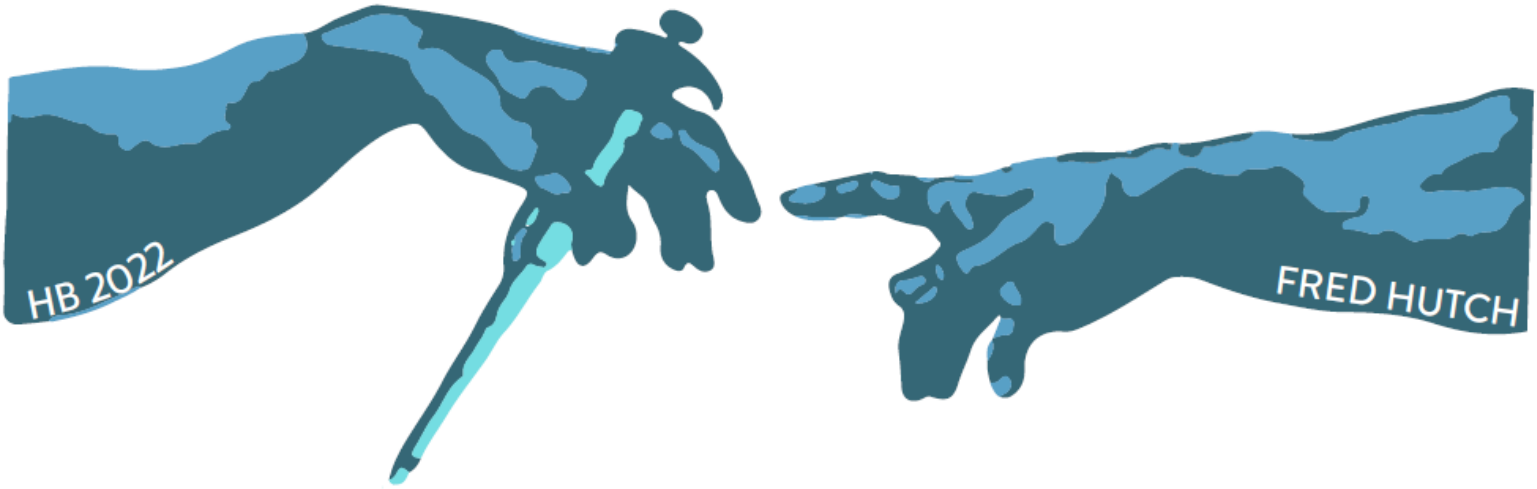
HUMAN BIOLOGY
2022-2023



About the cover: Artwork designed by Rachel Lex, Beronja Lab

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VIRAL MANIPULATION OF CHROMATIN

Our laboratory is focused on the mechanisms by which viruses hijack chromatin. Due to the major advancement in sequencing and imaging 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 blocking the host cell cycle and dampening downstream inflammation (Lynch *et al. Current Biology* 2021, 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 and composition of cellular chromatin. In conjunction with chromatin fractionation proteomics, we identified chromatin factor HMGB1

as well as linker histone H1 as key players in the chromatin distortion caused by protein VII. Projects in the lab use cell culture, microscopy, biochemistry and genomic techniques to assess these phenotypes and define the role of host chromatin factors in viral pathogenesis.

2. How does herpes simplex virus (HSV-1) exploit 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. Using a combination of techniques including CUT&Tag and electron microscopy, we recently discovered that HSV-1 cleverly takes advantage of structural changes to host chromatin that occur in response to infection in order to escape the nuclear compartment (Lewis*, Kelnhofer-Millevolte* *et al*, submitted 2022). Projects in the lab are currently focused on the mechanisms by which chromatin structure is altered during HSV-1 infection and how other herpesviruses contend with host chromatin during infection.

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Since starting as faculty at the Fred Hutch in July 2013, I have developed and refined a research program focused on phylodynamic analysis of pathogen sequence data with an intent of making inferences that are actionable to public health. I expanded this program from an initial focus on influenza virus evolution and my research now encompasses a number of viral systems, including Ebola, Zika, SIV, MERS-CoV, dengue, mumps and SARS-CoV-2. Since starting as faculty, I have published 83 papers of which 34 are `lab-lead', where either I or a direct trainee is first or last author. These lab-lead papers include 7 papers in Nature, Science or the New England Journal of Medicine. I've received funding from NIH, the Wellcome Trust, the Pew Charitable Trusts, the Bill and Melinda Gates Foundation and HHMI. This funding includes a MIRA R35 investigator award from NIGMS, a Pew Biomedical Scholar award, a Howard Hughes Medical Institute Investigator award and the Open Science Prize. I have mentored or am currently mentoring eight PhD students and six postdocs, as well as three undergraduate and four high school interns.

My research program focuses on using viral genome sequences to understand virus evolution and to gain insight into factors driving viral outbreaks, epidemics and pandemics. My lab uses genome data from seasonal influenza viruses to forecast strain turnover and inform the World Health Organization's seasonal vaccine strain selection process. We use genome data from emerging infections such as the West African Ebola epidemic, the Zika epidemic in the Americas and the SARS-CoV-2 pandemic to uncover the hidden history of viral spread unavailable through other avenues. Much of this work has been instantiated in the Nextstrain platform to conduct real-time genomic epidemiology and to share results broadly. This platform has seen substantial public health adoption to improve seasonal influenza vaccines, combat Ebola outbreaks and to understand and curb SARS-CoV-2 transmission.

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Have you ever wondered why some cancer patients have good responses and others do poorly? Why do two different individuals respond differently to cancer therapies? How can we improve the efficacy of cancer therapies? Why does resistance develop?

Our lab is answering these questions and identifying new drug targets for non-small cell lung cancer using functional genomics, biochemistry and molecular biology, analysis of clinical specimens, and preclinical in vivo modeling.

