LANDRY
CANCER BIOLOGY
CONSORTIUM

SPRING SYMPOSIUM

17 May 2018
12:00 – 6:30 PM

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About the Landry Cancer Biology Consortium

The Landry Cancer Biology Consortium provides a unique and essential service to the cancer biology community at Harvard: it brings this community together to realize its full potential.

Harvard University is home to 14 life sciences PhD programs—but no one gets a PhD in cancer. To drive new advances in multidisciplinary cancer research, and to introduce students at all levels to research and training opportunities across Harvard, the Landry Cancer Biology Consortium creates new courses, scientific events, and co-curricular activities, all designed to bring students and faculty together to share recent developments, address current challenges, and promote synergy in cancer research and treatment. In addition, through the Landry Cancer Biology Research Fellowship—a premier fellowship awarded to five exceptional PhD students each year—this program supports emerging leaders within the growing network of cancer biology researchers across Harvard.

This program is made possible by the generous support of the late C. Kevin Landry ’66 and his family, colleagues, and friends. This gift represents a transformative investment in some of the best and brightest young minds in cancer biology.

Symposium Schedule

Starting 11:30 am	Registration *
12:00 - 1:30 pm	Karin Grunebaum Foundation Poster Competition*
1:45 - 2:45 pm	Faculty Panel ** *"My Journey to Becoming a Professor"* **Sara Buhrlage, PhD** **Carla Kim, PhD** **Mo Motamedi, PhD**
2:45 - 3:30 pm	Featured Research ** *"Therapy Guided by Protein Dynamics in Single Cells"* **Galit Lahav, PhD**
3:30 - 4:30 pm	Keynote Address ** *"Choosing the Next Generation Of Cancer Targets: How Not to Be Wrong"* **William Kaelin, Jr MD**
4:30 - 6:30 pm	Reception * Poster Prizes Announced

*NRB 2nd Floor Lounge
**NRB 1031, 10th Floor Seminar Room

My Journey to Becoming a Professor
Faculty Panelists

Sara Buhrlage, PhD
Assistant Professor of BCMP

Sara Buhrlage, PhD, is an Assistant Professor in Dana-Farber's Cancer Biology Department and Harvard Medical School's Biological Chemistry and Molecular Pharmacology Department. Her research group focuses on the development of first-in-class inhibitors and prototype drugs for deubiquitylating enzymes (DUBs) that can be utilized to pharmacologically validate members of the gene family as new targets for cancer treatment and other diseases. DUBs have garnered significant attention recently as potential therapeutic targets in the field of oncology due to their removal of degradative ubiquitin marks from cancer causing proteins.

Prior to joining as a faculty member in July 2015, Dr. Buhrlage was a professional track scientist at Dana-Farber in the medicinal chemistry core laboratory. In this role she collaborated with Institute researchers to pharmacologically validate novel targets of disease and study mechanisms of oncogenesis and drug resistance.

Dr. Buhrlage completed a Doctor of Philosophy in organic chemistry in 2008, under the direction of Professor Anna Mapp, PhD, from the University of Michigan, where she successfully designed, synthesized and characterized small molecules that bind the transcriptional co-activator CBP and upregulate transcription when tethered to DNA. Following completion of her Doctor of Philosophy, Dr. Buhrlage trained for two years in medicinal chemistry at the Broad Institute. There, she led a team of six chemists performing lead optimization on a macrocycle inhibitor of the hedgehog protein, which resulted in analogs with superior potency, improved metabolic stability, excellent *in vivo* pharmacokinetics, and no *in vitro* safety liabilities.

Carla Kim, PhD
Professor of Genetics

Dr. Kim is Professor of Genetics at Boston Children's Hospital. Her laboratory has pioneered the use of stem cell biology approaches for the study of adult lung progenitor cells and lung cancer. Her work has contributed to a better understanding of stem cell biology in the lung, development of innovative approaches for examining the cellular and molecular basis of cancer and pulmonary disease, and identification of new therapeutic avenues for lung cancer.

