining this phenomenon. Which cell types cooperatively promote collective metastasis? What signals enable tumor cell clusters to survive and proliferate? How do clustered tumor cells coordinate their actions? To answer these questions, we use cutting edge tumor organoid, time-lapse microscopy, and single cell technologies to directly observe and manipulate tumor cell clusters. Our ultimate mission is to guide the innovation of new therapies for metastatic breast cancer patients.

Our research efforts are divided among three areas:

Molecular regulation of collective invasion

Tumors frequently invade collectively as clusters of cells into surrounding tissues, but the molecular phenotype of the cancer cells leading this process have remained unclear. Using organoid assays, we found that the molecular phenotype of invasive leader cells was distinct from the bulk tumor cells and conserved across breast cancer subtypes. We have now identified the molecular profiles of leader and non-leader cells, and are testing the function of specific genes in regulating their invasive behavior and interactions with the tumor microenvironment.

Mechanisms for cluster-mediated survival

Our recent studies establish the importance of a metastatic route involving the dissemination of tumor cell clusters. We have developed lineage tracing and microscopy platforms to track tumor cell clusters from their origin to distant sites. Our data reveal that tumor cell clusters have superior colonization relative to single tumor cells independent of their propensity to form emboli in vivo. We are now dissecting the molecular basis for these remarkable properties.

Dissecting intercellular signaling interactions in tumor cell clusters

A fundamental question is how collective cell behaviors emerge from the interactions of distinct cell types. Our studies have identified subpopulations of cells within tumor cell clusters with distinct molecular profiles and cell behaviors. We are interested in using molecular genetic tools and biosensors to interrogate how signals between subpopulations of tumor cells are integrated to generate collective behavior.

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CELL DIVISION AND THE UBIQUITIN-PROTEASOME SYSTEM IN CANCER DEVELOPMENT AND THERAPY

Research Interests: The Clurman lab studies protein degradation, cell division, and carcinogenesis. This research has led to fundamental discoveries about how cell division and protein ubiquitylation are regulated in normal cells, and their associations with cancer. Our approach has been to employ whatever methods are needed to make mechanistic and physiologic advances, and these methods have included biochemistry, cell biology, proteomics, and gene targeting in human cells and mice. As a result, our work has often revealed surprising findings in well-studied pathways. We have had a particularly sustained impact in studies on the Fbw7 ubiquitin ligase, which sits at the crossroads of our expertise in cell division, the mediation of protein degradation by the ubiquitin system, and tumorigenesis. We are moving our basic and mechanistic work towards therapeutic goals whenever possible.

Current Studies:

Fbw7 Tumor Suppressor – d Fbw7 is the substrate recognition component of an SCF-ubiquitin ligase that targets a remarkable group of proteins for degradation by the proteasome. Many Fbw7 substrates are critical oncoproteins, and Fbw7 is one of the most frequently mutated human tumor suppressor genes. Our lab has made numerous key contributions to understanding this complex pathway. One area involves our discovery of new substrates, including Myc, Notch, cyclin E, and Mediator, and their mechanisms of degradation. Another area has entailed the use of physiologic human and mouse gene targeting models to understand the normal and neoplastic functions of the various Fbw7 isoforms. Recently, we described the fundamental importance of

Fbw7 dimerization to its substrate interactions, and how dimerization may explain Fbw7's unusual mutational spectrum in cancer and lead to novel therapeutic strategies. Finally, we have developed new mouse models of Fbw7-associated cancer, including a new model of metastatic and chromosomally unstable colon cancer.

Cyclin E regulation and function – The cell cycle field exploded during my postdoc and early independent career, during which time a basic framework of cell division emerged, including cyclin E's central role in G0 to S-phase progression and tumorigenesis. We contributed substantially to this field and established new paradigms in cyclin E regulation and function. One area is cyclin E-degradation by the ubiquitin proteasome system, wherein we first made the surprising discovery that cyclin E autophosphorylation triggers its own ubiquitylation and destruction. We subsequently detailed the mechanisms through which cyclin E phosphorylations regulate its ubiquitylation by Fbw7 and establish its normal periodicity during the cell cycle. Another surprising discovery was our finding that cyclin E has a CDK-independent function in licensing replication origins, which was the first description of a CDK-independent cyclin function. We have also described the genomic instability caused by cyclin E deregulation in cancer and a homeostatic response that protects cells against this genomic instability. Finally, we have developed mouse models with targeted mutations of cyclin E phosphorylation sites to demonstrate the roles of phosphorylation-dependent cyclin E control in differentiation, genome stability, cell division, and tumorigenesis.

Cip/Kip CDK inhibitors – We have made many contributions to this field that have had a sustained impact. The first was the finding that cyclin E-CDK2 inhibited p27 by targeting it for proteolysis. The idea that a CDK inhibitor could be antagonized by the very kinase it inhibits was completely unexpected. Nonetheless, these data are now widely accepted and set the stage for many future studies of CDK inhibitor regulation. Another example is our work on the ubiquitin-independent degradation of the p21 CDK inhibitor by the proteasome. Although p21 is ubiquitylated, we found that its proteasomal degradation does not require ubiquitylation. Although controversial at the time, these data also established a new mechanistic paradigm which we subsequently fully characterized. We have also studied normal and neoplastic p27 functions models in novel mouse models, including a high throughput insertional mutagenesis screen, which revealed p27-collaborating oncogenes.

CDK2 functions and regulation – One continuing aspect of our work on CDK2 has focused on identifying CDK2 substrates, which began with our studies of cyclin E autophosphorylation. We subsequently developed novel proteomic methods using substrate thiophosphorylation and ATP analog substrate-sensitive CDKs, to discover more than a hundred new CDK2 substrates. We have now adapted this method to highly physiologic contexts, which has revealed even more new substrates, including the discovery that CDK2 regulates a host of chromatin-modifying enzymes to coordinate cell division and gene expression. In light of new therapies targeting CDK4/6, we have now adapted these methods to identify new CDK4/6 substrates. We have more recently focused on the importance of CDK2 inhibitory phosphorylation by Wee1, and made a human knockin cell line to show that CDK2 inhibitory phosphorylation has essential roles in cell cycle control, in DNA replication dynamics, and in genome instability. Most importantly, we found that the inability to inhibit CDK2 during stalled S-phase leads to massive DNA damage, which has formed the basis for an exciting new chemotherapy strategy.

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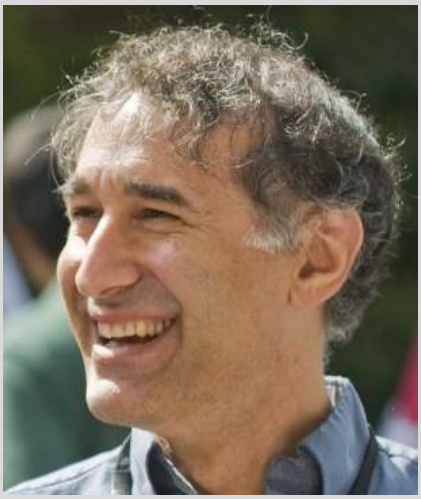

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HIV AND THE EVOLUTIONARY HISTORY OF VIRUS-HOST INTERACTIONS

The Emerman lab studies host-cell interactions of the human immunodeficiency virus (HIV) and related viruses in order to understand the molecular and evolutionary basis of virus replication and pathogenesis. We do this by studying the evolution and function of host antiviral genes. Our goal is to determine how HIV adapted to humans, and how ancient viral infections influenced the susceptibility or resistance of humans to modern lentiviruses.

Host restriction factors are potent, widely expressed, intracellular blocks to viral replication that are an important component of the innate immune response to viral infection. However, viruses have evolved mechanisms of antagonizing restriction factors. Through evolutionary pressure for both host survival and virus emergence, an evolutionary—arms race has developed that drives continuous rounds of selection for beneficial mutations in restriction factor genes. Because viruses can evolve faster than their hosts, the modern-day vertebrate innate immune system is optimized to defend against ancient, extinct viruses, rather than our modern viral threats. Thus, we use the evolutionary history of restriction factors and their functional interactions with viral proteins to understand modern viral pathogens such as HIV-1.

By using human polymorphisms in restriction factors as well as comparisons to other primates, we can identify “holes” in the human innate immune system that make us ill-adapted to infection by HIV. We are using this information combined with the evolution of restriction factors to create novel, more potent variants on existing antiviral proteins that we call “super restriction factors”.

We have developed a powerful new high-throughput screen for HIV restriction factors and host other host factors based on incorporation of CRISPR guide RNAs into HIV virions. We are using these screens to discover the interferon-stimulated genes that inhibit HIV, to discover human genes

that used for HIV for productive infection, and for uncovering pathways used by HIV to maintain itself in a latent state and avoid elimination by the immune system.

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VIRUSES AND CANCER

The Galloway Lab studies how human papillomaviruses (HPVs) and human polyomaviruses (HPyV), specifically Merkel Cell polyomavirus (MCPyV) contribute to the development of cancers, and how that information can be used to better prevent, diagnose or treat malignancies. High-risk papillomaviruses (HR-HPVs) are causative agents in nearly all cervical cancers and the majority of other anogenital and oropharyngeal cancers. By identifying the targets of the viral oncoproteins, E6 and E7, we have revealed key regulatory pathways that control proliferation, senescence, apoptosis and DNA damage repair. These pathways are also targets of somatic mutation in other epithelial tumors. We are particularly interested in the mechanisms by which the HR-HPV E6 and E7 proteins disrupt the repair of DNA damage. In addition to studying the mechanism we plan to exploit this vulnerability to develop therapeutic approaches to treat HPV associated neoplasia. Development of cervical cancer occurs over decades and the precursor intraepithelial lesions can be readily obtained. Analysis of the staged clinical lesions, coupled with ectopic expression of E6 and E7 in otherwise normal cells, allows us to distinguish the proximal consequences of E6/E7 expression from the myriad changes that result from genetic instability. Additionally, the requirement for HPV infection and gene expression in anogenital malignancy provides a clear target for prophylactic and therapeutic immune intervention.

A longstanding interest has been in characterizing the humeral immune response to HPV following natural infection or vaccination. We have mapped epitopes involved in virus neutralization and continue to characterize the breadth of the response. Current studies are characterizing the B cell memory response by identifying HPV-specific memory B cells, plasmablasts and the antibodies they express. These studies are important in determining the most effective vaccine regimens, whether fewer doses are effective and whether natural immune responses could be improved by vaccination. Understanding memory responses and the protective antibodies they generate will have broad implications for vaccine design. We are collaborating with others to conduct an efficacy and immunogenicity trial of a single dose of HPV vaccines in Kenya, and are also determining effective dosing regimens in HIV+ children in Peru.

We have also become interested in the role that the Merkel Cell polyomavirus (MCPyV) plays in the etiology of Merkel Cell carcinoma (MCC), a rare but aggressive skin cancer. Interestingly we demonstrated that antibodies to the common region of large and small T antigen are present in about 60% of patients with MCC and that increases in antibody titer are prognostic of recurrence. We also showed that MCPyV encodes another T antigen in an alternate reading frame to large T antigen, which we named ALTO. ALTO is evolutionarily related to the middle T antigen of rodent PyVs. A major focus is to determine the mechanisms by which the viral T antigens play in tumorigenicity and to develop suitable cell based and mouse models.

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National Cancer Institute, Outstanding Investigator Grant 2017 – 2024

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Washington State Life Sciences Hall of Fame 2017 –

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DNA VIRUS ADAPTATION TO HOST CELL DEFENSES

In response to myriad host defenses, viruses have evolved mechanisms that counteract cellular anti-viral factors. These host-virus conflicts result in an evolutionary "arms race," in which the structure and specificities of the participating genes change with surprising rapidity. Research in the Geballe lab focuses on identifying the factors, dissecting the mechanisms, and understanding the evolutionary pathways used by large DNA viruses, such as cytomegaloviruses and poxviruses, to enable viral replication in the face of host defenses.

Human cytomegalovirus (HCMV), a member of the herpesvirus family, typically produces few if any symptoms in otherwise healthy individuals, but often causes life-threatening infections in newborns, solid organ and hematopoietic stem cell transplant recipients, and other immuncocompromised patients. In addition to its medical importance, HCMV is also a useful model system for the study of viral mechanisms for circumventing host defenses such as the shut off of translation mediated by the interferon-induced, double-stranded RNA-activated protein kinase R (PKR). A genetic screen identified two essential HCMV genes that participate in maintaining translational capacity in the infected cell by inhibiting the PKR pathway. Comparisons between these HCMV genes and related ones encoded by nonhuman primate and rodent CMVs have revealed surprising specificity and complexity in the interactions and mechanisms by which these factors act. We are now dissecting the molecular basis for these differences and investigating additional critical roles these proteins play in the viral life cycle.

A broadly applicable strategy for studying viral mechanisms that counteract host defenses is to force viral adaptations in cell culture and then to sequence the resulting viruses to identify the genetic basis of the

adaptation. Such an experimental evolutionary approach revealed an unexpected ability of the model poxvirus vaccinia to adapt by amplification of a weak antagonist of the human PKR, followed by mutation and collapse back down to a single copy gene. Current efforts are underway to explore whether this “accordion-like” mechanism is a general property of large DNA viruses and to assess its role in adaptation of these viruses to other host defenses.

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REGULATION OF DISSEMINATED TUMOR CELL DORMANCY

Cyrus Ghajar directs the Laboratory for the Study of Metastatic Microenvironments (LSM^2). The goal of his lab is to understand how microenvironments within distant tissues regulate dormancy, re-emergence and therapeutic resistance of disseminated tumor cells (DTCs). Solving these puzzles is key to extending metastasis-free survival of cancer patients; with the ultimate goal of preventing metastasis altogether.

To accomplish this goal, the LSM^2 studies dormant DTCs within spontaneous metastasis models, and builds organotypic culture models to dissect how different microenvironments influence DTC fate. In addition to traditional laboratory techniques, we utilize intravital microscopy, live cell microscopy, ribosome profiling and proteomics to gather information on where dormant DTCs reside within a given tissue, the cellular and molecular constituents of these resident niches, and the dynamics by which DTCs operate within these niches.

Four major research directions are currently underway in the laboratory:

1. *Uncovering tissue-specificity of the dormant niche*: Dormant DTCs localize to microvessels regardless of where we find them, and even though the end result (i.e., quiescence) may be the same, the means to get there may differ. Specifically, it is well known that vascular beds possess very different properties depending on the tissue that they feed. This very well could result in (and in fact reflect) very different perivascular microenvironments, with different factors that affect disseminated tumor cell behavior in each organ. To elucidate the tissue-specificity of the perivascular niche, we engineer mimetics of microvascular beds of a number of different tissues, including those where disseminated breast tumor cells commonly emerge (e.g., brain, lung, bone marrow and liver) as well as those where they rarely do.