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. A central theme in the laboratory is understanding the mechanism and therapeutic targeting of cancer oncogenes in the RAS/MAPK pathway such as *KRAS*, *RIT1*, and *MET*.

Example projects in the lab include:

- Using genome-wide CRISPR screens in isogenic cell systems to identify oncogene-specific dependencies
- Determining how RAS-pathway oncogenes in lung cancer modulate gene expression and alternative splicing using RNA-seq
- Understanding the etiology of lung cancer in female never-smokers using exome sequencing
- Identifying new synthetic lethal drug targets using a paired guide RNA CRISPR approach
- Investigating the mechanism of RIT1 and YAP1 synergy in lung cancer
- Understanding how paralog synthetic lethality influences cancer cell therapeutic response and genome evolution

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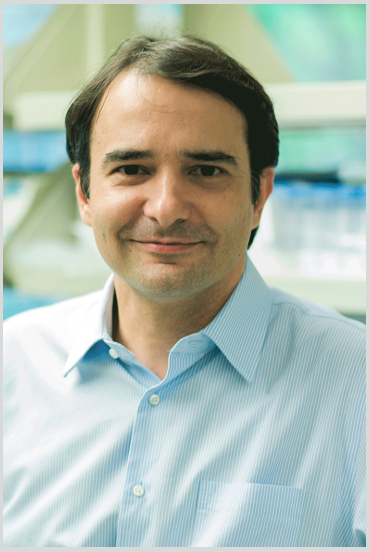

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REGULATION OF EPITHELIAL GROWTH IN DEVELOPMENT AND CANCER

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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Collective migration and metastasis by breast tumor cell clusters

My laboratory takes a particular interest in the social life of breast cancer cells. For decades, dogma has held that cancer cells must metastasize individually. However, a wealth of studies now reveal that tumor cells can also migrate and metastasize to distant organs as tumor cell clusters. Crucially, this collective pathway endows breast tumor cells with greatly augmented potential to migrate, proliferate, and evade cancer therapy. My group explores the molecular and cellular mechanisms regulating this collective process using time-lapse microscopy, 3D organotypic culture, novel animal models, and clinical studies of circulating tumor cells. Pairing cell biologic insight with translational impact, we aim to innovate new therapeutic approaches to effectively eradicate and prevent metastatic breast cancer.

SELECTED PUBLICATIONS

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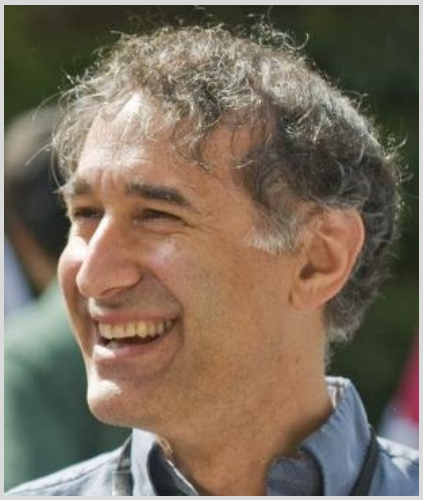

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** interested in taking a graduate student 2022-2023*

HIV AND THE EVOLUTIONARY HISTORY OF VIRUS-HOST INTERACTIONS, HIV Latency

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 host genes that impact HIV replication. Our goal is to determine how HIV adapted to humans to be become a pandemic virus and to help advance strategies to cure HIV infections.

We have developed a powerful new high-throughput screen for host genes that affect HIV replication based on incorporation of CRISPR guide RNAs into HIV virions. We extensively are use these screens to discover host genes that are used for HIV for productive infection across different viral strains and in different cell types. One of the major projects in the lab is to use similar screens for uncovering pathways used by HIV to maintain itself in a latent state in which it avoids elimination by the immune system. Such efforts are aimed at strategies towards HIV Cure.

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. We use a family of restriction factors called APOBEC3 proteins as and their antagonism with the lentiviral Vif protein as models to understand the cross-species adaptations that led to the birth of HIV-1 as human pandemic pathogen.

RECENT SELECTED PUBLICATIONS

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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 characterizing the basic features of an immune response to a subunit vaccine, 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 (MT) of rodent PyVs. In contrast to MT, ALTO has growth suppressive effect in MCCs that is tied to its ability to induce NFkB signaling. 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. We and others have identified ST as the major transforming protein of MCPyV and have identified a number of binding partners and genes that are regulated by ST expression.

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Professional Highlights

Fellow, Academy of American Association of Cancer Research 2022

Fellow, American Academy of Arts and Sciences 2019

Association of Women in Science (AWIS) Science Achievement and Leadership Award 2019

Paul Stephanus Memorial Endowed Chair 2018

National Cancer Institute, Outstanding Investigator Grant 2017 – 2024

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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.

Additional projects focus on the regulation of Kaposi sarcoma-associated herpesvirus reactivation from latency, the evolution of the pan-herpsevirus restriction factor MxB, and the regulation of CMV gene expression by polyamines.

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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 laboratory is to understand how microenvironments within distant tissues regulate the four "hallmarks" of disseminated tumor cells (DTCs): long-term survival, reversible growth arrest, immune evasion and therapeutic resistance. Solving these puzzles is key to extending metastasis-free survival of cancer patients; with the ultimate goal of preventing metastasis altogether.

Four major research directions are underway in our laboratory:

1. What puts DTCs to sleep? Do the underlying mechanisms change from tissue to tissue?

Breast cancer stereotypically yields metastases in the brain, lung, liver, bones and lymph nodes. But in a substantial fraction of patients, metastases may not emerge for five, 10, or even 20 years after treatment. How are DTCs kept at bay in the interim?