The success that Dr. Kim's lab has had investigating the molecular pathways that regulate lung stem cells and the role of stem cells in lung cancer is a testament to her potential to make new strides in understanding lung disease and basic biology. Working with genetically engineered mouse models that accurately represent human lung cancer, Dr. Kim's group was the first to identify cancer stem cell populations in the two most frequent types of lung cancer in patients (*Cell Stem Cell*, 2010 and *Cancer Cell*, 2014). Her lab's knowledge in lung stem cells has revealed a new combination therapy approach for particular subsets of lung cancer patients (Fillmore et al, *Nature*, 2015). Dr. Kim's lab has developed a 3D lung organoid system that makes it possible to derive specialized lung cells from lung stem cells (Lee et al, *Cell,* 2014). The organoid culture system developed by the Kim Lab grows tiny replicas of lungs, allowing them to model the complex interactions of lung stem cells and their neighboring cells. Most recently, the Kim Lab used the organoid cultures to define new types of mesenchymal cells that are required to support lung injury repair (Lee et al, *Cell,* 2017). This system can now be used to probe the role of lung stem cells and the diverse cell types with which they interact in lung cancer, in lung diseases such as cystic fibrosis and pulmonary fibrosis, and during lung development. These advances by the Kim Lab provide a whole new way to study lung diseases in the laboratory dish and to find new therapeutic interventions.

The impact of Dr. Kim's work has been acknowledged in a myriad of ways. From her publication in *Cell Stem Cell* winning Best Cancer Paper of 2010 to her receipt of the William Rippe Distinguished Award in Lung Cancer Research from the Lung Cancer Research Foundation, she has been widely acknowledged as one of the brightest researchers in lung stem cells. Dr. Kim received her Ph.D. in Genetics from the University of Wisconsin—Madison in 2002. She joined the Stem Cell Program at Children's Hospital Boston and established her laboratory in September 2006.

Mo Motamedi, PhD

Assistant Professor of Medicine

Mo Motamedi is an Assistant Professor of Medicine at Massachusetts General Hospital Center and Harvard Medical School. He completed his PhD from University of Alberta in Canada studying the molecular mechanism of replication-dependent homologous recombination using E. coli as a model. He then moved to Boston for his postdoctoral studies in the Department of Cell Biology at HMS, funded by two postdoctoral fellowships during is tenure. His postdoctoral studies in the Moazed Lab on the molecular mechanism of RNA-dependent epigenetic inheritance using the fission yeast as a model led to several publications and molecular insights including the Nascent Transcript Model, according to which non-coding RNAs (ncRNAs) tethered to chromatin provide a platform for the assembly of RNA processing and chromatin-modifying, -binding, and -remodeling complexes, which spread in cis to the neighboring chromosomal regions. This model established the first molecular blueprint for how long (lncRNAs) and small (sRNA) noncoding RNAs cooperate to regulate chromatin states – a mechanism which later was shown to be conserved in metazoans. His lab is now focused on the mechanisms by which chromatin and non-coding RNAs establish distinct transcriptional states as they transition into dormancy or quiescence using the fission yeast as a model. In recent years, he has expanded his research program into mammalian G0 cancer cells. Housed at the MGH Cancer Center, his vision is to see through the translation of one his lab's discoveries in yeast into real therapeutic targets in cancers. As a faculty, he received the the V Scholar, American Cancer Society, Howard M Goodman and other awards.

Therapy Guided by Protein Dynamics in Single Cells

Feature Research Speaker

Galit Lahav, PhD

Professor and Chair of Systems Biology

Galit Lahav received her PhD in 2001 from the Department of Biology in the Technion, Israel Institute of Technology. In 2003, she completed her postdoctoral fellowship at the Weizmann Institute of Science in Israel. She then spent a year at Harvard's Bauer Center for Genomics Research, and in the fall of 2004, she joined the Department of Systems Biology at Harvard Medical School. Her lab combines experimental and theoretical approaches to study the dynamics of signaling networks in human cells in order to understand cellular decision-making in individual cancer and healthy cells and why cells vary so dramatically in their response to irradiation and drugs.