2. *Understanding how the perivascular niche contributes to therapeutic resistance*. Breast tumor cells disseminate very early during tumor progression, and typically possess fewer genomic abnormalities than even the primary tumor. Because of this, it is unlikely that they inherently (e.g., due to somatic mutation) resist chemotherapy. Instead, we think the perivascular niche plays a large part in this, and are actively working to profile the perivascular niche to identify unique regulators of DTC survival, growth and drug resistance.

3. *Defining adaptive immune cell interactions with dormant DTCs*. We are working to understand whether the adaptive immune system recognizes dormant DTCs, and whether the ability of these cells to evade killing by the adaptive immune system depends on their growth status or their microenvironment.

4. *Devising regimens to specifically treat dormant DTCs*. Our goal is to leverage the above into new therapies to treat dormant DTCs, whether that entail “reinforcing” the dormant niche to keep them asleep, or compromising aspects of the niche that protect DTCs from therapy. We are also leveraging the models we have developed to screen for drugs that selectively target dormant cells. Our hope is that these approaches will lead to novel, effective therapies for dormant DTCs that ultimately prevent metastases from emerging.

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The Gujral lab is focused on understanding the molecular details of how cells respond to growth factors and how these signaling networks can be targeted therapeutically. To advance investigation of pathological signaling events among cells within the microenvironment, the Gujral lab is developing techniques for culturing organotypic slices of tumor tissue and monitoring the behavior of specific cell populations. Dr. Gujral discovered a pathway involving the growth factor Wnt5 and the receptor Fzd2. His lab explores how this pathway contributes to liver fibrosis and cancer metastasis by triggering changes in cell fate. The molecular networks that let cells respond to stimuli are complex, dynamic, interconnected, and context dependent. Therefore, the Gujral lab uses systems biology and computational biology along with cell biological, genetic, and pharmacological approaches to investigate tissue-specific signaling networks, identify molecular targets for drug discovery, and identify new uses for existing medicines.

Current Studies

Wnt Signaling in Disease: Metastasis is responsible for ~90% of cancer-associated death, yet progress has been slow in developing drugs that either specifically target metastasis or target cells with metastatic potential. The process of epithelial-to-mesenchymal transition (EMT) is associated normal embryonic development, but this process is aberrantly activated and contributes to metastatic potential in cancer. EMT is a reversible process in which epithelial cells adopt mesenchymal properties, which include changes in cell shape, proliferative capacity, and increased motility. Our lab identified the Wnt5-Fzd2 pathway as a key signaling network driving EMT and tumor metastasis in several challenging cancers, including liver, breast, lung, and colon. This pathway is one of several growth factor-mediated pathways that trigger EMT in both embryonic development and normal and transformed cell lines. Our lab uses a combination of cell biological, biochemical, and genetic approaches to uncovering the signaling networks by which growth factors stimulate EMT, which could guide the development of new therapies directed at cancer metastasis.

Developing New Technologies for Studying Signaling within a Tissue Microenvironment: A main focus of our lab is the development of new technologies that enable investigation of cellular interactions within the tissue microenvironment. Such methods need to (i) preserve the tissue microenvironment and maintain cell-cell interactions; (ii) study the properties of individual cells within this microenvironment, (iii) expose individual

cells within a tissue to stimuli in a spatially and temporally controlled manner, and (iv) evaluate the response of single cells within a tissue to stimuli. We are developing new experimental approaches to study cell-cell interactions and single cell responses in the complex tissue microenvironment. We are developing approaches relevant to investigating cancer within the tumor tissue context, as well as approaches that will enable new insights into physiological regulation of cells within normal or disease tissues.

Developing Computational Tools for Performing Network Pharmacology. Another area of investigation is in the application of computational strategies to explore novel pharmacological options. Many drugs affect multiple targets, and many effective therapies target multiple regulators of a physiological process or multiple mediators of an intracellular signaling pathway. Network pharmacology is a new field of science focused on targeting multiple steps in a physiological regulatory network. Goals of this field include facilitating the design of drugs with specific multi-target profiles and exploiting the existing polypharmacology of many currently used medicines. We are developing computational tools for evaluating the many potential clinical applications of kinase inhibitors, because kinases represent a core group of enzymes involved in most cellular responses to stimuli and represent an important target for existing therapeutics for cancer, inflammation, and other disease states. Additionally, most available kinase inhibitors lack specificity, making attributing the effects of an inhibitor to an individual kinase difficult. Finally, the physiological and pathological roles of many kinases remain to be discovered. Thus, the tools we are developing will enhance our understanding the basic biology of kinases as well as advance pharmacological exploitation of these key cellular regulators.

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Description of your research?

My laboratory's research is focused on studying the role of genomic alterations in cancer and expanding applications for precision medicine. We combine research in two complementary areas: (1) We develop and apply novel **computational methods** to comprehensively profile and study **cancer genomes** from patient tumors and (2) we develop approaches to use non-invasive **liquid biopsies**, such as **circulating tumor DNA** from blood, to monitor genomic changes in patients receiving therapy. Our goals are to uncover the genetic and epigenetic mechanisms of treatment resistance, identify blood-based genomic biomarkers, and translate these findings and innovations to advance cancer precision medicine.

We are interested in understanding the roles of tumor evolution and abnormal genome structure in cancer. We apply cutting-edge whole genome DNA sequencing technologies, particularly platforms that generate long-range genomic information, such as linked-read sequencing. These technologies enhance the reconstruction of genomic rearrangements and enable the study of alterations in non-coding genomic regions. Our recent work applying this technology to study advanced prostate cancer includes the discoveries of non-coding alterations to an enhancer of the androgen receptor and a genome-wide tandem duplication signature associated with CDK12-loss.

A key focus of our research is to accelerate the development of new approaches to exploit liquid biopsies for studying cancer. We leverage insights from the analysis of tumor genomes to inform the design of circulating tumor DNA applications to study treatment response in cancer patients. We have established strong collaborations with experimental and clinical scientists at Fred Hutch and UW to apply our approaches to study treatment resistance in prostate and other cancers.

The development of novel computational algorithms for analyzing human cancer genomes is a primary research focus of our group. Probabilistic, machine learning algorithms that we have developed include TITAN, HMMcopy, and ichorCNA for predicting genome-wide alterations from tumor and cell-free DNA sequencing data. These software tools are widely used by the cancer research community. We are actively developing new methods to expand our suite of tools to analyze and discover new signatures in tumor and circulating tumor DNA.

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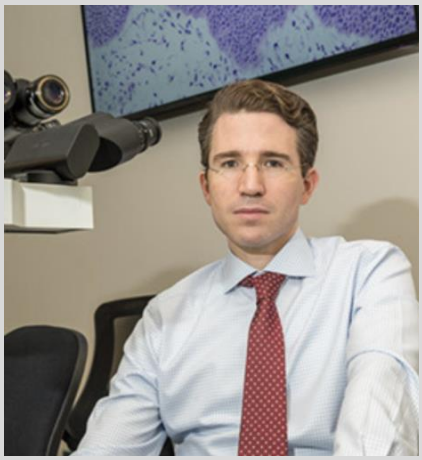
Michael Haffner, MD, PhD



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I am a physician-scientist and as a practicing pathologist I gain a lot of insights and motivation for my laboratory-based research from observations made as part of my clinical practice.

A defining feature of almost all solid tumors is that cancer cells show dramatic changes in cell shape and size compared to benign cells. Yet, we know little about the principles that govern cell shape and their alterations in cancer. Every cell has a structural scaffold, a skeleton, termed the cytoskeleton. It consists of a complex interlinked network of proteins that supports the cell and helps to maintain its shape. To invade other tissues, cancer cells need to alter their cytoskeletal properties to become malleable enough to change their shape and to move through tissues. However, the processes by which cancer cells become such “shape shifters” are not well understood. To explore this further, my laboratory will focus on elucidating alterations of the cytoskeleton in solid tumors with a particular focus on prostate cancer.

For instance, we have recently described a novel protein, named AIM1 that regulates cytoskeletal organization by binding to actin, a major component of the cytoskeleton. When AIM1 is present, the cells' scaffolding keeps it rigid and correct shape. When AIM1 is lost, cells can remodel their cytoskeleton more frequently, change their shape and become capable of invading and migrating to distant locations. Notably, AIM1 function is disrupted in many solid tumors and genomic alterations of *AIM1* are associated with aggressive tumor growth.

The goal of these studies is to understand the mechanisms that govern changes in cell architecture to explore such cancer specific changes for therapeutic targeting.

I am very interested in having a graduate student join my laboratory in 2019-2020.

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My lab studies the structure and dynamics of the nuclear envelope (NE) to understand how changes in this compartment cause human genetic diseases and drive cancer pathogenesis. Our previous work characterized a new NE dynamic in cancer cells where the nuclear membrane ruptures, which causes mislocalization of proteins and even organelles, and then either repairs or collapses. Remarkably, cancer cell nuclei that undergo many rounds of nucleus rupture and repair are viable, but these NE dynamics can increase genome instability, cause massive chromosome rearrangements frequently found in human tumors, and activate innate immune pathways that lead to inflammation and metastasis. However, we are just at the beginning of redefining the NE as a dynamic structure and determining how defects in NE stability impacts cell function. My lab uses a combination of fluorescent microscopy, biochemistry, and genomics tools to investigate the following questions:

1) What controls NE rupture and repair in cancer cells?

Our current model of NE rupture, based on observations in cancer cells, *in vivo* migratory cells, and cells from laminopathy patients, is that disorganization of the nuclear lamina leads to areas of weak membrane that are prone to both chromatin herniation and membrane rupture when force is applied to the nucleus. However, our understanding of why the lamina becomes disrupted and what types of forces cause instability are rudimentary. In addition, the mechanisms of NE repair are almost completely uncharacterized. To understand these mechanisms better, we are taking a candidate approach to ask how previously characterized nuclear lamina proteins affect NE stability and a large-scale approach to identify new factors that affect nuclear lamina structure and NE membrane dynamics.

2) Why is the NE around micronuclei so unstable?

Our previous work found that when cancer cell chromosomes missegregate and recruit their own NE at the end of mitosis, forming compartments called micronuclei, they make a highly unstable NE that is prone to rupture and collapse. We showed that NE rupture in micronuclei frequently causes significant DNA damage and subsequent work demonstrated that the fragmented chromatin can undergo massive rearrangements in the next cell cycle. Yet why micronuclei fail to assemble a stable NE and why they can't repair after rupture are still unknown. We are taking both hypothesis-driven and proteomics approaches to address this question to increase our fundamental knowledge about NE assembly mechanisms and identify new methods to prevent NE rupture in cancer cells.

3) What are the consequences of losing nucleus compartmentalization at the cellular level and how does this contribute to human disease?

We currently use a cultured cell system to study the cellular mechanisms that cause NE instability. However, rapidly dividing cells in culture are likely desensitized to the compartmentalization defects associated with transient NE rupture, since mislocalized proteins and organelles can be removed during mitosis. Thus we are also developing new tools to study the consequences of NE instability, such as increased DNA damage, impairment of nuclear processes, including gene expression, impairment of cellular functions, and changes in transformation potential, in disease-relevant cell types. Our overall goal is to define causal links between NE rupture, misregulation of cellular functions, and disease phenotypes to identify potential therapeutic opportunities.

Selected Publications

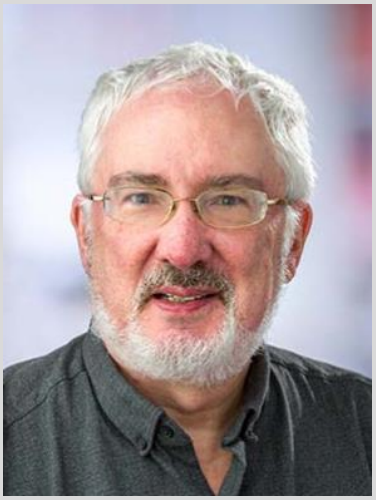
Emily M Hatch "Nuclear envelope rupture: little holes, big openings." *Curr Opin Cell Biol* **52,** 66–72 (2018).

Emily M Hatch and Martin W Hetzer "Nuclear envelope rupture is induced by actin-based nucleus confinement." *JCB*, 2016 Oct 10; 215 (1): 27.

Emily M Hatch, Andrew H Fisher, Thomas J Deerinck, Martin W Hetzer "Catastrophic nuclear envelope collapse in cancer cell micronuclei." *Cell*, 2013 Jul 3;154(1):47-60.

Jesse D Vargas, Emily M Hatch, Daniel J Anderson, Martin W Hetzer "Transient nuclear envelope rupturing during interphase in human cancer cells." *Nucleus*, 2012 Jan-Feb;3(1):88-100.

David Hockenbery, MD.



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Members of Dr. Hockenbery's laboratory study programmed cell death (apoptosis) pathways, and the role of cell metabolism in apoptosis, oncogene functions, and environmental/dietary risk factors, including excess nutrient supply. We have identified small molecule inhibitors of Bcl-2 and published the first structure of a Bcl-xL homodimer. My lab has also published studies of c-Myc regulation of mitochondrial gene expression and central carbon metabolism, and discovered a novel pathway of ubiquitin-mediated protein turnover in mitochondria, known as MAD (for mitochondria-associated degradation). We are currently pursuing metabolomic studies directed toward identifying unique metabolic pathways in chemoresistant breast cancers, and biochemical markers of mitochondrial injury in serum and urine samples. **The following projects are currently active in the lab:**

a) Fructose selectively stimulates growth and metastasis of triple-negative breast cancer cells.

b) Activation of the Fanconi Anemia/BRCA DNA damage response by endogenously produced aldehydes.

c) Metabolic dependencies on nicotinamide metabolism in glioblastomas (collaboration with Patrick Paddison).

d) Activation of oxidative metabolism in Fbw7-deficient colon cancers (collaboration with Bruce Clurman).

e) Functional genomics and metabolomics of Tasmanian Devil Facial Tumor cell lines.

f) Fnip1 function in lymphocyte development and energy stress (collaboration with Brian Iritani).