In 2013, we established that dormant breast cancer cells localize to the outer surface of microvessels- a microenvironment called the perivascular niche. There, endothelial-derived factors effect quiescence. Specifically, we reported that endothelial derived thrombospondin-1 suppresses DTC outgrowth in lung and in bone marrow. Interestingly, although DTCs are found on microvessels within the brain as well, brain endothelium does not express thrombospondin-1. This is but one minor reflection of how vascular and perivascular environments vary by tissue. Our hypothesis is that these unique perivascular environments effect dormancy in unique ways. To elucidate the tissue-specificity of the perivascular niche and of dormancy, 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. What do DTCs rely on for survival? DTCs transit into totally 'foreign' microenvironments, where they are able to survive in patients for a decade or more. What biologies are they relying on to survive?

To address this question, we have adopted a high-content/high-throughput approach leveraging organotypic vascular niches, reporters of quiescence and apoptosis, and a 5,500 compound library. Our findings have revealed— unsurprisingly— that the drugs that target quiescent *vs.* proliferative tumor cells are completely different. By combining sequencing with therapeutic pressures, we have begun to unravel the pathways targeted by these compounds, with the hopes that this will inform us about the unique molecular and metabolic requirements of dormant DTCs. The goal is to define safe and specific therapies we can apply to eradicate dormant DTCs.

3. How do dormant disseminated tumor cells resist therapies?

It is assumed commonly that quiescent DTCs do not respond to genotoxic therapy because such therapies only target rapidly dividing cells. We challenged this notion, showing that chemotherapeutic regimens employed in the treatment of invasive breast cancer select for perivascular tumor cells in the bone marrow. Using organotypic vascular niches, we discovered that endothelium protects DTCs from chemotherapy, and that the mechanisms are cell cycle-independent, relying instead on interactions between integrins and molecules present within the vascular niche. Targeting integrins sensitized the vast majority of DTCs to chemotherapy, yielding drastic enhancements to metastasis-free survival in pre-clinical models.

This finding has motivated us to measure how chemotherapy impacts other non-dividing cells; especially endothelium. We hypothesized that chemotherapy causes DNA damage within the endothelium, and that this damage is linked to a vascular secretome that paradoxically protects DTCs from chemotherapy. We are now working to thoroughly define this secretome, and the signaling that links it to DNA damage. Targeting what we have called the chemotherapy-associated vascular secretome at its root or at its stem may yield efficacious therapies that can be applied with or without integrin inhibitors to eradicate DTCs.

4. How do dormant DTCs escape immunity?

We have used model antigens and immune-competent models to establish that dormant DTCs evade immunity, and uncover intrinsic and extrinsic mechanisms that allow them to do so. These findings have motivated us— in collaboration with Stan Riddell's Lab— to explore engineered T cell receptor (TCR)- and chimeric antigen receptor (CAR)-based approaches to target dormant DTCs. Our goals are to: (i) identify the formant of immunotherapy best suited to eradicate dormant DTCs, (ii) re-engineer these cells so that they are optimized to traffic to and eradicate rare cell populations, and (iii) profile *human* DTCs so that we can identify realistic (neo)antigens that we can target with engineered T cells.

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** very interested in taking a graduate student 2022-2023*

Leveraging Signaling Networks for Drug Discovery

The complexity of cellular communication is one of the greatest challenges in science. Today, this complexity represents a tremendous barrier to understanding disease and personalizing therapy. The Gujral lab is focused on understanding at the molecular level how cells respond to stimuli, both physiological stimuli and those that contribute to disease. The molecular networks by which cells respond to stimuli are complex, dynamic, and interconnected. Therefore, studying these networks requires multiple interdisciplinary approaches. Our lab integrates state-of-the-art methods from several fields, including advanced computer science, bioinformatics, pharmacology, biochemistry, and cell biology, to investigate tissue-specific signaling networks, identify molecular targets for drug discovery, and identify new uses for existing medicines.

Of particular interest is how cells in tissues communicate. In multicellular organisms, tissues and organs are composed of many different types of cells with distinct functions. Solid tumors can be considered a pathologic type of tissue. We now realize that to truly understand biology and disease we must understand not just individual types of cells, whether healthy or diseased, but also the tissue environment. In non-cancerous tissue, such environments are called the "tissue microenvironment"; in solid tumors, this environment is called the "tumor microenvironment." These microenvironments include the main functional cell type, such as epithelial cells or cancer cells, as well as extracellular molecules organized into an extracellular matrix and a diverse and often changing set of other cells, such as stem cells, immune cells, fibroblasts, fat cells, and blood and lymphatic vessels. Reciprocal signaling between the main functional cells and the microenvironment is constantly occurring and changes based on physiological state and therapeutic intervention.

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.

Signaling Networks Within Tissue Microenvironment. Traditionally, signaling networks have been studied and dissected in the context of single-cell and single-cell populations, like some of the studies described above.

On the other hand, it is also well-established that the cellular microenvironment, which is highly dynamic and heterogeneous, exerts critical influences on signaling networks. However, the community has lacked experimental approaches to study cell-cell interactions and single-cell responses in complex tissues. To address these challenges, the Gujral lab is developing new strategies that will enable studies of signaling networks in the tissue microenvironment. Specifically, we have been developing methods for maintaining thin sections of mouse and patient-derived tumor slices for mechanistic and drug discovery studies. These preparations are called organotypic tissue slices, and they preserve the organization and heterogeneity of the cells and extracellular structures within the tumor tissue. The tumor tissue slices are 200 – 250 µm thick, representing ~10 layers of cells and include both the cancer cells, normal cells, and immune cells in this tumor microenvironment. We have optimized the conditions to maintain the viability of organotypic tumor tissue slices for several weeks in culture. We have also developed methods for the delivery of small molecules using an active flow-based perfusion system. We demonstrated the utility of this *ex vivo* model system for medium-throughput cytotoxic and immuno-oncology drug screening studies.