Lahav has been recognized for her excellence in both teaching and research through several awards and honors including the Vilcek prize for Creative Promise in Biomedical Science and an Excellence in Mentoring award from Harvard Medical School. She is a dedicated mentor to new faculty at Harvard and organizes workshops and courses on leadership and managements skills for Harvard Junior Faculty. She is also highly committed to furthering the advancement of women in science.

Choosing The Next Generation Of Cancers Targets: How Not To Be Wrong

Keynote Speaker

William Kaelin, Jr, MD
Professor of Medicine

Dr. Kaelin is a Professor in the Department of Medicine at the Dana-Farber Cancer Institute, Harvard Medical School, and Associate Director, Basic Science, for the Dana-Farber/Harvard Cancer Center. He obtained his undergraduate and MD degrees from Duke University and completed his training in internal medicine at the Johns Hopkins Hospital, where he served as chief medical resident. He was a clinical fellow in Medical Oncology at the Dana-Farber Cancer Institute, during which time he was a McDonnell Scholar.

Dr. Kaelin is a member of the American Society of Clinical Investigation and the Institute of Medicine (IOM). He recently served on the National Cancer Institute Board of Scientific Advisors, the AACR Board of Trustees, and the IOM National Cancer Policy Board. He is a recipient of the Paul Marks Prize for cancer research from the Memorial Sloan-Kettering Cancer Center and the Richard and Hinda Rosenthal Prize from the AACR. In April 2010, Dr. Kaelin was named one of five recipients of the prestigious Canada Gairdner International Award, and he also was elected to the National Academy of Sciences.

A Howard Hughes Medical Investigator since 1998, Dr. Kaelin's research seeks to understand how, mechanistically, mutations affecting tumor-suppressor genes cause cancer. His long-term goal is to lay the foundation for new anticancer therapies based on the biochemical functions of tumor suppressor proteins. His work on the VHL protein helped to motivate the eventual successful clinical testing of VEGF inhibitors for the treatment of kidney cancer. Moreover, this line of investigation led to new insights into how cells sense and respond to changes in oxygen, and thus has implications for diseases beyond cancer, such as anemia, myocardial infarction and stroke.

Karin Grunebaum Foundation Poster Competition

1) **Rebecca Herbst**
   Longitudinal mapping of CD4 T cell heterogeneity in cancer identifies IL-33 as a driver of tumor immunosuppression

2) **Charlie Evavold**
   Host-derived Gasdermin D pores mediate opposing cell fates

3) **Marie Siwicki**
   Investigating neuroimmune interactions in the tumor microenvironment

4) **Liang Wei Wang**
   Epstein-Barr virus induces oncogenic one-carbon metabolism to support B cell transformation

5) **Paris Bentley**
   The Role of the SMC5/6 Complex in the Papillomavirus Lifecycle

6) **Jee-Eun Choi**
   SIRT6 acts as a tumor suppressor by inhibiting the growth of CD34+ tumor propagating cells in squamous cell carcinoma

7) **Jennifer Hsiao**
   MTAP as a tumor suppressor in melanoma

8) **Jessica Spinelli**
   Metabolic recycling of ammonia generates a localized pool of glutamate that promotes mitochondrial translation in breast cancer cells

9) **Renee Geck**
   Arginine catabolism is a metabolic vulnerability in triple-negative breast cancer

10) **Golnaz Morad**
    Using proteomics profiling to elucidate the interactions of breast cancer-derived exosomes with the blood brain barrier

11) **Benika Pinch**
    Targeting the Prolyl Isomerase Pin1 with Covalent Inhibitors

12) **Haley Manchester**
    Identifying new therapeutic combinations for NRAS-mutant melanomas

13) **Chelsea Powell**
    Chemically Induced Degradation of Anaplastic Lymphoma Kinase (ALK)