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MOUSE MODELS OF BRAIN TUMORS

The main goal of my laboratory is the use of genetically-accurate mouse models of glioblastoma to understand the molecular basis for the genesis of these tumors and their response to standard therapy. In the process, we have developed preclinical trial infrastructure and imaging that supports the development of novel therapeutic approaches. My laboratory developed the RCAS/tv-a system of post-natal somatic cell-type specific gene transfer to study cancer formation in mice, and used this system to model the formation of gliomas and medulloblastomas. We demonstrated that stem cells are more sensitive to transforming events than differentiated cells, that Akt activity is elevated in human glioblastomas, and that deletion of *PTEN* (as occurs in human glioblastoma) in mice was causal in glioma formation and progression. Brain tumor cells that are resistant to radiation therapy occupy the perivascular niche and have stem-cell characteristics driven by a combination of Akt and notch activities. We have shown that nitric oxide produced by endothelial cells promote stem-cell characteristics in perivascular cells through cGMP, PKG and Notch signaling. We have further characterized therapeutic DNA Damage Response pathway in gliomas and compared this data with that from human GBM. Finally, one recent breakthrough in the field is the molecular subdivision of the GBMs. Our contribution to this was demonstrating proteomic evidence that specific signaling pathway activity characterizes these subgroups and that the mouse models were specific mimics of the molecular GBM subgroups. The human and mouse glioma data has led to molecularly-stratified clinical trials for GBM patients.

The biology of immunotherapy response in gliomas
We have been using our immunocompetent models of gliomas to better understand the immunologic response to the tumor and to its response to standard therapy. Different glioma subtypes have different immune characteristics, a feature mimicked by our mouse models. The Abscopal effect, where radiation or other localized therapy induces the body's immune system to recognize and target tumors outside the field of therapy as foreign. We have created experimental paradigms that have bilateral tumors of different subtypes,

or where one tumor is treated and the immunologic response measured on the other side. Ultimately, we hope to better understand when and how checkpoint inhibitors, CAR T cells and oncolytic vectors can be used in combination with standard therapy.

The biology of stem-ness in tumors and its consequences in gliomas *in vivo*
One topic in this area is the issue of stem-ness in tumor cells and what drives this character. The work evolves from our previous work showing that stem like cells are located in the PVN and are driven by NO signaling mentioned in the abstract among others.

Mathematical and mouse modeling
My laboratory has a long-standing collaboration with Franziska Michor of the Computational Biology department at the Dana Farber. We combine mathematical modeling with mouse modeling to understand the likelihood of events in the evolution of gliomas development or in optimizing therapy based on parameters obtained from mouse models. In these projects we have: 1. identified the most probable cell of origin for PDGF-induced gliomas, 2. determined the order of genetic events in the evolution of these tumors, 3. identified the first events in gliomas formation, and identified an optimized schedule for delivery of radiation therapy based on parameters obtained from our PDGF-induced gliomas model.

The biology of therapeutic response in gliomas
Many laboratories are studying the biology of these tumors (and other tumor types), but few are trying to understand the biology of how these tumors respond to therapy. This is conceptually important because the disease that kills people in the western world is a treated and recurrent tumor, not an untreated tumor. Therefore, we have spent effort in developing the technologies to understand how these tumors respond to standard therapy using the same rigor that we have studied the biology of the tumor in the first place.

MRI and bioluminescence imaging and preclinical trial drug development
In order to perform preclinical trials in mice, we need to identify tumors, quantify their size, and follow them over time non-invasively. One approach that we have used is by MRI scanning with T2 weighted images or with T1 weighted images with and without contrast as is done in people. However, MRI only measures anatomic structure and not biologic processes. Therefore, we have developed bioluminescence imaging strategies for use in preclinical trials of brain tumor-bearing mice. We initially developed a reporter mouse that expressed luciferase from the E2F1 promoter that measures proliferation and a Gli responsive promoter measuring SHH signaling. We are now developing genetic backgrounds that activate luciferase expression by cre recombinase activity that will allow us to “see” the tumor cells *in vivo* that have been deleted for PTEN, or that have knocked down INK4a/arf. This will allow us to easily identify mice with tumors and to count live tumor cells *in vivo* non-invasively.

The glioma tumor microenvironment
Gliomas are composed of not only tumor cells per se but also reactive astrocytes, microglia, endothelial cells and pericytes. Multiple lines of evidence indicate that many if not all of the cells that make up the stroma in these tumors contribute to the tumor biology and may be valid therapeutic targets.

Novel models of gliomas subtypes and ependymomas
We have also developed a modified version of the RCAS/tv-a system that achieves loss-of-function combined with lineage tracing using short hairpins and florescent tags. This system is able to mimic the mesenchymal GBMs by combining knockdown the combination of NF1 and p53 while lineage tracing each of these two events from specific cell types, with a penetrance of essentially 100%. We are using this model to understand the evolution of mesenchymal GBM from proneural ones and understand the complexity of these tumors. This type of lineage tracing allows us to appreciate the cellular heterogeneity in ways that germline strategies are unable to. We also have developed a new model of ependymoma by expressing a commonly occurring gene fusion (C11orf95/RELA) with this system.

PDGFR inhibition as a therapeutic strategy for PDGF-driven GBM
PDGF signaling characterizes the proneural subgroup of GBM and is sufficient to induced similar tumors in mice. One might think that inhibition of PDGFR would be a good therapeutic strategy for at least the proneural GBM subgroup. However, several trials of PDGFR inhibitors have been done in humans with GBM and none have been successful. A simple explanation is that the patients were not stratified to PDGFR active tumors prior to enrolling in these trials. However, there are several additional more interesting possibilities as to why this might be the case, and we are investigating under what circumstances PDGFR inhibition might be effective. One contributing factor is likely to be cellular heterogeneity of these tumors where subclones of cells within the tumor express PDGFR while others express EGFR in humans, and in mice similar results can be seen. A second contributing factor in the resistance to PDGFR inhibition is the fact that most of the gene expression changes that accompany the oncogenic transformation of olig2 expressing cells by PDGFR in vivo are not reversed by PDGFR inhibitors in vivo, even when that inhibition achieves a full cycle arrest. Additionally, mutant forms of PDGFR alpha found in some GBM appear to reduce effect of PDGFR inhibition. Finally, we have found that additional alterations found in human gliomas such as loss of Ink4a/arf, p53 or PTEN enhance oncogenic character of these tumors and prevent PDGFR inhibition of achieving full cell cycle arrest.

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RESEARCH FOCUS

The focus of our group is the study of the tumor microenvironment with an emphasis on the role of the neutrophil lineage. We are currently investigating the role of immune cells within the lung tumor microenvironment in a comprehensive fashion, beginning with how they have been recruited, and ending with a detailed understanding of the mechanism(s) by which a specific immune cell effector has impacted tumor growth.

We have recently developed a program to personalize immune modulatory therapies for non-small cell lung cancer (NSCLC) patients. Using a combination of mouse models of lung cancer and human NSCLC specimens, we have been able to determine the exact composition of the immune response to lung cancer. Our findings show that neutrophils are the most common immune cell population present in human NSCLC and inversely correlate with $CD8^+$ cellular content and access to malignant tumor. Ongoing studies in our lab have been designed to understand the mechanistic basis of neutrophil antagonism of lymphocyte responses and to investigate the efficacy of neutrophil depletion in conjunction with immune checkpoint inhibitor therapy.

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Our goal is to comprehensively delineate the fundamental role of mRNA translation in normal cell physiology, cancer etiology, and cancer progression. Armed with this knowledge we are defining the next generation of therapeutic vulnerabilities in disorders associated with translation deregulation such as prostate cancer and bladder cancer.

Dissecting the functional interface between transcription and translation in genitourinary malignancies. The process by which mRNA is translated into a protein is a highly energetic and meticulous process that is essential for life. However, protein synthesis can also be usurped by cancer to drive cellular transformation, uncontrolled proliferation, evasion of apoptosis, metastasis, and drug resistance (Hsieh et al. Cancer Cell 2010, Hsieh et al. Nature 2012, Hsieh et al. Science Signaling 2015). Work from our laboratory indicate that transcription factors and chromatin remodelers utilize the translation apparatus to shape the cellular proteome (Liu and Horn et al. Science Translational Medicine 2019). Interestingly, this relationship can be co-opted to drive specific cancer behavior at a molecular, cellular, and organismal level in prostate and bladder cancer. A major focus of the lab is to understand how the translation apparatus controls cancer initiation and progression in prostate and bladder cancer.

Key questions:

1) How does the translation apparatus interface with chromatin remodelers or transcription factors in the context of genitourinary malignancies?

2) What are the key downstream translational drivers necessary for cancer phenotypes?

3) How can we therapeutically disrupt oncogenic translation?

Understanding mechanisms of oncogenic mRNA specific translation. mRNA specific translation is the mechanism by which distinct mRNAs are preferentially translated to control cellular phenotypes. This can be mediated through the protein synthesis apparatus or changes in mRNA sequence and structure. Our laboratory has been fascinated by the untranslated regions (UTRs) of mRNAs, which are necessary for efficient protein synthesis and possess a plethora of somatic mutations in cancer. Surprisingly, their functionality remains poorly understood (Schuster and Hsieh Trends in Cancer 2019). Thus, we are deeply investigating how somatic alterations in the UTRs impact cancer phenotypes.

Key questions:

1) What are the underlying *cis*-regulatory mechanisms that enable oncogenic mRNA specific translation?

2) How do somatic mutations to transcribed but non-coded portions of the genome impact post-transcriptional gene regulation and cancer phenotypes?

3) How can we screen for the functionality of cancer-associated somatic mutation in UTRs?

Advance staged bladder cancer as a platform for biological and therapeutic discoveries. In 2015, our laboratory along with Drs. Ming Lam (UW Urology), Jonathan Wright (UW Urology), Bruce Montgomery (UW Oncology), and Funda Vakar-Lopez (UW Pathology) nucleated the first bladder cancer focused rapid autopsy program in the world. We have used the precious resource of late stage tumor specimens to interrogate the genomic underpinning of aggressive bladder cancer and to develop patient derive xenografts and primary cell-based models. Through this work, we have identified distinctions between upper tract urothelial carcinoma and lower tract urothelial carcinoma as well as the potential therapeutic implications of druggable genetic lesions in patients with metastatic bladder cancer (Winters et al. JCI Insight 2019).

Key questions:

1) What are the distinctions of transcriptionally defined bladder cancer subtype across tumors in a patient with metastatic bladder cancer and how do these distinctions impact outcomes?

2) How does the proteomic landscape of metastatic bladder cancer differ between unique organ sites?

Advancing our understanding of mRNA specific translation through collaboration: RNA modifications and translation control in normal cell physiology. Chemical modifications to RNA such as a methylation of adenines and isomerization of uridines have been shown to impact the process of mRNA translation. Work from our laboratory in collaboration with the Bellodi Lab (Lund University) and the Paddison Lab (Fred Hutch) have demonstrated a central role for these types of modifications in shaping the cellular proteome. Importantly, these processes are essential for the maintenance of normal stem cell physiology and the dynamic transitions that occur during erythrocyte differentiation (Guzzi et al. Cell 2018, Kuppers et al. bioRxiv 2018). In addition, through work with the Beronja Lab (Fred Hutch) we are unraveling the critical role of mRNA specific translation in cell fate choice.

Key questions:

1) How do m6A modifications enable the select translation of mRNA essential for the various stages of erythrocyte differentiation?

2) How are key cell fate regulators dynamically and translationally controlled in basal epithelial cells of the skin *in vivo*?

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CANCER TARGET DISCOVERY AND DEVELOPMENT

Our lab's research program has a long-standing interest in using mouse models to address the cellular, molecular, and genetic mechanisms of tumor progression. The functions of oncogenes and tumor suppressor genes are remarkably conserved between mice and humans and mouse models have and will continue to provide fundamental insights into the causes, prevention, and treatment of human cancer.

More recently we have pivoted direction towards more translation approaches, specifically discovering and developing novel drugs and drug targets for the treatment of cancer. Using arrayed well-based siRNA high throughput screens, we are identifying the complement of genes that are required for survival of cancer cells but not normal cells. By combining this functional genomic data with small molecule drug screens and genomic characterization within the same cells, we arrive at a set of prioritized targets and novel drug candidates. Because we can do this in patient derived tumor cells we can anchor results to patient treatment history thereby enhancing successful clinical translation. . To date, we have identified novel targets for head and neck cancer, pancreatic cancer, breast cancer and ovarian cancer. We are collaborating with clinician scientists, computational biologists and patient advocates at a number of cancer centers and precision medicine initiatives to broaden and deepen this cancer drug target search engine. We are confident that this grass roots and cross disciplinary approach will accelerate the discovery of safer, more effective cancer treatments in the near future.

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EPIGENETIC REPROGRAMMING OF PANCREATIC CANCER

The central theme of our research group is to study how the dysregulation of chromatin modifying enzymes contributes to pancreatic cancer pathogenesis and, further, whether these pathways present liabilities that could be exploited for cancer therapy.

Although chromatin-remodeling proteins are frequently dysregulated in human cancer, little is known about how they control tumorigenesis. This question is particularly relevant given that oncogenic transformation often involves epigenetic rewiring to meet the demands of uncontrolled proliferation, survival and metastasis. An imbalance in chromatin dynamics can lead to cancer by inactivating tumor suppressors, activating oncogenes, or by reactivating pathways that inhibit differentiation or favor stem cell self-renewal.

A challenge of the next decade will be to not only chronicle the altered expression and mutations of chromatin factors but to also define the phenotypic ramifications and the epigenetic abnormalities for each in cancer. Exploring chromatin factor dysregulation in cancer also provides a tractable system to address a more fundamental question of how tumor cells evolve when epigenetic barriers are altered, what characteristics are selected for to enhance tumor cell growth and the plasticity of these tumor cells in response to environmental perturbations.

A more in depth study of the chromatin factors that are lost or gained during tumorigenesis and how they remodel the epigenome are likely to form the basis for innovative approaches to cancer therapy and the development of novel biomarkers.

Currently, the laboratory is investigating the following questions:

1) What are the epigenetic barriers to the development and pathogenesis of pancreatic cancer?

2) What is the role of epigenetic dysregulation in defining transcriptional subtypes of pancreatic cancer?
3) How does the aberrant expression of developmental programs drive pancreatic cancer growth, progression and metastasis?