Systems pharmacology approaches to dissect signaling networks. Network pharmacology is a new field of science focused on targeting multiple steps in a regulatory signaling network. The 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. Given that kinases represent one of the largest target families in drug development, as well as critical components of all signaling networks, we are developing computational tools for evaluating potential clinical applications of kinase inhibitors. Through these efforts, we aim to enhance our understanding of the basic kinase biology as well as advance pharmacological exploitation of these key cellular regulators. To this end, our lab has established a series of machine learning approaches, called KiR, KInhibition, KiDNN, and KiRNet, that use large scale drug-target profiling efforts, machine learning approaches, and broadly-selective chemical tool compounds to pinpoint specific nodes (kinases and associated networks) underlying a given phenotype such as the growth of cancer cells or release of cytokines. Using a combination of these approaches, we are able to **1**) identify specific signaling nodes that are important for a given phenotype; **2**) predict response to ~500 FDA-approved or clinical grade kinase inhibitors as single agents and rank order up to13 million drug combinations *in silico*. In the past few years, we have applied a combination of the above systems-based approaches to broad areas of biology, ranging from the studies of **malaria**, **COVID-19,** and **prostate cancer**. These studies identified new molecular regulators and potential therapeutics, highlighting the potential of these computational tools for unbiased biological discovery.

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The University of British Columbia, 2014, Ph.D. in Bioinformatics

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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 for non-invasive **liquid biopsies**, such as **circulating tumor DNA** from blood, to monitor genomic changes in cancer patients. Our goals are to uncover the genetic and epigenetic alterations in 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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All cells in the human body have the same DNA sequence; but a cell in the heart looks and functions very differently than a cell in the prostate. These differences are dictated by an additional layer of information that is encoded by modifications of the DNA molecule that do not change the DNA sequence. Such epi-genetic marks (literally meaning on top of the genome) define the function and behavior of all cells.

Of the many epigenetics marks that are currently known, DNA methylation, which involves the addition of a chemical group directly on the DNA molecule, is the best studied. DNA methylation is essential for cellular differentiation and determines the fate of a cell. Although essential for every cell in the human body, cancers find unique ways to highjack and change these epigenetics marks. Prostate cancers in particular show major DNA methylation changes. We therefore reasoned that by understanding the pattern of DNA methylation changes in prostate cancer, we can identify unique vulnerabilities that would allow us to treat prostate cancers more effectively.

These studies led us to unmask a novel type of prostate cancer that is characterized by highly distinct DNA methylation changes. Whereas the DNA of normal cells and most prostate cancers is peppered with methylation marks, this type of prostate cancer shows a dramatic loss of DNA methylation. This new subclass of prostate cancer, which makes up around 10-15% of advanced tumors. Further, these tumors show a more aggressive behavior. However, we hypothesize that the loss of DNA methylation likely generates unique vulnerabilities in these cancer cells that we can target with specific therapies. We are therefore determined to identify the “Achilles’ Heel” of these epigenetically unique tumors.

I am very interested in having a graduate student join my laboratory in 2022-2023.

Michael Haffner, MD, PhD

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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, and activate signaling 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 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 is limited. 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. We are also collaborating with cancer researchers to examine NE stability in *in vivo* and treatment contexts.

2) Why are 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 massive DNA damage and cytoplasmic chromatin is thought to be a key trigger of innate immune and invasion signaling. Yet why micronuclei fail to assemble a stable NE, why they can't repair after rupture, and the mechanism of DNA damage are still unclear. We are both taking a hypothesis-driven approach and developing new tools to address these questions.

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

Although loss of nucleus compartmentalization has been shown to cause substantial changes in genome organization and cell signaling, we expect that many additional responses occur that have gone undetected. Thus we are developing new tools to comprehensively identify the effect of NE instability on the proteome and transcriptome, as well as at the level of chromatin structure. Our overall goal is to define causal links between NE rupture, misregulation of cellular functions, and cancer development to identify potential therapeutic opportunities.

Selected Publications

Anna E Mammel, Heather Z Huang, Amanda L Gunn, Emma Choo, Emily M Hatch. Chromosome length and gene density contribute to micronuclear membrane stability. *Life Sci Alliance.* 2021, Nov 17. https://www.biorxiv.org/content/10.1101/2021.05.12.443914v1

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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). **The following projects are currently active in the lab:**

a) In vivo CRISPR-Cas9 screens to interrogate tumor metabolism specific to obese hosts.

b) Functional genomics and metabolomics of Tasmanian Devil Facial Tumors, a transmissible cancer..

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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. Our contribution to molecular subdivision of gliomas 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. In addition, in order to validate our finding mouse tumors, we have been using large human tumor datasets and creating the computational tool oncoscape for interactive dimension reduction of molecular and clinical data allowing cohort creation and iterative refinement.

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.

Gene fusions and oncogenesis
Gene fusions occur in many cancers. Some are transcription factors, others are components of signal transduction pathways. Signaling component fusions include those fused to the kinase domain of one of the Trk receptors. We have demonstrated the ability for many of the Trk fusions to induce tumors in mice, and are working on their mechanism of action currently. Transcription factors are also common components of gene fusions. YAP fusions are found in many, and in some cases are found in the majority of specific tumor types. We have demonstrated the ability for many of the YAP fusions to induce tumors in mice and the mechanism of YAP fusions in oncogenesis.

Meningioma models
Meningiomas are the most common of the intracranial tumors. There are rare YAP fusions in pediatric meningiomas. We have demonstrated of the ability for the YAP-MAML2 fusion to induce meningiomas in mice, and that these mouse meningiomas share both histology and RNA expression patterns with the human counterparts. Moreover, constitutively active YAP works in mice to induce meningiomas as well, Demonstrating that the YAP pathway is a main driver in the most common subtype of these tumors. We are continuing to use these mouse models to identify therapies against tumors with these fusions in patients.