14) Jacob Layer
POLD2 Promotes Error-Prone Repair and Chromosomal Translocations

15) Caitlin Nichols
Loss of heterozygosity of essential genes represents a novel class of cancer vulnerabilities

16) Jonathan Lee
An Integrated Genome-wide CRISPRa Approach to Functionalize lncRNAs in Drug Resistance

17) Amy Schade
Cyclin D-CDK4 relieves cooperative repression of proliferation and cell cycle gene expression by DREAM and RB

18) Carmen Sivakumaren
Targeting the PIP4K2 lipid kinase family in cancer using novel covalent inhibitors

19) Andrew Giacomelli
Saturation mutagenesis of *TP53*

20) Nathan Schauer
Covalent USP7 inhibitors for wild-type p53 malignancies

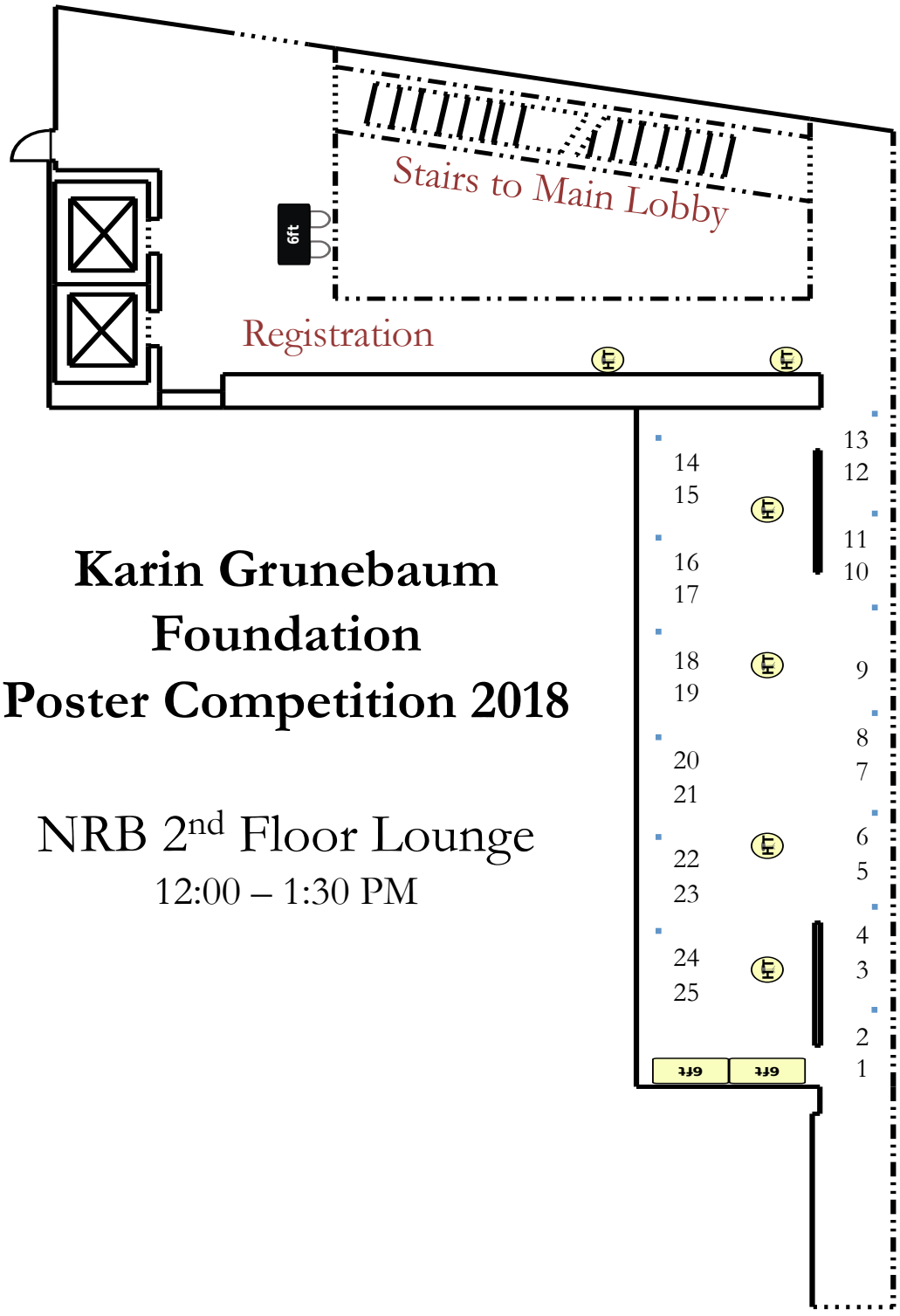
21) Thomas Howard
MDM2 and MDM4 are Therapeutic Vulnerabilities in Malignant Rhabdoid Tumors