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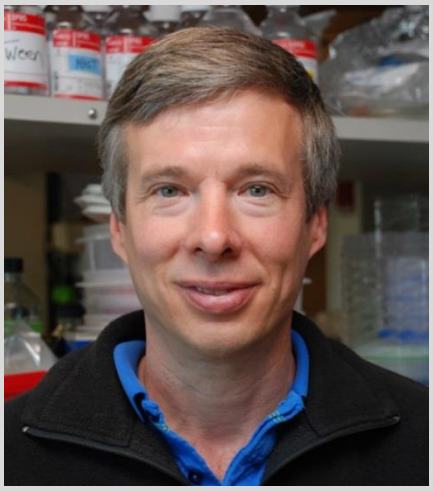

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GAP JUNCTIONAL REGULATION AND CANCER BIOMARKERS

The Lampe laboratory investigates the control of cell growth both at the cell biological/mechanistic level and through cancer biomarker discovery. We study the cell biology connecting gap junctions and intercellular communication with the control of cell growth, the cell cycle and, how the relationship is disrupted during wounding and carcinogenesis. Our interest in gap junctions as potential biomarkers of cancer and, more recently, the advent of new screening methodologies has expanded our efforts into broad proteomic screens for potential cancer biomarkers using high density antibody array technologies to discover proteomic, autoantibody and glycomic biomarkers of cancer. We study potential biomarkers for pancreas, colon, breast and lung cancer. Our colon cancer biomarkers are most advanced and are involved in industry sponsored studies hopefully heading towards regulatory approval. The lung cancer work has the best chance to have a large impact in the way existing screening programs currently function. Our ongoing cell/molecular research involves the regulation of gap junction assembly and function. Gap junctions allow for diffusion of small molecules (<1000 MW) between adjacent cells via matched cell-to-cell membrane channels. Vertebrate gap junctions are composed of proteins derived from the connexin gene family. We have shown that gap junction formation and degradation are highly regulated via protein kinases at various stages of the assembly process and the cell cycle. The most widely expressed gap junction protein connexin43(Cx43) is regulated via phosphorylation at over a dozen sites. Current studies include determination of the cellular localization of different connexin phosphorylation events and the specific serine substrates that are phosphorylated within connexins during cell division, development, skin wounding, cardiac stress and carcinogenesis. Thus, we link the activation of specific kinases to phosphorylation on a particular residue within the connexin protein and to connexin function in tissue including ovary, eye, skin and heart.

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The aim of my lab is to identify molecular drivers and biological properties of prostate cancer that may be exploited for the development of new and effective treatments. In our research we employ cutting-edge technologies including mouse and human prostate epithelial transformation systems; functional genomics; multi-omic data integration; high-throughput screening; small molecule drug discovery; and immuno-oncology to develop new approaches to stratify and treat prostate cancer.

The research in the lab is divided into three main areas:

1. Functional characterization of drivers of prostate and bladder cancer: Next-generation sequencing has enabled the large-scale profiling of aberrant genetic events associated with cancer. However, functional annotation of this rich information in relevant, genetically-defined cancer models is limited. To address this, we use a forward genetic approach with mouse and human epithelial organoid transformation systems. Benign epithelial cells are modified to stably express specific oncogenic factors, cultured briefly in the permissive environment of organoid cultures, and transplanted into immune-deficient mice. The resultant tumors are characterized to gain insight into the mechanisms by which the interaction of specific oncogenic events generate certain phenotypes of prostate and bladder cancer.

2. Immunotherapeutic targeting of prostate cancer differentiation-specific antigens: We have established a platform integrating RNA-seq and proteomics to nominate tumor-associated antigens enriched in subtypes of advanced prostate cancer with limited systemic expression in normal tissues. Candidate cell surface and intracellular antigens undergo multi-level validation including immunohistochemistry of microarrays of metastatic prostate cancers and benign human tissues. From the validated targets, we engineer and test both humoral and cellular immunotherapies in cell line- and patient-derived xenograft models of advanced prostate cancer.

3. Disrupting the protein stability of Myc and androgen receptor (AR) in advanced prostate cancer: Myc and AR are transcription factors with essential roles in the pathogenesis and maintenance of typical prostate cancer. We have developed Myc and AR reporter assays that facilitate the dynamic assessment of both subcellular protein localization and protein turnover. We are interrogating these assays with genome-wide CRISPR loss-of-function and gain-of-function screens to uncover the genetic/biologic pathways that modulate Myc and AR localization and stability. These reporters have also been used to complete high-throughput, high-content imaging screens with diverse chemical libraries to identify small molecule Myc and AR protein destabilizers. Lead identification is ongoing in collaboration with prominent medicinal chemistry and prostate cancer biology colleagues.

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USE OF IN VIVO APPROACHES TO STUDY CANCER INITIATION, PROGRESSION AND RESPONSE TO THERAPY

Our laboratory investigates the mechanisms through which cancer-mutated genes drive tumorigenesis. We focus on small cell lung carcinoma (SCLC), a highly aggressive neuroendocrine cancer. Typically, SCLC has metastasized by the time of diagnosis, and survival rates are dismal. We identified major driver genes mutated in human SCLC using next-generation sequencing approaches. To explore key activities of SCLC-mutated genes we use mouse genetics and functional studies. We have generated a panel of new mouse models of SCLC. These models, along with derived cell lines, are employed to understand how mutations in certain genes promote SCLC and to identify vulnerabilities conferred by these mutations. Genomic analyses and functional genomics, including genome scale CRISPR inactivation and cDNA overexpression screens are used in these efforts.

We have become particularly interested in understanding cancer-mutated genes that alter chromatin, as in general, there is a poor understanding of how such mutations drive cancer. We use mouse models to explore tumor suppressor roles for histone methyltransferases, acetyltransferases and other cancer-mutated chromatin regulating genes.

Our ultimate aim is to translate an increased understanding of the basic biology of SCLC driver genes to the development of novel therapies for a cancer type greatly in need of new therapies. Working with our clinical colleagues at the Seattle Cancer Care Alliance, we have generated a panel of patient derived xenograft (PDX) models of SCLC. Both PDX and genetically engineered mouse models are used for studies that try to link mutations in key SCLC driver genes to therapy response.

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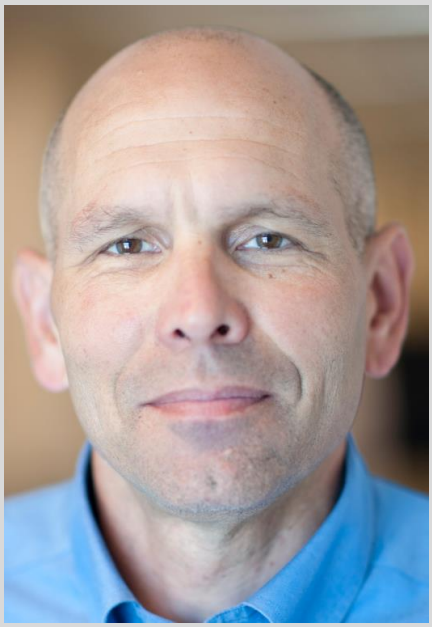

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**** very interested in taking a graduate student 2019-2020***

EXPLOITING CANCER GENOMICS AND THE TUMOR MICROENVIRONMENT TO GUIDE ONCOLOGY TREATMENT

The Nelson laboratory focuses on prostate cancer as a „test case" that dramatically illustrates the variation in cancer behavior between individuals and the need for prevention, better screening methods and the potential for personalized approaches to revolutionize oncology care. Prostate cancer is the most common malignancy in men with more than 160,000 new cases diagnosed yearly in the US. There is a clear genetic predisposition with higher rates of aggressive cancer in certain families. Environmental and dietary factors also contribute. Major technological advances in DNA sequencing now provide an opportunity to comprehensively detail every molecular change that occurs in a given tumor. This information has the potential to avoid "one-size-fits-all" treatments, eliminate ineffective therapy, and tailor interventions to individual tumor vulnerabilities.

Areas of current work include:

Developing New Therapeutic Strategies for Early and Late Stage Cancer
We aim to determine the molecular features that associate with response and resistance mechanisms to pathway-targeted agents and conventional chemotherapy. Several clinical (translational) trials are underway including studies incorporating neoadjuvant therapies and large-scale tumor genome sequencing. Tissue samples are acquired pre- and post-therapy and molecular correlates of direct drug effects are identified to define tumor and host signatures: (a) predictive of therapeutic response and (b) predictive of disease outcome (relapse). Mechanism-based assessments of specific oncogenic mutations serve to focus further drug development. We are exploring new minimally-invasive approaches to assess the molecular composition of

tumors using circulating tumor cells and cell-free tumor DNA biomarkers that allow for iterative sampling of tumor biology.

Characterization of the Cellular Androgen Receptor (AR) Program
A major focus of the lab has been the identification of down-stream "effector" genes that are responsible for cellular events (e.g. proliferation) after androgen receptor (AR) activation. The AR is the first known example of a „precision medicine" target that continues to be the major focus of treatment in advanced prostate cancer. We have identified a network of genes that are regulated by androgens in prostate cancer cells. Systematic studies involving the genetic and pharmacological modulation of these genes are designed to determine their cellular function with the aim of identifying those genes involved in proliferation, anti-apoptosis, differentiation, and treatment resistance. Genes with prostate-restricted expression serve as therapeutic targets for immunological and pharmacological strategies.

Determining the Role of the Tumor Microenvironment (TME) in Cancer Biology
The macro and microenvironments within which malignant neoplasms arise can exert profound influences on tumor behaviors that range from a complete reversion of the malignant phenotype to the promotion of tumor cell invasion and metastatic growth. In addition to tumor cells, the architecture of most solid tumors includes an assortment of non-malignant cell types derived from distinct developmental lineages that carry out structural or functional roles including fibroblasts, muscle cells, nerves, and vasculature. We have determined that components of tumor microenvironments (TME) also contribute to *de novo* and acquired treatment resistance. In current practice, the majority of cancer-directed therapeutics do not exclusively target malignant cells, but also injure benign cells in the local, and potentially the distant host microenvironments. Such collateral damage is quite evident for non-specific therapies that involve DNA-damaging modalities such as genotoxic drugs and ionizing radiation. Ongoing work centers on characterizing a DNA Damage Secretory Program in the TME that is comprised of a remarkable spectrum of proteases, growth factors and cytokines. The composite effects of this program promote tumor cell proliferation, metastasis, and also resistance to therapeutics.

Cancer Predisposition.
Prostate cancer is one of the most heritable malignancies: it is estimated that ~50% of prostate cancer risk is due to genetic factors. In addition to common polymorphisms that influence cancer predisposition, we have recently determined that rare highly-penetrant cancer predisposition genes are frequently mutated in men with aggressive/advanced prostate cancer. These predisposition genes link prostate cancer with other heritable cancers such as breast and ovarian cancer in the context of *BRCA1/2* mutations and colon cancer in the context of mismatch repair gene mutations. Importantly, mutations in these DNA repair genes identify families at risk for cancer and support precision oncology strategies that exploit responses to specific therapeutics.

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HIV-1 TRANSMISSION AND PATHOGENESIS

Dr. Overbaugh's laboratory has a long-standing interest in understanding the mechanisms of HIV transmission and pathogenesis and the role of innate and adaptive immunity in these outcomes. The lab seeks to understand what immune responses contribute to whether a productive infection is established and why certain viruses are selected in that process. In the case of antibody responses, much of the work is on defining the types of antibodies that are associated with protection in HIV exposed humans. The lab also studies the functional properties of these antibodies, including their ability to mediate killing of infected cells and neutralize virus. The lab has made the surprising finding that infants more rapidly develop neutralizing antibody responses to HIV than adults and the HIV-specific antibodies have less somatic hypermutation. Efforts are underway to identify the target of these responses and to define the evolutionary pathway that leads to their emergence of HIV antibody responses in infants. Similar studies are focused on individuals who are superinfected with a second strain of HIV and develop robust antibody responses.

The studies of innate immunity seek to define host cells factors that target the replication of circulating, transmitted variants of HIV. These studies include defining the interferon induced genes that respond to HIV as well as determining which of these factors restrict viral replication, particularly transmitted strains. Her laboratory has more recently begun exploring similar questions for Zika virus, focused on understanding if there are differences in the factors that restrict different strains of Zika.

Much of the HIV research in the lab is focused on populations in Africa because this is where the AIDS epidemic is most severe. The laboratory is part of a larger team, comprising researchers in both Seattle and Kenya (The Kenya Research Project), that is studying the molecular epidemiology of HIV transmission.

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**** very interested in taking a graduate student 2019-2020 for our epitranscriptomics program***

FUNCTIONAL GENOMICS OF STEM CELL AND CANCER CELL BIOLOGY

The Paddison Lab uses genomic screening technologies to probe human gene activity in mammalian stem and progenitor cells and cancer cells.

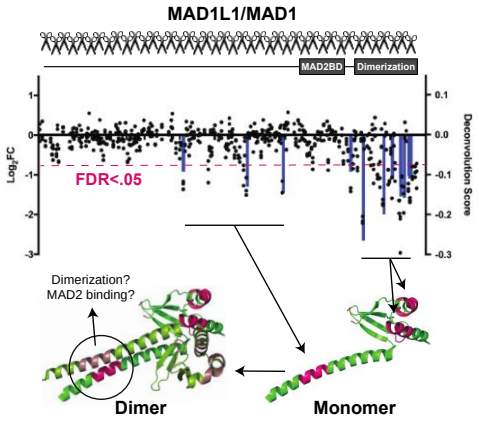

Fig 1. Identifying predicted and new essential protein domains using mutagenic sgRNA tiling libraries.

Functional Genomics

Functional genomics is the study of the function of genes contained within an organism's genome, or, put another way, an approach to figure out what roles genes have in an organism. In the last fifteen years, two powerful homology-based gene targeting technologies have come along that have revolutionized functional genomics in mammals. These are RNAi and CRISPR-Cas9. The Paddison Lab routinely uses both to power studies regarding the underlying biology of human stem and progenitors, by targeting each gene in the genome and determining their contribution to a phenotype of interest.

In addition, we have leveraged the mutagenic properties of CRISPR-Cas9 to test models of the human genome's protein coding structure. Although the human genome is currently predicted to contain 5494 conserved protein family (Pfam) domains (e.g., methyltransferase-like domain), most of these domains have not been validated while ~45% of protein coding regions lack Pfam domains altogether. However, by tiling sgRNAs across protein-coding genes we are able to identify essential

domains and motifs in phenotypically constrained regions. As a result, genes required for particular phenotypes (e.g., viability) can produce a unique mutational signature, with constrained regions scoring as phenotypic "peaks" (Fig. 1). We are currently applying this technique to >400 genes in the human genome spanning the epigenome, mitotic factors, and the spliceosome.

Biological Focus Areas

Precision oncology. The promise of "precision oncology" relies on decoding the molecular signatures of tumors to make predictions about effective therapies. The prevailing wisdom is that precision therapies will arise from identifying and targeting "drivers" of oncogenic transformation (e.g., mutated oncogenes). However, this approach has met with limited clinical success, particularly for some of the most devastating and difficult to treat cancers. Glioblastoma multiforme (GBM) is the most aggressive and common form of brain cancer in adults: approximately 90% of GBM patients die within two years of diagnosis with current standard of care therapy. We used GBM stem-like cells in combination with functional genomic screening to identify novel GBM therapeutic targets (**Fig. 2**).