RNA splice variants
Many genes have multiple splice forms, many of which are predominant in embryonic development and cancer. we have shown that one specific splice variant of TrkB, TrkB.T1 is expressed throughout the embryo during development and the predominant form in most cancers. We have also shown that forced expression of this splice variant is sufficient to cause cancer in many cell types post-nataly when this splice variant is normally shut off. We are working to determine if TrkB.T1 might be a therapeutic target for several tumor types.

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RESEARCH FOCUS

The focus of our group is the study of the lung tumor microenvironment with an emphasis on the role of the neutrophil lineage. We have determined that the immune system has a limited number of ways to respond to the presence of cancer. Currently, we are developing strategies to reliably identify these immune response subtypes and generating therapeutic strategies to address them. Ultimately, we plan to personalize immune based therapies for lung cancer patients based on the dominant aberrancy in their immune response.

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** very interested in taking a graduate student 2022-2023*

The goal of the Hsieh Lab at the Fred Hutchinson Cancer Research Center 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 cancer.

Dissecting the functional interface between transcription and translation in cancer. 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 indicates that transcription factors 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 cancer.

Key questions:

1) How does the translation apparatus interface with transcription factors and other regulators of gene expression in the context of genitourinary malignancies?

2) What are the key downstream translational drivers necessary for cancer phenotypes such as lineage plasticity?

3) How can we therapeutically disrupt oncogenic translation?

Andrew Hsieh, MD

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 mRNA metabolism and efficient protein synthesis (Schuster and Hsieh Trends in Cancer 2019, Lim et al. Nature Communications 2021). Surprisingly, their functionality remains poorly understood particularly in the disease context. Thus, we are deeply investigating how UTR dynamics tune gene expression to impact the multistep process of cancer initiation and progression.

Key questions:

1) How are UTRs usurped to promote cancer pathogenesis?

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

Advance stage 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 this 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, Jana et al. JCI Insight 2021). There are also ongoing projects focused on dissecting translation deregulation in bladder cancer.

Key questions:

1) To what extent and how does bladder cancer heterogeneity influence disease aggressiveness and response to therapeutics?

2) How is the translation apparatus usurped in urothelial cells to drive the process of transformation?

Advancing our understanding of translation regulation in normal cell physiology through collaboration. 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. Nature Communications 2019). In addition, through work with the Beronja Lab (Fred Hutch) we are unraveling the critical role of mRNA specific translation in cell fate choice (Cai et al. Cell Stem Cell 2020).

Key questions:

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

2) How is mRNA-specific translation directed in basal epithelial cells of the skin to control self-renewal and differentiation?

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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 PANCREATICOBILIARY CANCER

The central theme of our research group is to study how the dysregulation of chromatin modifying enzymes contributes to pancreaticobiliary 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 pancreaticobiliary cancer?

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

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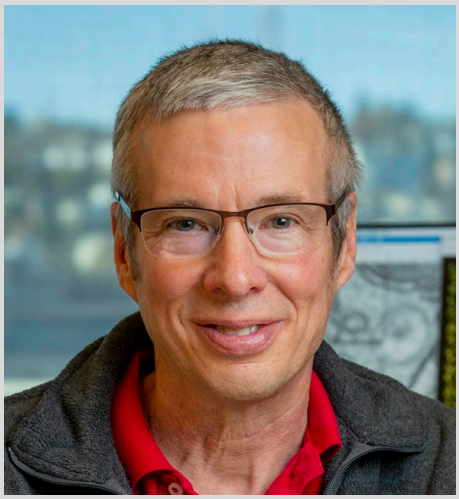

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CANCER BIOMARKERS

The Lampe laboratory investigates the control of cell growth both at the cell biological/mechanistic level and through cancer biomarker discovery. The advent of new screening methodologies has expanded our efforts into broad proteomic screens for potential cancer biomarkers using high density antibody array technologies produced by our lab 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 real impact on lung cancer mortality through better methods of screening. We already have discovered and preliminarily validated excellent markers for both non-small cell (NSCLC) and small cell (SCLC). We need to formally validate them in larger sample sets. For NSCLC this means testing them in both prediagnostic (i.e., National Lung Screening Trial) and diagnostic sample sets combined with Computed Tomography (CT) imaging as part of our funded Lung SPORE project 4. For SCLC we have a panel of excellent autoantibody markers that have been twice validated that we will further validate in NLST and WHI samples via a recently funded NCI R01 grant. Some of these autoantibodies are to proteins inappropriately expressed in SCLC and thus are targets for both imaging (e.g., immunoPET) and immunotherapy (CAR-T and targeted drug delivery).

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The aim of my lab is to identify molecular drivers and biological properties of prostate and bladder 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 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, test, and optimize 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. 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 optimization is ongoing in collaboration with prominent medicinal chemistry and prostate cancer biology colleagues.

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UNDERSTANDING BLOOD CELL FORMATION IN HEALTH AND DISEASE

Our laboratory seeks to understand the molecular basis of blood cell formation in health and disease. We focus on defining how cancer-associated mutations impact hematopoietic stem cell function, how they drive disease development and response to therapy. Our goal is to develop novel treatments for blood disorders including bone marrow failure (BMF), myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML).

Hematopoiesis is the continuous production of mature blood cells that is essential for life. This includes red blood cells that oxygenate our cells, platelets that clot wounds, and white blood cells that fight infections and provide us with life-long immunity. This process is dynamic and is sustained by a very rare group of cells known as hematopoietic stem cells (HSCs). They possess the ability to both self-renew (to make a carbon copy of itself) and differentiate into functional progenies, and this decision is controlled by both cell-intrinsic and cell-extrinsic factors. How this is regulated at the molecular level is still not completely understood. Our laboratory seeks to dissect how this process is regulated by transcriptional and post-transcriptional mechanisms. Our objective is to to find novel treatments to life-threatening blood diseases such as myelodysplastic syndromes (MDS), bone marrow failure and acute leukemias.