22) Manav Gupta
Treatment of SMARCA4/BRG1 mutant lung cancers with ATR inhibition

23) Matthew McBride
The SS18-SSX fusion oncoprotein hijacks BAF complex targeting and function to drive synovial sarcoma

24) Maurizio Fazio
Chromatin remodeler SATB2 reactivates neural crest developmental program in melanoma leading to invasion and drug resistance

25) Roodolph St. Pierre
SMARCE1 de novo Missense Mutations in Coffin-Siris Syndrome Perturbs mSWI/SNF Structural Assembly at Neurodevelopmental Genes



Karin Grunebaum Foundation Poster Competition 2018

NRB 2nd Floor Lounge

12:00 – 1:30 PM

Student Poster Abstracts

Longitudinal mapping of CD4 T cell heterogeneity in cancer identifies IL-33 as a driver of tumor immunosuppression

Rebecca Herbst
Aviv Regev Lab

Antitumor immune response plays an important role in disease progression and presents an exciting opportunity for new immunotherapy based cancer therapies. However, we have limited understanding about the diversity of tumor infiltrating immune cells and the dynamics of this heterogeneous population during tumorigenesis. Here, using single cell RNA sequencing, we longitudinally profiled conventional CD4 T (Tconv) and Treg cells in a genetic mouse model of lung adenocarcinoma. We demonstrate that tumor-associated Treg and Tconv cells are highly diverse and dynamic, shifting towards increasingly immunosuppressive phenotypes as the tumor progresses. Interestingly, these phenotypic shifts are associated with T cell clonal expansion. Furthermore, we identify ST2 as a marker of effector Tregs, and show that Treg-specific ablation of ST2 enhances CD8+ T cell infiltration of tumors, potentially identifying it as an attractive therapeutic target in cancer. Collectively, our data demonstrates the power of high-resolution and longitudinal profiling of CD4 T cell to identify and target immunomodulators of tumoral T cells.

Host-derived gasdermin D pores mediate opposing cell fates

Charlie Evavold
Jonathan Kagan Lab

Interleukin-1 (IL-1) family cytokines are cytosolic proteins that exhibit inflammatory activity upon release into the extracellular space. These factors are released following various cell death processes, with pyroptosis being a common mechanism. Recently, it has been recognized that phagocytes can achieve a state of hyperactivation, which is defined by their ability to release IL-1β while retaining viability, yet it is unclear how IL-1 can be released from living cells. Herein, we report that the pyroptosis regulator gasdermin D (GSDMD) promotes IL-1β release from living macrophages that have been exposed to inflammasome activators, such as bacteria and their products or host-derived oxidized lipids. Cell- and liposome-based assays demonstrated that GSDMD pores were required for IL-1β transport across an intact lipid bilayer. These findings identify a non-pyroptotic function for GSDMD, and suggest host-derived membrane pores may serve as direct conduits for unconventional protein secretion. Our ongoing studies aim to determine how this family of pore-forming proteins may augment cell death and protein secretory phenotypes in cancer and the immune system.