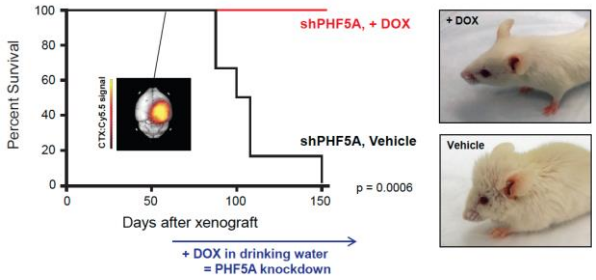

Fig 2. Inhibition of PHF5A causes human GBM tumor regression and survival in immune-compromised mice. PHF5A is important for 3' splice site recognition and was identified as a key GBM vulnerability driven by MYC activity.

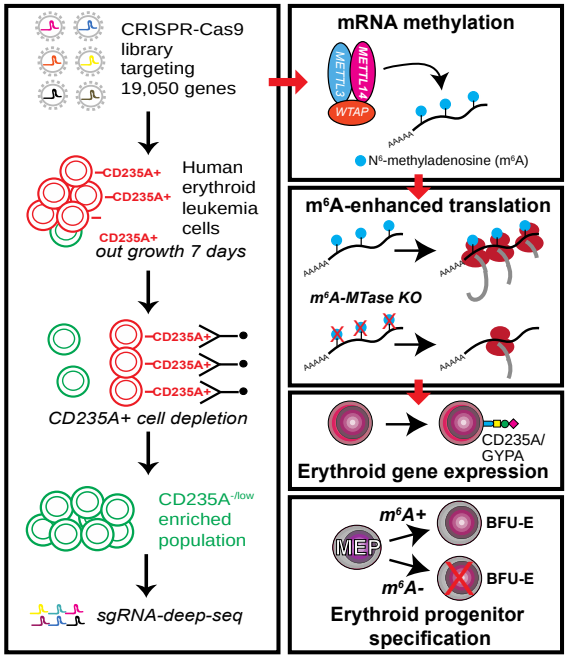

Fig. 3: Overview of screen for regulators of erythroid gene expression program and roles for m^{6}A mRNA marking during lineage specification.

Epitranscriptomics. Epitranscriptomics generally pertains to chemical modifications of mRNA occurring during or after gene transcription. N6-methyladenosine (m^{6}A) is among the most frequent post-transcriptional chemical modifications found in mammalian mRNA. In cell-based models, m^{6}A has been suggested to participate in numerous types of mRNA regulation (e.g., turnover, splicing, translation, or miRNA targeting). However, while m^{6}A-mRNA likely exists in most if not all eukaryotes, physiologically relevant roles for m^{6}A-mRNA have yet to be well established in mammals. Recently, in collaboration with Dr. Beverly Torok-Storb (Clinical Research Division), we performed a genome-wide CRISPR-Cas9 genome-wide screens to identify genes required for human erythroid (red blood cell) lineage specification. Among the novel hits were m^{6}A mRNA regulatory machinery, including core methyltransferase subunits *METTL14*, *METTL3*, and *WTAP*. Through a collaboration with Dr. Andrew Hsieh's group (Human Biology), we have now shown that m^{6}A mRNA marks promote the *translation* of a network genes required for human erythropoiesis, including factors that control epigenetic patterning in chromatin (**Fig. 3**) (Kuppers et al., 2019).

Regulation of Cell Identity and Cell Growth. Cancer cells may arise from maligned development programs, hijacking molecular pathways that are normally involved in developmental processes such as cell fate determination. The existence of cancer stem cells, which may play vital roles in tumor progression, maintenance, and recurrence, underscores this notion. One of the current projects in lab in this space is understanding how normal and cancer-causing progenitor cells enter and exit quiescent-like states. Through single cell RNA sequence analysis and functional genomic screens, we have identified classes of genes that block or promote entry into quiescent-like states. The results from these studies will better help define our notions of cell cycle regulation and maintenance of progenitor cell identity.

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INITIATION AND PROGRESION OF HUMAN CANCER

Work in my lab is focused on identifying and understanding the molecular events associated with the initiation and progression of human cancer. The clinical significance of these events in malignant and pre-malignant human breast tumors is of primary interest. Our involvement in collaborative research provides a unique opportunity for translational of basic science discoveries to questions that can be posed in large, clinical and population-based studies.

The current model of carcinogenesis is that of a multi-step accumulation of genetic changes within cells that supplant normal controls on cell division and lead to increased cell proliferation. Abnormalities of the cell cycle in a highly proliferative cell population may then lead to additional molecular alterations. In fact, derangements in the cell cycle may not only contribute to uncontrolled cell growth but may be causal factors in the development of cancer. In collaboration with epidemiologists and basic scientists, we were one of the first groups to identify the loss of cell cycle inhibitor $p27^{kip1}$ as an important indicator of poor prognosis in breast cancer. Follow up in a phase III Southwest Oncology Group (SWOG) clinical trial showed that, in women with steroid receptor positive tumors but not in women with steroid receptor negative tumors, loss of nuclear p27 protein expression is a marker of poor prognosis for women treated with adriamycin. Recent data evaluating mislocalization of p27 to the cytoplasm we found that cytoplasmic p27 contributed to lapatinib resistance in Her2+ breast cancer cells by suppressing apoptosis. Our results suggest that p27 localization may be useful as a predictive biomarker of therapeutic response in patients with Her2+ breast cancers.

Steroid receptor status is one of the main differentiating characteristics of breast cancer and lack of ER, PR and HER2 expression in tumor cells ('triple negative' breast cancer (TNBC)) is associated with a specific constellation of risk factors such as inherited BRCA1 mutations, high rates in African American women, aggressive tumor characteristics, poor outcome, and lack of response to hormonal treatment. In breast cancers of young AA and Caucasian women in a population-based case-control Atlanta study, we found high-frequency

gene copy number alterations (CNAs) in genomic locations that were subtype-specific, and of the highest frequency in TNBC compared to the other subtypes of breast tumors, and at specific genomic sites in tumors of AA women. We are using these data to guide the evaluation of gene copy number, methylation and LOH alterations of specific genes in the genomic regions identified.

Although others and we have successfully identified molecular markers of tumorigenesis using conventional approaches, single gene and protein changes are also unlikely to reflect the complexity of the molecular changes present in tumor cell populations. Using global approaches, we are now in a position to elucidate the molecular components, and the connections between the components, that coincide with the acquisition of malignant traits. We are conducting discovery projects in diverse populations including women exposed to radiation from the Chernobyl nuclear accident and Latina women from the U.S. and Latin America.

We were awarded funding from the Breast Cancer Research Foundation in 2017 to evaluate the effect of radiation on global tumor mutations, gene copy number changes, gene expression and DNA methylation, as well as inherited genetic contributions in radiation exposed women in the Chernobyl region of Russia.

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NEOPLASTIC EVOLUTION IN SPACE AND TIME
CONTROLLING COMPLEXITY AND CONTEXT

Personal Statement

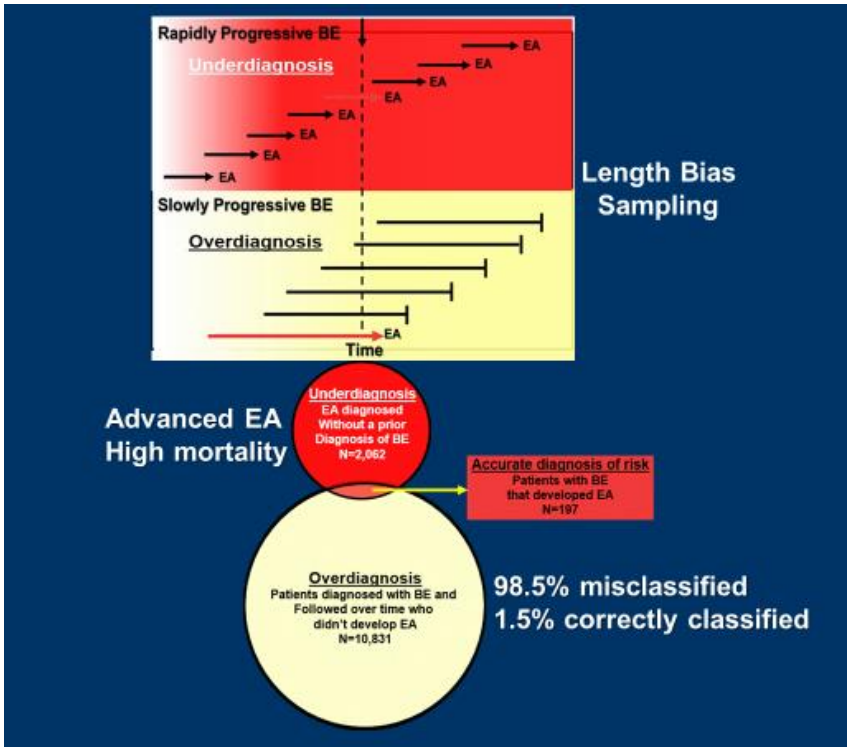
Our team has been funded by our Program Project Grant (P01 CA091955), a NCI Provocative Question Grant (R01 CA179949) and a NIH Challenge Grant (RC1 CA146973). Advances that we made with these NIH grants are described below.

Background. I was trained as a geneticist (PhD). Lee Hartwell and I discovered yeast cell cycle mutants when I was an undergraduate researcher in the University of Washington Department of Genetics. This discovery led to two groundbreaking papers that became the basis for Lee's well deserved Nobel Prize.

I became interested in cancer from the research of Drs. Stan Gartler and the late Phil Fialkow in the University of Washington Department of Genetics. They both taught me that cancer was a disease of somatic genome evolution. This concept was later articulated eloquently by the late Dr. Peter Nowell in his 1976 *Science* classic article. The insights of these pioneers led me to enter medical school to study cancer as an evolutionary process. The lessons of every class help guide me to a subspecialty (gastroenterology), in which endoscopy allowed access to visually detect and biopsy neoplastic lesions of the GI tract where genomic and evolutionary insights could be investigated to prevent or detect cancer early when it was curable.

Research focus. The focus of our Fred Hutch research team and our collaborators is Barrett's esophagus (BE) and its evolution to esophageal adenocarcinoma (EA). However, the vast majority of patients with BE do not

progress to EA and BE instead remain in a benign state that patients die with, not of. Conversely, EAs typically arise in individuals who were not known to have BE before presentation of an advanced, symptomatic EA. The outcomes of population screening for BE and EA have been well documented in Denmark by Hvid-Jensen et al (Figure 1) as well as many other investigators.



This conundrum has also been documented in many other "premalignant" conditions and cancers, including those of the breast and prostate, as well as many other organs. NIH sponsored a "Think Tank" on the topic. I was a member of this Think Tank, which resulted in two major publications:

(1) "*Overdiagnosis and overtreatment in cancer: an opportunity for improvement*." Esserman LJ, Thompson IM Jr, **Reid B** JAMA. 2013 Aug 28;310(8):797-8 and

(2) "*Addressing overdiagnosis and overtreatment in cancer: a prescription for change."* Esserman LJ, Thompson IM, **Reid B**, Nelson P, Ransohoff DF, Welch HG, Hwang S, Berry DA, Kinzler KW, Black WC, Bissell M, Parnes H, Srivastava S. Lancet Oncol. 2014 May;15(6):e234-42. doi: 10.1016/S1470-2045(13)70598-9.

Our research on BE is designed to elucidate and control the evolution of BE and EA over time and space in the esophagus. The goals include (1) accurately distinguishing aggressive BE that evolves rapidly to EA from benign BE that does not progress to EA over the lifetime of a patient and who will instead die of unrelated causes. Other goals include (2) elucidation of the evolutionary mechanisms that cause some BE to rapidly progress to EA while others remain indolent. These mechanisms may also be aberrant in other "CIN" cancers. Finally, (3) we seek to elucidate the evolutionary mechanisms that can render EA resistant to all known therapies, including immunotherapy.

Challenges for EA control.

The fundamental challenges of BE and EA include (1) *complexity* of the EA genome compared to the typical non-progressing BE genome, (2) the *speed* with which this genomic complexity evolves and (3) absence of a *known effective treatment for advanced EA*. The complexity of the cancer genome has recently become widely appreciated through advances in DNA sequencing and genomics but there have been no major advances in treatment of advanced EAs.

The clinical implications of the speed at which cancers evolve has been the subject of discussion for decades. Cancers that evolve rapidly will be harder to detect because rapid progression decreases the "window of opportunity" for early detection of EA (Figure 1). In this case, slowly or non-progressing BE will be easy to detect because they persist for sufficiently prolonged periods that the patient will die of competing causes of mortality before EA develops. This concept has been called "length bias sampling" (Figure 1). The outcomes in BE are poor with only a small proportion of patients who benefit from currently available approaches to prevention, early detection or therapy for EA (Figure 1). Length-bias sampling has also been implicated in screening for

many other types of cancer, but very few studies have compared the temporal evolutionary progression to cancer with a control population of patients who do not progress to cancer. Our team has done this in BE. Some of our results have been published during the current review period and others are currently being analyzed.

Although there was initial optimism that EA might respond to immunotherapy, subsequent studies have reported that EA and many other cancers with high rates of aneuploidy and chromosome instability are resistant to current immune therapies. Our goals are to provide research advances that will improve current outcomes for prevention, early detection and therapy of EA. We have either recently completed or are currently analyzing three large data sets. I have also participated in a number of Think Tanks and other venues to generate new concepts, ideas, initiatives and approaches to address these vexing challenges that characterize many cancers in addition to EA.

Below I review three major studies that our team has been engaged over the past 5 years. The first two studies have been completed and published. The third is currently in analysis.

Study 1 (Early Detection) was a case-cohort study design that used high density (1M) SNP arrays to track evolving somatic genomes in space and time in 248 patients with BE. As progressors approached the diagnosis of EA, they developed chromosome instability with gain or loss of whole chromosomes or large regions of chromosomes, whole genome doublings and massive chromosome instability. This genome instability increased diversity in the Barrett's epithelium that resulted in evolution to EA over a 2-4 year period (Figure 1). In contrast, non-progressors had only localized regions of genomic changes including 9p loss or copy neutral LOH, and damage in fragile sites, including frequent losses of CDKN2A, FHIT, and WWOX that remained remarkably stable over time (Figure 1) (NIH P01 CA091955, NIH Challenge Grant RC1 CA146973).