We employ multi-disciplinary approaches to understand how aberrant RNA processing and chromatin regulation drives pathogenesis of blood cancers. Our research program integrates multiple disciplines

including basic and translational hematology, transcriptional regulation, and RNA biology. Current projects in the lab include:

- Understanding and targeting aberrant transcriptional and RNA processing pathways in hematologic malignancies.
- Dissecting the transcriptional, epigenetic and RNA splicing landscape in blood cell development.
- Identification and development of novel therapeutic targets in blood disorders.

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USE OF IN VIVO APPROACHES TO STUDY LUNG 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 that responds well to chemotherapy but rapidly becomes chemoresistant. 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 (e.g. see Augert et al, 2020, *Cancer Cell*; Grunblatt et al, 2020, *Genes and Development*; and Jia et al, 2018, *Cancer Discovery*). 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. We collaborate closely with our clinical colleagues at the Seattle Cancer Care Alliance to generate and study patient derived xenograft models of SCLC and identify therapeutic strategies to target subsets of SCLC (see Augert et el, 2019 *Science Signaling* and Norton et al, 2021 *Genes and Development*). Genomic analyses and functional genomics, including genome-scale CRISPR inactivation and cDNA overexpression screens are used in these efforts. We perform genetic screens in cell culture and in vivo to identify oncogenes and tumor suppressor genes and to identify new therapeutic approaches that can be tested in our in vivo models. Our ultimate aim is to translate an increased understanding of the basic biology of SCLC driver genes to the development of novel more effective therapies.

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TARGETING ONCOGENIC SIGNALING THROUGH CONTROL OF PROTEIN HOMEOSTASIS

Deregulated signal transduction pathways rewire proteomic, transcriptional, and epigenetic networks to drive cell growth and survival in cancer. While these pathways identify targetable vulnerabilities in cancer, there remains a shortage of targeted agents that disrupt critical signaling nodes and/or overcome drug resistance. The Nabet lab is dedicated to targeting oncogenic signaling networks through control of protein homeostasis to advance new therapeutic strategies in cancer.

The focus of the Nabet lab is to develop and deploy novel chemical biology strategies to inhibit, degrade, or activate target proteins in cancer. We pioneered a technology known as the degradation tag (dTAG) system that employs small molecule degraders to harness the cell's ubiquitin-proteasome machinery to rapidly eliminate tagged proteins. We leverage dTAG and develop novel proximity-inducing technologies to discover, validate, and gain insights into clinically relevant targets within oncogenic signaling networks. We believe in open-source team science and work closely with medicinal chemist collaborators worldwide to translate our findings by developing small molecule inhibitors, degraders, and dimerizers to target these proteins. Through these efforts we work to deepen our biological understanding of the molecular circuits that drive cancer cell survival and growth as well as translate these insights into therapeutic strategies for unmet medical needs.

Our current research is focused on:

1. Targeting vulnerabilities that coordinate resistance to signaling disruption in cancer.
Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest human cancers, and development of targeted therapeutics for oncogenic drivers of PDAC has been difficult to achieve. While directly targeting mutant KRAS is a major research focus, intrinsic and acquired resistance emerges in response to KRAS disruption. We employ the dTAG system to define the transcriptional regulators responsible for adaptive survival to disruption

of KRAS signaling and aim to identify combination approaches targeting these factors to improve the durability of responses in PDAC.

2. Evaluating the clinical potential of targeted degradation as a therapeutic strategy in cancer.
Targeted protein degradation is a breakthrough approach, whereby rather than inhibiting protein function, proteins are pharmacologically eliminated through the proteasome. To leverage the advantage of degraders for eliminating all protein function, we employ dTAG for target discovery and validation and collaborate with medicinal chemists to develop direct-acting small molecule degraders for therapeutic targets with enzymatic and non-enzymatic activities. We aim to characterize small molecule degraders in translationally relevant cancer models to gain new insights into the biological activities of targeted proteins and to provide a framework for preclinical evaluation of targeted disruption of these factors.

3. Developing next-generation strategies to control target protein activity.
Developing strategies to activate target proteins such as tumor suppressors using pharmacological approaches has been a major challenge. We focus on developing novel technologies for protein stabilization through recruitment of enzymes capable of inducing post-translational modifications to activate a target protein. We aim to expand the spectrum of proximity-induced interactions that can modulate protein activity for therapeutic gain.

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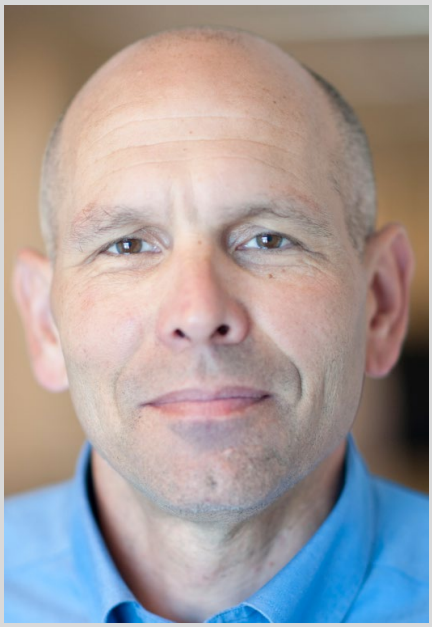

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EXPLOITING CANCER GENOMICS AND THE TUMOR MICROENVIRONMENT TO GUIDE ONCOLOGY TREATMENT

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 230,000 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 (ctDNA) 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 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 cellular functions to identify roles 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 viral transmission and pathogenesis and the role of innate and adaptive immunity in these outcomes. Much of this work has focused on HIV, but more recently has expanded to other emerging global pathogens, Zika and SARS-CoV-2. The lab seeks to understand what immune responses contribute to whether a productive HIV 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.