Investigating neuroimmune interactions in the tumor microenvironment

Marie Siwicki
Mikael Pittet Lab

Neuroimmune interactions are increasingly appreciated as significant mediators of the immune responses. In the context of cancer, the immune response is now considered a hallmark of disease, influencing tumor initiation, progression, and response to treatment, notably in the era of immune checkpoint blockade therapy. However, a complete picture of factors influencing generation and efficacy of the anti-tumor immune response is lacking. A more comprehensive understanding of tumor-associated immune processes is thus needed. By interrogating immune interactions with the peripheral nervous system in the context of tumor biology, we aim to elucidate important and under-appreciated forces influencing the immune response in the tumor microenvironment, whether pro-tumoral or anti-tumoral. We aim to 1) Decipher contribution of different branches of the peripheral nervous system to the tumor microenvironment; and 2) Determine functional relevance of neuroimmune interactions to tumor progression or control.

Epstein-Barr virus induces oncogenic one-carbon metabolism to support B cell transformation

Liang Wei Wang
Benjamin Gewurz Lab

Epstein-Barr virus (EBV) transforms quiescent, non-dividing B-cells into continuously proliferating lymphoblastoid B-cell lines (LCL), which is a particularly useful characteristic for modeling lymphomagenesis in both in vitro and in vivo contexts. Knowledge of how EBV remodels host metabolic pathways to support rapid B-cell outgrowth and to overcome redox stress remains incomplete. To gain insights into EBV-induced metabolic dependency factors, we constructed a proteomic map of B-cell transformation. Primary B-cells from 12 human donors were profiled by tandem-mass-tag mass spectrometry at rest and at 9 timepoints after EBV infection. This approach, termed quantitative temporal viromics, generated expression profiles of >6500 host and 17 viral proteins and highlighted EBV-induced pathways. Notably, EBV infection triggered B-cell mitochondrial remodeling, with the one- carbon (1C) metabolism pathway among the most strongly upregulated. Mitochondrial 1C metabolism utilizes folate-dependent chemical reactions to generate formate, glycine and reduced NADPH from serine, a non-essential amino acid. Epstein-Barr virus-encoded nuclear antigen 2 (EBNA2) and c-MYC were critical for upregulation of key mitochondrial 1C enzymes, including methylene tetrahydrofolate dehydrogenase 2 (MTHFD2), which is highly expressed during fetal development but not in most adult tissues. Chemical and CRISPR genetic analyses underscored the functional significance of MTHFD2 and mitochondrial 1C metabolism in EBV-driven B-cell growth and survival. MTHFD2 was important for intramitochondrial NADPH generation, and compartment-specific perturbation of mitochondrial NADPH levels diminished LCL growth and survival. Isotope tracing studies delineated roles for EBV-induced mitochondrial 1C metabolism in purine and thymidylate syntheses and NADP+ reduction. To fuel 1C metabolism, EBV upregulated import and de novo synthesis of serine, each representing a non-redundant dependency factor. Glycine derived from 1C metabolism also supported de novo glutathione synthesis, which together with NADPH facilitated redox homeostasis. Our results highlight mitochondrial 1C metabolism as a key EBV-induced metabolic dependency factor and potential therapeutic target.