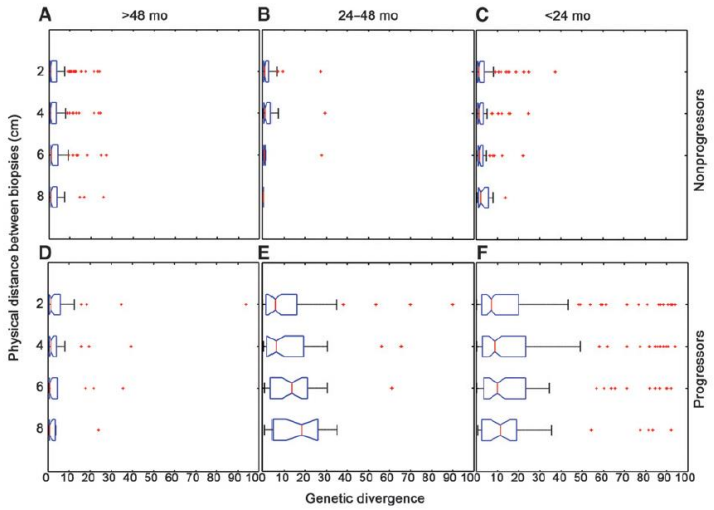


Figure 1. Genetic divergence in a case-cohort study of evolving BE genomes over space and time using 1M SNP arrays.. Non-progressors (N=169) maintain a consistently low level of large chromosome alterations. In contrast, progressors developed marked chromosome instability (CIN) including whole genome doublings (WGD) and genome-wide gains and losses of whole chromosomes, and large regions of chromosome beginning 24-48 weeks before the diagnosis of EA,

Study 2 (Prevention). Our recent study of chemoprevention with aspirin or other NSAIDs was funded in response to the NCI Provocative Question: "Given the evidence that some drugs commonly and chronically used for other indications, such as an anti-inflammatory drug, can protect against cancer incidence and mortality, can we determine the mechanism by which any of these drugs work?" (Funding: P01 CA091955, NIH Provocative Question Grant R01 CA179949).

We hypothesized that use of aspirin and other NSAIDs exerted their protective effects through an evolutionary mechanism: decreasing the mutation rate in the BE epithelium. We tested this hypothesis in a well-designed study of NSAID users vs. nonusers with input collaboration and joint authorship from epidemiology and biostatistics colleagues. This study found that differences in 96-trinuceotide somatic base substitutions between

NSAID users and nonusers were highly significant ($p<3x10^{-16}$). The study also found that two Cosmic Signatures (1 and 17) were dominant in BE and were sensitive to NSAID use and smoking status. We also found that NSAID use decreased copy number alterations, especially copy gains, which are more common in EAs than BE. Finally, a pathway analysis found that NSAID users had significantly lower diversity of functional mutations in genes across nine pathways compared to non-users (p=0.007). Interestingly, the two pathways with the greatest reduction in mutations are known to interact with the immune system. These results have been recently published (Galipeau et al *Genome Medicine Feb 27; 10(1): 17, 2018)* and published in the Science Spotlight (http://www.fredhutch.org/en/news/spotlight/2018/04/hb_galipeau_genomemedicine.html). (Funding: NIH P01 CA091955; NCI Provocative Question R01 CA179949)

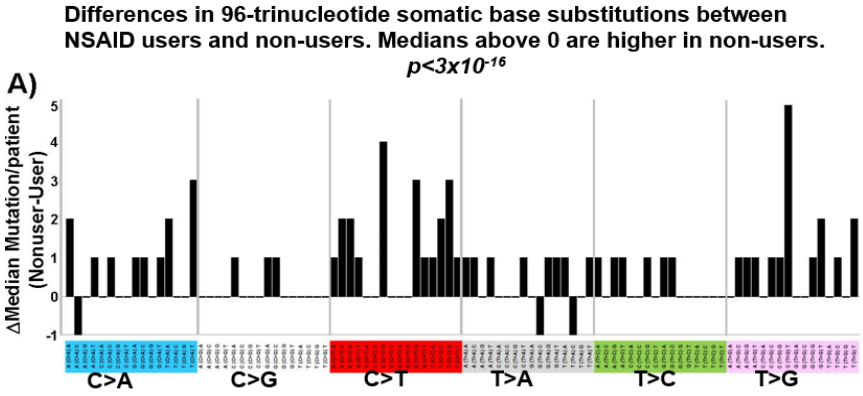
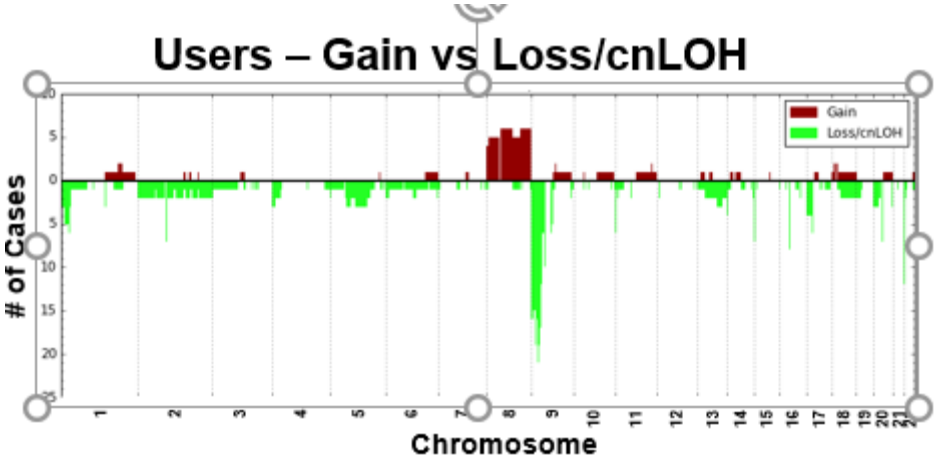


Figure 2A. NSAID use and somatic mutations. Differences in 96-trinucleotide somatic base substitutions between NSAID users and non-users. The difference in median mutation load at each 96 trinucleotide somatic base substitution is shown between NSAID non-users and users. Medians above zero are higher in NSAID non-users.



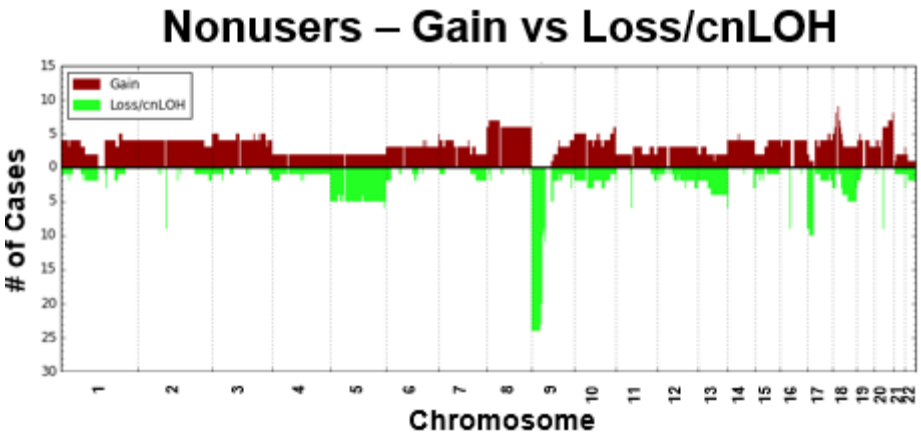


Figure 2B. NSAID use and copy number alterations, Red: Number of patients with allele specific copy gain, balanced gain, or high gain. Green: Any homozygous deletion, loss, or cnLOH. X axis: chromosome location. Y-axis: Number of samples with any event (41 user and 41 nonusers samples). Homozygous deletions are too small to be represented in this plot.

Study 3 (Early Detection and Therapy). This is a well-designed case-control study using Whole Genome Sequencing (WGS) to compare mutation rates in patients who p78rogressed from BE to EA compared to control patients with BE who did not progress. This study is currently in analysis. However, we have already discovered that patients that progress to EA continue to accumulate mutations in the BE segment while patients who do not progress to EA do not develop more mutations over time (Figure 3) (Funding: P01 CA091955).

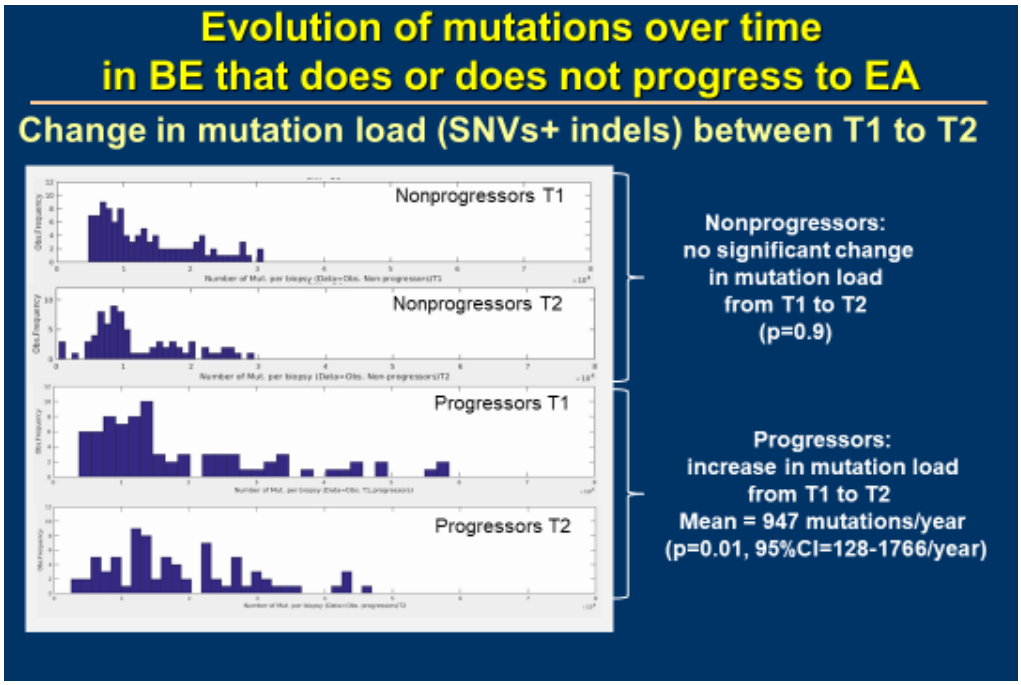


Figure 3. Whole Genome Sequencing in a case control study of 40 patients with Barrett's esophagus who progressed from BE to EA and 40 control patients with BE who did not progress to EA. The total mutation load (SNVs plus indels) remained stable in patients who did not progress to EA. However mutation load increased over time in patients who progressed to EA. The mean increase in mutation load was 947 mutations per year in progressors. This study is still being analyzed.

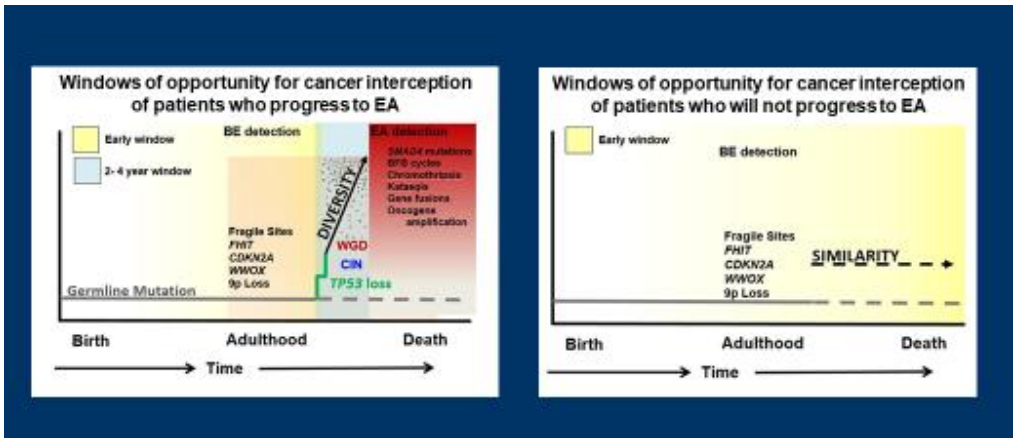


Figure 4. Summary: Progressors have about a 2-4 year window of opportunity for detection of TP53 mutation, CIN and WGD. In contrast non-progressors have similar early abnormalities but do not develop TP53, CIN and WGD.

Think Tanks, Leadership, Citizenship

Over the course of my career, I have been privileged to meet and work with investigators representing a broad range of disciplines to improve outcomes for patients who have or are at risk for developing cancer. These are all listed in my CV, but they include:

2004	NCI/NHGRI Workshop – "Exploring Cancer Through Genomic Sequence Comparisons"
2005-2007	NCI Translational Research Working Group – Co-chair Funding and Organization Committee
2008	Santa Fe Institute Working Group "Integrating Evolutionary Theory into Cancer Biology"
2008	Program Committee for NCI Translational Science Meeting
2008	NCI Think Tank: A New Look at Evolution and Evolutionary Theory in Cancer
2008	NCI Think Tank: The Coding, Decoding, Transfer, and Translation of Information in Cancer
2008	NCI/AACR Think Tank: Charting the Future of Cancer Prevention
2009-2013	NCI Intergovernmental Personnel Act Mobility Program with Division of Cancer Prevention
2010	NCI Think Tank: Rethinking the Role of Infectious Agents in Cancer
2011-2016	Esophageal Cancer Disease Working Group in the Cancer Genome Atlas (TCGA)
2011	National Cancer Institute, Gastric and Esophageal Cancer Meeting "Esophageal and Gastric Cancers, Solving Tough Problems"
2011	Center for Evolution and Cancer (Founding Member)

2011	International Evolution and Cancer Conference
2011- 2012	NCI DCP Think Tank "Defining the Molecularly-Informed Natural History of Occult Neoplasms Meeting," Organizing Committee
2014	Preventing Overdiagnosis, University of Oxford, UK
2014	MD Anderson Advisor: Epidemiology and Cancer Prevention Meeting
2014	EDRN Scientific Workshop, Panel Discussion Leader – Integrative Biology Session
2016	AACR Cancer Prevention Summit
2017	Publication of TCGA Esophageal Cancer Sequencing
2017	Special Session AACR National Meeting: Cancer Interception in Space and Time
2017	NCI Think Tank: Pre Cancer Atlas
2017	Introductory presentation, NCI Think Tank: PreCancerAtlas (PCA)
2017	Think Tank Panel Discussion: "Evolutionary Biomarkers: A Key Strategy for Precision Cancer Medicine"
2018	Think Tank: "Evolutionary Biomarkers II: Advancing the Field via Innovative Strategies and Novel Clinical Trials"

In summary, our team focuses on BE and EA as complex, adaptive systems that arise through genome instability, selection and evolution. We collaborate widely with other disciplines including epidemiologists, statisticians, math modelers, clinicians and others to improve the care of these patients.