She is currently extending her work on antibodies to the study of SARS-CoV-2. There the focus is on identifying the key antibody responses to infection and their association with outcome.

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 and SARS-CoV-2 and is conducting screens to identify antiviral host factors to these important global pathogens.

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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FUNCTIONAL GENOMICS OF STEM CELL AND CANCER CELL BIOLOGY

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.

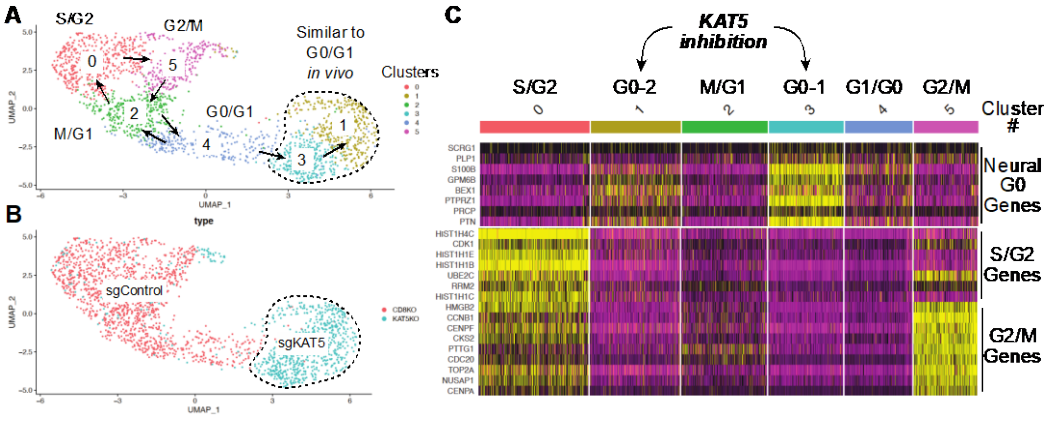

Figure 1: Inhibition of histone acetylase KAT5 in brain tumor stem-like cells (GSC-0827) causes emergence of a G0-like state observed in GSC-0827 tumors and human NSCs, we have dubbed "Neural G0". **A/B.** ScRNA-seq of sgControl vs sgKAT5 in GSC-0827 cells 5 days post-nucleofection. **C.** Gene expression analysis of gene clusters from **A** (yellow= higher expression). KAT5 was a top hit is a recent screen attemepting to identify gene which when inhibited trigger quiescence in human brain stem-like cells.

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 (**Fig. 1**). The results from these studies will better help define our notions of cell cycle regulation and maintenance of progenitor cell identity. In collaboration with Drs. Anoop Patel and Eric Holland, we recently received NIH funding for study of tumor quiescent states and genes that regulate tumor cell quiescence ingress and egress.

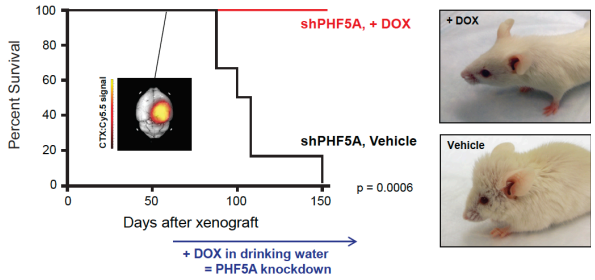

Figure 2. Inhibition of PHF5A causes human GBM tumor regression and survival in immune-compromised mice. PHF5A is important for 3' splice site recognition and was identified as a key GBM vulnerability driven by MYC activity.

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**).

Epitranscriptomics. Epitranscriptomics generally pertains to chemical modifications of mRNA occurring during or after gene transcription. N6-methyladenosine (m^6A) is among the most frequent post-transcriptional chemical modifications found in mammalian mRNA. In cell-based models, m^6A has been suggested to participate in numerous types of mRNA regulation (e.g., turnover, splicing, translation, or miRNA targeting). However, while m^6A-mRNA likely exists in most if not all eukaryotes, physiologically relevant roles for m^6A-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^6A 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^6A mRNA marks promote the *translation* of a network genes required for human erythropoiesis, including factors that control epigenetic patterning in chromatin (Kuppers et al., 2019). We have now extended this work to characterize all transcripts that are m^6A marked during erythroid lineage formation using a new m^6A-seq technique (Kuppers, in preparation).

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Host-pathogen interactions and genetic diversity of *Helicobacter pylori*

In the mid 1990's, a bacterium, *Helicobacter pylori*, was linked to stomach cancer, the fourth leading cancer killer worldwide. *H. pylori* establishes lifelong infection in the stomach of half the human population world-wide. The consequences of this infection range from undetected gastritis (inflammation of the stomach) to ulcer disease and gastric cancer. Our lab is interested in the mechanisms by which this bacterium can establish and maintain a chronic infection in the stomach and the molecular cross-talk between the host and the bacterium 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 in both 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 bacterial properties related to pathogenesis and patient outcome. This includes efforts to track genetic changes that accumulate during chronic infection of humans using noninvasive samples (like stool), which we test with molecular methods (like ddPCR) to track virulence and antibiotic resistance genes.

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 specific proteins, as well as cell morphology more broadly, 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 to understand how *H. pylori* builds its helical shape.