The Role of the SMC5/6 Complex in the Papillomavirus Lifecycle

Paris Bentley
Peter Howley Lab

Papillomaviruses cause proliferative epithelial lesions and the high-risk subtypes are the causative agent of cervical cancer. These small DNA viruses largely rely on interactions with host cell machinery to complete their replication cycle. The papillomavirus E2 protein is the major replicative protein of papillomaviruses. It influences transcription of viral genes and is required for viral DNA replication and persistence of the viral genome in infected cells. Because E2 lacks enzymatic activity, many of its functions are mediated by interactions with host cell machinery. Proteomic experiments have identified an interaction of the host SMC5/6 complex with the E2 proteins of various papillomavirus types. The SMC5/6 complex is a member of the cohesin and condensin, structural maintenance of chromosomes family of proteins and is conserved in all eukaryotes. It is activated by the DNA damage response and is essential for DNA double-strand break repair through homologous recombination. Here, I investigated the role of SMC5/6 in various E2 functions. The papillomavirus replication cycle is tightly linked to the differentiation state of the host cell, where viral DNA undergoes three modes of DNA replication. My studies confirm the interaction with E2 and suggest that SMC5/6 may play different roles in the different types of viral DNA replication. Experiments utilizing a cell line derived from a cervical intraepithelial neoplasia, harboring episomal copies of high-risk HPV31b DNA, indicate that the SMC5/6 complex is required for maintenance of viral episomes in host cells. This suggests a possible mechanism by which E2 ensures long-term persistence of HPV.

SIRT6 acts as a tumor suppressor by inhibiting the growth of CD34+ tumor propagating cells in squamous cell carcinoma

Jee-Eun Choi
Raul Mostoslavsky Lab

Head and neck squamous cell carcinoma (HNSCC) is one of the most common cancers in the US. As such, a better understanding in HNSCC carcinogenic process and contributing molecular mechanisms may pinpoint to new therapeutic interventions for the patients. Notably, despite extensive genetic studies, little is known on the metabolic features of HNSCC. Metabolic reprogramming to aerobic glycolysis has recently emerged as a key event during carcinogenesis. Our lab has shown that the histone deacetylase SIRT6 inhibits HIF-1α- and c-Myc-dependent transcription, both of which are critical in metabolic reprogramming of cells, thereby acting as a tumor suppressor in the context of colon cancer. Based on these results, we aimed to assess whether SIRT6 loss facilitates squamous cell carcinogenesis, specifically affecting glucose metabolism and stemness of cancer cells. In an *in vivo* model of cutaneous squamous cell carcinogenesis with SIRT6 deletion (*K14-cre+; Sirt6*$^{fl/fl}$), tumor onset was faster in *Sirt6*$^{fl/fl}$ mice compared to WT animals, and those tumors from *Sirt6*$^{fl/fl}$ mice were bigger. SIRT6 loss also positively regulates tumor maintenance without continuous proliferative cues, suggesting that SIRT6 deletion could impact on the tumor propagating cells (TPCs). Strikingly, loss of SIRT6 increased the percentage of TPCs (a6^{high}/CD34+), most of which were highly glycolytic. Administration of a glycolytic inhibitor (dichloroacetate) *in vivo* effectively inhibited tumor growth and maintenance, resulting in much fewer percentages of TPCs. Our results indicate that enhanced glucose metabolism is crucial in maintaining and expanding TPCs. In human HNSCC cell lines, SIRT6 negatively regulates glucose metabolism and cell growth *in vitro* and *ex vivo*, nicely recapitulating the *in vivo* observations. Glucose isotope tracing experiments *in vitro* suggest that pentose phosphate pathway and generation of antioxidant molecules are main metabolic pathways affected by SIRT6. Transcriptomic analysis of TPCs from *in vivo* skin tumors revealed critical gene expression signatures, where TPCs exhibit decreased differentiation, increased stem-like, and enhanced antioxidative/redox markers. SIRT6 loss even further up-regulated stemness genes and the genes that generate antioxidant molecules, providing molecular mechanisms to explain a more aggressive tumorigenic phenotype in SIRT6-deleted tumors. Overall, our studies indicate that high glycolytic flux (which can contribute to the pentose phosphate pathway and antioxidant molecules) is crucial in TPCs. Our studies clearly demonstrate that SIRT6 acts as a tumor suppressor by regulating glucose metabolism and stemness of tumor cells. This study has further therapeutic implication that targeting antioxidant defense pathways could effectively eradicate TPCs, critical population maintaining a whole tumor.