SELECTED PUBLICATIONS (17 from a total of 193)

1. Hartwell, L.H., Culotti, J.C., Pringle, J.R. and Reid, B.J. Genetic control of the cell-division cycle in yeast: A model. Science 183:46-51, 1974. This was first genetic model of the eukaryotic cell cycle. This model became the basis for the 2001 Nobel Prize in Physiology or Medicine.

2. Galipeau, P.C., Cowan, D.S., Sanchez C.A., Barrett, M.T., Emond, M.J., Levine, D.S., Rabinovitch, P.S. and Reid, B.J. 17p (p53) allelic losses, 4N (G2/tetraploid) populations, and progression to aneuploidy in Barrett's esophagus. This was the first manuscript to report the spatial and temporal evolution of *TP53* loss, chromosome instability and genome doublings during progression to cancer. Proc Nat Acad Sci USA, 93: 7081-7084, 1996. This manuscript and our other research was prominently referenced in a recent publication reporting that the sequence of gains or losses of chromosome arms followed by genome doublings occurs commonly in human cancers (Carter et al Nature Biotechnology 2012; 30: 413). It is now recognized that nearly 40% of all human cancers studied by TCGA have undergone a whole genome doubling (Zach et al Nature Genetics 2013; 45: 1134).

3. Barrett, M.T., Sanchez, C.A., Prevo, L.J., Galipeau, P.C., Wong, D.J., Paulson, T.G., Rabinovitch, P.S. and Reid, B.J. Evolution of neoplastic cell lineages in Barrett's esophagus. Nature Genetics, 22: 106-109, 1999. This was the first model of genomic clonal evolution based on analyses over space and time in individuals at risk for cancer. It showed that clonal evolution was branching in contrast to linear models that have dominated clinical and cancer thought for more than a century. These branches underlie many aspects of cancer that we find frustrating clinically, including relapse after therapy, underdiagnosis and overdiagnosis. The method used in manuscripts 2 and 3 was used to construct phylogenetic trees based on somatic genomic alterations in advanced cancers (Gerlinger et al NEJM 2012; 366:883) and many others subsequently.

4. Reid, B.J., Levine, D.S., Longton, G., Blount, P.L. and Rabinovitch, P.S. Predictors of Progression to Cancer in Barrett's Esophagus: Baseline Histology and Flow Cytometry Identify Low and High Risk Patient Subsets. American Journal of Gastroenterology, 95:7 1669-76, 2000. To our knowledge, this was the first prospective cohort study to evaluate somatic genomic alterations (tetraploidy {whole genome doubling}, aneuploidy) as predictors of progression from a premalignant condition to cancer.

5. Maley, C.C., Galipeau, P.C., Li, X., Sanchez, C.A., Paulson, T.G., Blount P.L. and Reid, B.J. The combination of genetic instability and clonal expansion predicts progression to esophageal adenocarcinoma. Cancer Research 64: 7629-33, 2004. Prior to this manuscript, there was an intense debate in the literature as to whether genomic instability or clonal expansion was the key driver in progression to cancer. This manuscript found that both interacted to produce an increased risk of evolving to esophageal adenocarcinoma.

6. Vaughan, T.L., Dong, L.M., Blount, P.L., Ayub, K., Odze, R.D., Sanchez, C.A., Rabinovitch, P.S. and Reid, B.J. Non-steroidal anti-inflammatory drugs and risk of neoplastic progression in Barrett's oesophagus: a prospective study. Lancet Oncol 6: 945-52, 2005. This manuscript found that use of aspirin and other NSAIDs was associated with decreased risk of progression to somatic genomic abnormalities (tetraploidy and aneuploidy) as well as esophageal adenocarcinoma.

7. Maley, C.C., Galipeau, P.C., Finley, J.C., Wongsurawat, V.J., Li, X, Sanchez, C.A., Paulson, T.G., Blount, P.L., Rabinovitch, P.S. and Reid, B.J. Genetic clonal diversity predicts progression to esophageal adenocarcinoma, Nature Genet 38: 468-473, 2006. This paper reported that clonal genetic diversity, a measure derived from evolutionary biology, was an independent risk factor for progression from Barrett's esophagus to esophageal adenocarcinoma. This manuscript was subsequently cited in a study of breast cancer that highlighted the importance of genetic diversity as a cause of intratumor heterogeneity to more effectively plan cancer treatment strategies (Park et al J. Clin Invest 2010; 120:636). This concept that somatic genetic diversity is the fuel for cancer evolution is now well established for many other human cancers.

8. Galipeau, P.C., Li, X., Blount, P.L., Maley, C.C., Sanchez, C.A., Odze, R.D., Ayub, K, Rabinovitch, P.S., Vaughan, T.L. and Reid, B.J. NSAIDs Modulate CDKN2A, TP53, and DNA Content Risk for Progression to Esophageal Adenocarcinoma. PLoS Medicine 4: 342-54, 2007. This was a ten-year prospective cohort study in which we found that 9p LOH, 17p LOH and DNA content abnormalities, assessed by STR polymorphisms and DNA content flow cytometry, identified individuals with Barrett's esophagus at high and low risk for progression to esophageal adenocarcinoma. We also found that the 10 year risk of progression to cancer could be reduced from 80% to 30% by use of aspirin and other NSAIDs even in very high risk patients with advanced genomic abnormalities in their Barrett's esophagus.

9. Reid, B.J., Li, X. Galipeau, P.C. and Vaughan, T.L. "Barrett's Oesophagus and Oesophageal Adenocarcinoma: time for a new synthesis" Nat. Rev. Cancer 10: 87-101, 2010. PMCID: PMC2879265. In this manuscript, we reviewed the world literature on Barrett's esophagus and esophageal adenocarcinoma to identify the overarching problems and critical barriers to progress in prevention and early detection of this highly lethal cancer. We found that current clinical and research paradigms lead to overdiagnosis of slowly or non-progressing Barrett's esophagus and underdiagnosis of life threatening early esophageal adenocarcinoma. We then proposed a global research strategy to develop risk models to reduce both over- and underdiagnosis. The research and risk models for the general population and primary care settings have been addressed using the international BEACON consortium of which Dr. Reid is a founding member.

10. Esserman, L.J., Thompson, I.M. and Reid, B.J. Addressing the Emergence of Overdiagnosis and Overtreatment in Cancer: An Opportunity for Improvement and Change. Journal of the American Medical Association 310: 797-798, 2013. PMID 23896967. Altmetric, which tracks blogs, social media, government policy documents, newspapers, magazines, and downloads, ranked this manuscript as 31st of the top 100 scholarly articles that received the most public attention in 2013. This article was also featured on the front page of the New York Times (July 30, 2013).

11. Li, X., Galipeau, P.C., Paulson, T.G., Sanchez, C.A., Arnaudo, J., Liu, K., Sather, C.L., Kostadinov, R.L., Odze, R.D., Kuhner, M.K., Maley, C.C., Self, S.G., Vaughan, T.L., Blount, P.L., and Reid B.J. Temporal and spatial evolution of somatic chromosomal alterations: A case-cohort study of Barrett's

esophagus. Cancer Prev Res 7(1): 114-127, 2014. PMCID: PMC 3904552. This was the first manuscript to evaluate the evolving neoplastic genome over both space and time in patients who do and do not progress to cancer. The article became the basis for an article by Yael Waknine in MEDSCAPE (December 23, 2013). Barrett's that did not progress to cancer typically had only small, localized changes that remained stable without generating diversity over long periods of follow-up. In contrast, Barrett's esophagus that progressed to cancer was characterized by ***punctuated*** gains or losses of whole chromosomes or chromosome arms beginning about four years prior to the diagnosis of cancer followed by ***catastrophic genome doublings*** beginning about two years prior to cancer diagnosis. Recent studies have inferred a similar sequence of events in many other cancers based on data at one point in space and time (See for example, Carter et al Nature Biotechnology 30: 413, 2012). These recent papers acknowledge the seminal contribution of Galipeau et al Manuscript 2 above, which showed that 17p LOH could be detected in diploid biopsies and was highly associated with genome doubling events that were rapidly followed by progression to aneuploidy.

12. Li, X., Paulson, T.G., Galipeau, P.G., Sanchez, C.A. Liu, K., Kuhner, M.K., Maley, C.C., Self, S.G., Vaughan, T.L., Reid, B.J., and Blount, P.L. Assessment of Esophageal Adenocarcinoma Risk Using Somatic Chromosomal Alterations in Longitudinal Samples in Barrett's Esophagus. Cancer Prev Res 2015 September 8:845-856. PMCID:PMC 4560605. This manuscript reported that a risk model based on chromosome instability and evolution from Barrett's esophagus to esophageal adenocarcinoma as reported in the above manuscript (11 in "Selected publications") outperformed histopathologic assessment of dysplasia and flow cytometric DNA content in predicting future progression from Barrett's esophagus to esophageal adenocarcinoma.

13. Reid, B.J., Paulson, T.G., and Li, X. Genetic Insights in Barrett's Esophagus Adenocarcinoma. 2015 Gastroenterology 2015 Oct;149(5):1142-1152. PMCID:PMC 4589483.

14. Reid, B.J, Culotti, J.G., Nash, R.S., Pringle, J.R. Forty-five years of cell-cycle genetics. Mol Biol Cell. 2015 Dec 1;26(24):4307-12. PMCID:PMC 4666127. This invited retrospective reviewed the history of the events that led to Lee Hartwell's 2001 Nobel Prize in physiology or medicine. It provided a vehicle to report the contributions of a large number of scientists that enriched our research on the yeast cell cycle and made an excellent eukaryotic model system.

15. Kuhner MK, Kostadinov R, Reid BJ. Limitations of the Driver/Passenger Model in Cancer Prevention. Cancer Prev Res (Phila). 2016 May;9(5):335-8. doi: 10.1158/1940-6207.CAPR-15-0343. PubMed PMID: 26932841; PubMed Central PMCID: PMC4856031. This manuscript challenged the concept that mutations could be categorized into two classes (passengers/drivers) and proposed a 4 tier model.

16. Integrated genomic characterization of oesophageal carcinoma. Cancer Genome Atlas Research Network. Nature. 2017 541(7636): 169-175. doi: 10.1038/nature20805. PubMed PMID: 28052061.

17. Galipeau, P.C., Oman, K.M., Paulson, T.G., Sanchez, C.A., Zhang, Q., Marty, J.A., Delrow, J.J., Kuhner, M.K., Vaughan, T.L., Reid, B.J., and Li, X. NSAID use and somatic exomic mutations in Barrett's Esophagus. Genome Medicine Feb 27; 10(1):17, 2018.

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NOVEL TARGETED THERAPIES IN LIVER CANCER

Liver cancer is one of the fastest growing cancers in the United States and world-wide both in incidence and mortality. Intrahepatic cholangiocarcinoma (ICC) is the second most common subtype of liver cancer and has almost tripled in incidence in the last 40 years (Saha, et al, 2016). As the incidence of ICC continues to rise, it is imperative that we improve our understanding of the pathogenesis underlying this disease as well as our therapeutic options in this highly lethal malignancy. We have developed, to our knowledge, the most comprehensive set of model systems to study ICC in the world, which includes a rich panel of human ICC cell lines, patient-derived xenografts as well as genetically engineered mouse models (GEMMs). Our laboratory utilizes this unique resource to study pathogenic mechanisms of this disease and to identify therapeutic approaches with the goal of translating our work into novel clinical trials. Specifically, our laboratory has three major areas of focus:

1. ICC Pathogenesis: In our recent work establishing novel GEMMs and in vitro stem cell systems, we identified a molecular program by which mutant IDH blocks differentiation of liver stem cells through the suppression of the master transcription factor HNF4α to promote ICC (Saha, et al. Nature, 2014). We are developing additional GEMM systems to determine how expression of different ICC oncogenes and tumor suppressor mutations in specific cell types within the liver can impact regenerative responses to injury within the liver, and how these mutations may cooperate to drive tumorigenesis.

2. Genomics of drug sensitivity in ICC: The identification of readily targetable driver mutations in ICC, including isocitrate dehydrogenase (IDH) mutations and fibroblast growth factor receptor 2 (FGFR2) fusions, promises to change the clinical paradigm for ICC to incorporate reflex genetic profiling of all ICC patients over the next decade. While novel targeted agents against these presumptive driver mutations are currently in early clinical development, we are using our genetically-faithful preclinical model systems (ICC GEMMs, PDXs and novel patient-derived cell lines) to fully characterize the nature of the response to these targeted therapies in order to understand how best to deploy them in the clinic.

Beyond direct inhibition of mutant IDH or FGFR2, we hypothesize that the widespread changes in cell differentiation state, metabolism and epigenetic control triggered by these driver mutations may confer additional vulnerabilities that can be targeted. Therefore, we have subjected our ICC cell line models to high throughput drug screens to identify synthetic lethal interactions in specific genetic subsets of this disease. As an example, this work has already led to the discovery of a specific dependency of IDH mutant ICC on SRC kinase activity and a hypersensitivity of these cells to SRC family kinase inhibitors (Saha, et al., *Cancer Discovery* 2016). Our research will further explore the mechanisms underlying these synthetic lethal interactions and work to translate our findings into novel clinical trials for these patients.

3. Predicting resistance mechanisms to targeted therapy in ICC: Although we have seen dramatic responses to targeted therapies in molecular subsets of ICC, prior experience indicates that acquired resistance is likely to eventually arise in response to any targeted therapy. Again taking advantage of our rich collection of model systems, we are using a systematic approach to predict and overcome these resistance mechanisms and will validate our findings using tissue samples obtained from patients undergoing targeted therapy clinical trials in parallel.

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Goyal L*, Saha SK*, Liu LY, Siravegna G, Leshchiner I, Ahronian LG, Lennerz JK, Vu P, Desphande V, Kambadakone A, Mussolin B, Reyes S, Henderson L, Sun JE, Van Seventer EE, Gurski JM, Baltschukat S, Schacher-Engstler B, Barys L, Stamm C, Furet P, Ryan DP, Stone JR, Iafrate AJ, Getz G, Porta DG, Tiedt R, Bardelli A, Juric D, Corcoran RB, Bardeesy N, Zhu AX. Polyclonal secondary FGFR2 mutations drive acquired resistance to FGFR inhibition in FGFR2 fusion-positive cholangiocarcinoma patients. Cancer Discovery 2017 Mar;7(3):252-263. doi: 10.1158/2159-8290.CD-16-1000. Epub 2016 Dec 29. *These authors contributed equally.