3. **Host tissue responses to chronic infection**: We have developed several bacterial 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 morphological 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, testing both wild-type mice and genetically modified mice with altered immune pathways or stomach epithelial differentiation. This allows us to look at the relative fitness of different bacterial mutants, their location within the gastric epithelium, 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 linked to disease and as potential lead compounds drug development. To this end we use interdisciplinary approaches ranging from chemical synthesis and medicinal chemistry to pharmacology, genetics and cell biology. The compounds we are studying have been identified from large collections of synthetic, drug-like compounds and from natural sources. Most drug screens are phenotypic and unbiased in terms of specific targets. While screening compound libraries is a significant part of what we do most of our efforts go into target identification, mechanistic studies to understand the biology, pharmacology, and potential clinical efficacy of lead compounds.

The clinical use of several therapeutically important 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 mechanosensory cells of the inner ear. 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 screening hit 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 recently completed phase I safety trials and is about to enter phase 2 efficacy trials in patients with non-tuberculous mycobacterium lung infection. Preliminary studies with ORC-13661 indicate that the compound also protects mammalian auditory hair cells against cisplatin-induced death.

We previously identified inhibitors of yeast (*S. cerevisiae*) and human NAD-dependent deacylases—protein hydrolases that cleave acyl groups, including acetyl groups from the ε-amino group of lysine residues—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 regulation to DNA damage responses and modulators of specific sirtuins have been suggested as therapeutic agents for a variety of human diseases. In collaboration with Toni Bedalov's laboratory (Clinical Research Division, Fred Hutch), we are working to optimize our sirtuin-2 inhibitors using medicinal chemistry strategies for use as therapeutics in germinal center-derived lymphomas. Sirtuin 2, or SIRT2, plays a unique role in the biology of B-cell development. Immature B-cell precursors must 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.

In addition to lab-based research, we are involved in a long-term collaboration with New Mexico State University under a U54 grant funded by the National Cancer Institute's Center to Reduce Cancer Health Disparities. This program carried out education and outreach activities in New Mexico as well as the Yakima Valley of Washington State. The partnership also supports collaborative projects in cancer biology and health disparities research.

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Immunotherapy using CAR-T cells has shown impressive efficacy in B cell malignancies but has largely been ineffective when targeting antigens on epithelial cancers, which account for 80-90 percent of all cancers. The Srivastava lab is focused on using clinically relevant animal models of human cancers to define the mechanisms limiting the activity of CAR- and TCR-engineered T cells in solid tumors and to evaluate strategies to overcome these barriers. A major focus of the lab is in understanding the principles that govern basic T cell trafficking, persistence, and activity and applying these principles to engineer more effective adoptive T cell therapies for human cancers. Our long-term goal is to work with clinical colleagues and industry partners to translate the most promising strategies to the clinic.

Developing genetically-engineered mouse models to study CAR-T cell therapy for solid tumors
Transplantable and tumor xenograft models lack clinically relevant tumor microenvironments (TME), making it difficult to study the mechanisms that limit activity of transferred T cells in solid tumors. We recently adapted the $Kras^{LSL\text{-}G12D/+}p53^{fl/fl}$ (KP) genetically engineered mouse (GEM) model of non-small cell lung cancer, which mimics the initiation, progression, and immunosuppressive TME of human lung cancer, to express a target for CAR-T cells, and demonstrated that this model mirrors many of the barriers to effective CAR-T cell therapy observed in patients, including poor CAR-T cell infiltration into tumors and acquired dysfunction. Using this model, we identified a novel lymphodepletion regimen that induces immunogenic tumor cell death and activates tumor macrophages to express T cell-recruiting chemokines, resulting in improved CAR-T cell infiltration, remodeling of the TME, and increased tumor sensitivity to anti-PD-L1 checkpoint blockade and improved survival. However, the infiltrating CAR-T cells still became dysfunctional over time, suggesting that additional strategies to recruit greater numbers of T cells that retain function are needed to achieve durable efficacy. Current research in the lab is focused on:

1. Improving trafficking of engineered T cells to solid tumors

2. Engineering T cells to resist the development of exhaustion

3. Preserving the function of engineered T cells in immunosuppressive tumor microenvironments

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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 demand for 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 existing cancer therapies function by interfere with metabolic pathways, 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, cell culture, 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 our work has shown that bolstering aspartate levels in cancer cells in vivo can increase tumor growth. 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. Current work entails understanding metabolic adaptations that allow cancer cells to tolerate tissue specific oncogenic mutations in aspartate synthesis pathways and mechanistically understanding how aspartate limitation constrains cell proliferation.

2. Determining the roles of synthesis and salvage in proliferative metabolism

While metabolism is classically viewed through the lens of efficient ATP production, maximization of biomass synthesis in proliferation requires balancing many additional metabolic factors, including substrate acquisition, redox metabolism, nitrogen shuttling, one-carbon cycles, etc. Alterations to metabolic systems can then impinge upon others to cause metabolic constraints, and so the mechanisms cancer cells use to balance their metabolic demands are of high interest for new therapeutic targets. One underappreciated avenue available to these cells is to scavenge macromolecules directly, either to use as is or to disassemble and rebuild, to bypass the complex demands of synthesis. We are broadly interested in accounting for which metabolites are synthesized versus salvaged and are currently investigating the mechanisms by which cancer cells can uptake and incorporate environmental lipids to support their biosynthetic demands.

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. Our work 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. In our current work, we are evaluating the presence of novel reactive metabolite drivers of oncogenic signaling in cholangiocarcinoma. We are also using biochemical and functional genomics approaches to identify uncharacterized pathways that support cancer cell metabolism.

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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 coheson-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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Dr. Vasioukhin's laboratory has a long-standing interest in understanding the mechanisms of normal tissue homeostasis, transformation, tumor progression and metastasis. The laboratory focuses on mouse prostate gland epithelium and skin epidermis as primary in vivo research model systems. It is also extensively using primary human organoid cultures. The laboratory employs a combination of mouse genetic, molecular, and microscopic methods to uncover the mechanisms underlying cancer initiation and progression, as well as to explore new avenues for therapeutic treatments.

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