Saha SK, Gordan JD, Kleinstiver BP, Vu P, Najem MS, Yeo J, Shi L, Kato Y, Levin RS, Webber JT, Damon LJ, Egan RK, Greninger P, McDermott U, Garnett MJ, Jenkins RJ, Rieger-Christ KM, Sullivan TB, Hezel AF, Liss AS, Mizukami Y, Goyal L, Ferrone CR, Zhu AX, Joung JK, Shokat KM, Benes CH, Bardeesy N. (2016). Isocitrate dehydrogenase mutations confer dasatinib hypersensitivity and SRC-dependence in intrahepatic cholangiocarcinoma. Cancer Discovery 2016 Jul;6(7):727-39. doi: 10.1158/2159-8290.CD-15-1442. Epub 2016 May 26.

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Saha SK*, Parachoniak CA*, Ghanta KS, Fitamant JF, Ross KN, Najem MS, Gurumurthy S, Akbay EA, Sia D, Cornella H, Miltiadous O, Walesky C, Desphande V, Zhu AX, Hezel AF, Yen K, Straley K, Travins J, Popovici-Muller J, Gliser C, Ferrone CR, Apte U, Llovet JM, Wong KK, Ramaswamy S, Bardeesy N. (**2014**). Mutant IDH inhibits HNF4a to block hepatocyte differentiation and promote biliary cancer. *Nature,* 513:110-4. *These authors contributed equally.

Borger DR, Goyal L, Yau T, Poon RT, Ancukiewicz M, Deshpande V, Christiani DC, Liebman HM, Yang H, Kim H, Yen K, Faris JE, Iafrate AJ, Kwak EL, Clark JW, Allen JN, Blaszkowsky LS, Murphy JE, **Saha SK**, Hong TS, Wo JY, Ferrone CR, Tanabe KK, Bardeesy N, Straley KS, Agresta S, Schenkein DP, Ellisen LW, Ryan DP, Zhu AX. (**2014**). Circulating oncometabolite 2-hydroxyglutarate is a potential surrogate biomarker in patients with isocitrate dehydrogenase-mutant intrahepatic cholangiocarcinoma. *Clin Cancer Res,* 20:1884-90.

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HOST-PATHOGEN INTERACTIONS AND GENETIC DIVERSITY OF HUMAN PATHOGEN, *HELICOBACTER PYLORI*

In the mid 1990's, a bacterium, *Helicobacter pylori*, was linked to gastric cancer, the third leading cancer killer worldwide. *H. pylori* establishes lifelong infection in the stomach of half the human population world-wide. The consequence of this infection ranges from undetected gastritis, to ulcer disease, and gastric cancer. Our lab is interested in the mechanisms by which this bacterium can establish and maintain a chronic infection and the molecular cross talk between the host and the bacteria during the decades-long infection that can lead to disease. Our current projects include:

1. *H. pylori* genomic diversity: *H. pylori* clinical isolates show extensive heterogeneity both in sequence and the presence and absence of whole genes. Even in the context of a single human stomach there exist multiple clones with unique gene complements. We are currently investigating how this diversity is generated and the consequences of this diversity on patient outcome. This includes new efforts to track genetic changes that accumulate during chronic infection of humans and the phenotypic consequences of theses genetic changes.

2. Cell wall modification and cell shape: Shape mutants (straight or slightly curved rods instead of helical rods) have stomach colonization defects in our mouse infection model. Most of these cell shape factors alter the peptide content of the peptidoglycan cell wall. We are testing motility in viscous solutions, susceptibility to various stresses and peptidoglycan-mediated innate immune signaling to tease out how these proteins and cell morphology contribute to survival in the stomach. To understand how changes in

cell wall peptides drive shape changes at size scale of the cell, we are using super-resolution microscopy of cell shape proteins and cell wall synthesis probes combined with mathematical modeling.

Host tissue responses to chronic infection: We have developed a number of mutant libraries including random transposon mutant libraries and a sequenced defined mutant library encompassing most non-essential genes. We are using these libraries in a variety of in vitro and in vivo systems to probe *H. pylori* phenotypes important for pathogenesis. We use gastric epithelial tissue culture cells and primary gastric tissue organoids to monitor wild-type and mutant bacteria binding to host cells and stimulation of host cell signaling pathways including those activating innate immunity and host cell shape changes. To understand bacterial-host interactions in the complex environment of the stomach, which includes many cell types, we employ a mouse model of infection with wild-type and genetically modified mice that perturb immune pathways or stomach differentiation. This allows us to look at the relative fitness of different mutants, their location and their ability to induce host inflammation and pathology associated with gastric cancer

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CHEMICAL BIOLOGY, DRUG DISCOVERY AND TRAGET VALIDATION

The overarching goal of research in the Simon laboratory is the development of small molecules as mechanistic probes for a variety of cellular processes and as potential lead compounds drug development. To this end we use interdisciplinary approaches ranging from chemical synthesis and medicinal chemistry to genetics and cell biology. The compounds we are studying have been identified from large collections of synthetic, drug-like compounds and from natural sources. A majority of drug screens are phenotypic and unbiased in terms of specific targets. While screening compound libraries is a significant part of what we do the majority of our efforts go into target identification, mechanistic studies to understand the biology and pharmacology of lead compounds and efforts to improve their activity through chemical synthesis of analogs.

The clinical use of several effective drugs is limited because of drug-induced hearing loss. Aminoglycoside antibiotics (e.g. amikacin) and platinum-based cancer drugs (e.g. cisplatin) are among drugs that can cause significant and in many cases irreversible hearing loss due to selective toxicity to the auditory hair cells. The mechanism of hair cell death, also called ototoxicity, is poorly understood. In collaboration with Ed Rubel's laboratory at the Bloedel Center for Hearing Research and Department of Otolaryngology and Dave Raible's laboratory in the Department of Biological Structure both at the University of Washington, we carried out a screen using zebrafish mechanosensory hair cells as a model for mammalian auditory hair cells. This screen identified a family of small molecule inhibitors of aminoglycoside-induced hair cell death. Dr. Rubel's laboratory showed that our lead compound, PROTO1, also protects rat auditory hair cells and preserves hearing following doses of kanamycin that induce significant hearing loss in control animals. We have optimized the protective activity as well as pharmacological properties of PROTO compounds through medicinal chemistry and structure activity relationship (SAR) studies. An optimized analogue of PROTO-1 called ORC-13661 was recently

granted Investigational New Drug (IND) status by the Food and Drug Administration (FDA). Phase I safety trials started in early 2018.

We previously identified inhibitors of yeast (*S. cerevisiae*) and human NAD-dependent deacetylases using yeast cell-based phenotypic screens. In humans, there are seven NAD-dependent deacetylases called the sirtuins. These ubiquitous enzymes have been shown to play roles in functions ranging from transcriptional modulation to DNA damage responses and modulators of specific sirtuins have been suggested as therapeutic agents for a variety of human disease. In collaboration with Toni Bedalov's laboratory (FHCRC), we are working to optimize our sirtuin-2 inhibitors using medicinal chemistry strategies for use as anti-lymphoma therapeutics. Sirtuin 2, or SIRT2, plays a unique role in the biology of B-cell development. Immature B-cell precursors have to undergo genetic rearrangements to mount an antigen-specific response to pathogens. The genetic rearrangements, such as V(D)J recombination and somatic hypermutation, would normally be perceived as DNA damage and lead to apoptosis were it not for the suppression of the DNA damage response regulator p53 and upregulation of the transcriptional repressor BCL6. SIRT2-mediated deacetylation of p53 and BCL6 accomplishes these functions. In B-cell lymphoma, mutation of histone acetyl transferases (HATs) and consequent hypo-acetylation of p53 and BCL6 accomplishes the same ends allowing DNA damage to go undetected. Inhibition of SIRT2 is B-cell lymphoma harboring HAT mutations restores p53 and BCL6 acetylation homeostasis and leads to cell death. We hope small molecule SIRT2 inhibitors will be effective B-cell lymphoma therapeutics.

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Understanding the Metabolic Constraints of Cell Proliferation

Cancer cells have altered cell metabolism compared to the parental cells from which they arise. To maintain aberrant proliferation, cancer cells enact changes in metabolic fluxes to support the increased synthesis of proteins, nucleotides and lipids needed to replicate cell biomass and divide. Thus, exploiting the metabolic differences between normal cells and cancer cells is a promising approach to improve cancer therapy. Indeed, many chemotherapeutic agents are cytotoxic due to their ability to interfere with metabolic pathways in cancer cells. The fact that these treatments can be curative provides strong evidence that cancer cells have targetable metabolic vulnerabilities, making it critical to better understand how cell metabolism supports proliferation and to determine which metabolic pathways are required for cancer growth. My laboratory uses mass spectrometry, isotopic tracing, metabolic flux measurements, and cancer models to broadly understand how metabolism supports cell proliferation.

The current goals of my laboratory range from testing metabolic targets in preclinical cancer models to discovery of novel metabolic interactions and pathways:

1. Investigating mechanisms of aspartate metabolism for cancer therapy

The amino acid aspartate is a critical substrate for protein and nucleotide synthesis that must be synthesized intracellularly to support cancer cell proliferation. Aspartate production is metabolically costly, and my work has shown that bolstering aspartate levels in cancer cells in vivo can increase tumor growth rate. These data indicate aspartate is an endogenous metabolic limitation for tumor growth and that any further suppression of aspartate would therefore inhibit cancer growth. Since aspartate levels are dependent on various synthesis pathways and metabolic fates in different conditions, all of which can modified by changes in gene expression and signaling, we will seek to understand how different cancer relevant biological processes including

signaling cascades, metabolic adaptations, and drug sensitivities converge on aspartate metabolism to promote tumor growth and sensitize tumors to therapies.

2. Determining the roles of coenzyme homeostasis to support proliferative metabolism

While metabolism is classically viewed through the lens of efficient ATP production, maximization of biomass synthesis in proliferation requires balancing several other coenzyme systems in addition to ATP. My work has shown that maintenance of NAD+/NADH by mitochondrial respiration is essential for supporting proliferation, however, there are many other coenzyme systems in cells, e.g. NADP+/NADPH, Biopterins, ubiquinone/ubiquinol, etc., that are interrelated and are also used to support biosynthesis. Further complication arises when considering that these coenzymes are also compartmentalized into organelles which have distinct roles in using these coenzymes to support local and global metabolism. Thus, we will determine how modifying coenzyme homeostasis causes network effects throughout metabolism and how these consequences can affect cell proliferation.

3. Discovery of metabolic products and pathways

The canonical map of metabolic reactions is often perceived as a complete list of all reactions that occur in all human cells. However, there is no reason to assume that this list is comprehensive in all tissue types and conditions. Recent work by me and others has identified previously uncharacterized metabolites in a subset of cancer cells, providing a proof of concept that there may be many metabolites yet to be discovered. Using state-of-the-art mass spectrometry and a novel isotopic tracing technique we will seek to identify new metabolic products, and potentially entire new metabolic pathways, with the hope of identifying biomarkers and metabolic modifiers of disease.

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TRANSCRIPTIONAL REGULATION OF MYOGENESIS AND NEUROGENESIS IN NORMAL DEVELOPMENT AND DISEASE

Cell specification, differentiation, and trans-differentiation:
The conversion of a non-muscle cell into a skeletal muscle cell by the expression of the transcription factor MyoD was the first demonstration of genetically engineered trans-differentiation. We have been using this as a model system to study how a single initiating event, in this case the expression of the MyoD transcription factor, can orchestrate the chromatin and transcriptional changes necessary to switch cell specification, and how this process might be subverted in rhabdomyosarcomas, cancers that express MyoD but do not differentiate into muscle cells. Similar to myogenesis, neurogenesis is regulated by the related NeuroD transcription factors. We have been able to demonstrate that non-neuronal cells can be converted into neurons by the forced expression of NeuroD family members, and we are comparing MyoD and NeuroD factors to determine how they achieve distinct transcriptional programs despite having very similar DNA binding regions. These studies are beginning to show how master regulatory factors drive programs of cell differentiation.

DUX4 regulation of totipotency in development, muscular dystrophy, and cancer:
We have identified the double-homeodomain transcription factor DUX4 as a gene that drives expression of the totipotent signature in the early cleavage stage embryo at the time of the initial wave of zygotic gene activation. Mis-expression of DUX4 in skeletal muscle causes facioscapulohumeral dystrophy (FSHD), a common form of muscular dystrophy. DUX4 mis-expression in muscle is caused by the inefficient epigenetic repression of the DUX4-containing D4Z4 macrosatellite repeat on chromosome 4, either because of deletions that decrease the number of the macrosatellite units in the array or mutations in SMCHD1, a cohesion-family member protein that epigenetically represses repetitive regions, including the D4Z4 repeat array. Recently, we have also identified DUX4 expression in many solid cancers where it promotes immune evasion by down-regulating MHC Class I protein expression, possibly related to a

normal role in the immune evasion of the early embryo. Future work seeks to further understand the role of DUX4 in normal development, FSHD muscular dystrophy, and cancer; as well as seeking mechanisms of suppressing DUX4 expression for therapeutic interventions.

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CELL POLARITY AND CELL ADHESION IN MAMMALIAN DEVELOPMENT AND CANCER

Individual cells in all multicellular organisms need to communicate with each other to coordinate their behavior to ensure the survival of the entire organism. Cell-cell adhesion is one of fundamental cellular functions pivotal for the formation of metazoan organisms. It is necessary for integration of individual cells into the tissues and organs. Cell adhesion mechanisms are regulated by intrinsic cell polarity pathways which govern the position of cell-cell and cell-substratum adhesion structures. In turn, cell adhesion structures can physically separate different plasma membrane domains and support and reinforce cell polarity. Together the cell adhesion and cell polarity systems regulate each other to assemble individual cells into intricate 3-dimentional structures necessary for proper development and homeostasis of multicellular organisms. We are interested in cell polarity and cell adhesion mechanisms because many of these cellular functions are perturbed or disrupted in human cancer. This makes cancer cells unable to properly read their cellular neighbors and overall microenvironment and adjust and control their behavior. Thus, the general aim of our research is to understand the mechanisms responsible for orchestrating cellular behavior that help individual cells to work together to maintain normal tissue homeostasis and prevent cancer. Our laboratory is pursuing research in two major directions:

1. We study cell polarity and cell adhesion mechanisms and their role in normal mammalian development and cancer.
2. We are working on the identification of molecular mechanisms and the development of novel therapeutic approaches in human prostate cancer.

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