Metabolic enzyme MTAP as a tumor suppressor in melanoma

Jennifer Hsiao
David Fisher Lab

Methylthioadenosine phosphorylase (MTAP) is an enzyme that is expressed in virtually all normal tissues but lost in many cancers. MTAP deficiency can be due to either deletion of the MTAP gene or methylation of the MTAP promoter. In normal cells, MTAP catalyzes the conversion of methylthioadenosine (MTA), produced during polyamine biosynthesis, to adenine and 5-methylthioribose-1-phosphate, which is subsequently converted to methionine. Although recent genome wide association studies (GWAS) have associated the MTAP locus with melanoma risk, the molecular mechanisms linking MTAP loss to increased tumorigenesis are not yet fully understood. In this study, we hypothesized that loss of MTAP and the resulting accumulation of MTA would have an effect on microphthalmia transcription factor (MITF), the master regulator of melanocytes that has been shown to be an oncogene in melanoma. We present a novel signaling mechanism in which loss of MTAP and subsequent accumulation of MTA induces expression of MITF via inhibition of the phosphodiesterase PDE4D3 by MTA. Inhibition of PKA abolishes the induction of MITF, suggesting that the PKA pathway is hyperactivated when MTAP levels are low. We show that downregulation of MTAP expression leads to increased proliferation of melanoma cells. Using a melanoma xenograft mouse model, we observed that downregulation of MTAP expression leads to increased tumor growth in vivo. These data all point to MTAP as a tumor suppressor in melanoma, and indicate that MTAP loss is an alternative mechanism of dysregulating MITF, a known oncogene in melanoma.

Metabolic Recycling of Ammonia Generates a Localized Pool of Glutamate that Promotes Mitochondrial Translation in Breast Cancer Cells

Jessica Spinelli
Marcia Haigis Lab

Although ammonia (NH_3) is a ubiquitous by-product of tumor metabolism, the fate of NH_3 in cancer had never been investigated. Plasma NH_3 is maintained below 50 mM in healthy adults to evade toxicity associated with hyperammonemia. Conversely, in the tumor microenvironment (TME), NH_3 accumulates to sub-millimolar levels. Therefore, we hypothesized that cancer cells are uniquely poised to tolerate NH_3. In this study, we investigated the fate of NH_3 in cancer cells as either (1)- a toxic metabolic waste product or (2)- a nitrogen source for biosynthetic reactions.

To determine the fate of NH_3 in cancer cells, we developed an LC-MS assay that enabled detection and distinction of NH_3 isotopologues ($^{14}NH_3$ and $^{15}NH_3$) in cellular lysates to facilitate metabolic tracing studies (Spinelli *et al.,*Sci Rep, 2017). Using this assay in combination with metabolomics, we tracked the fate of $^{15}NH_3$ in breast cancer cells. We found that NH_3 generated in metabolic reactions is recycled with 60% efficiency through reductive amination catalyzed by glutamate dehydrogenase (GDH), generating glutamate and downstream amino acids. Assimilation occurs via the "reverse" activity of GDH, in which NH_3 is the kinetic limitation of this reaction. Therefore, assimilation is specific to physiological niches that have high NH_3 levels, such as the TME. We then measured NH_3 recycling *in vitro, in vivo* xenograft models, and in primary tumors resected from breast cancer patients at Massachusetts General Hospital. Thus, we demonstrated that tumor cells scavenge the metabolic by-product NH_3 as a nitrogen source for biomass (Spinelli *et al*., Science, 2017).

Next, we characterized the effect of NH_3 on breast cancer growth and survival. NH_3 was not toxic to tumor cells, contrary to normal cells. GDH-mediated NH_3 assimilation stimulated proliferation in breast cancer cells, suggesting that amino acids are a limiting factor for proliferation. Since NH_3 is initially assimilated by GDH in the mitochondria, we investigated whether subcellular compartmentalization of amino acids played a role in proliferation. Through metabolic tracing, rapid immunoprecipitation of mitochondria for metabolomics, and genetic perturbations, we identified that glutamate generated downstream of NH_3 assimilation is compartmentalized between the mitochondria and cytosol through the activities of the GOT isozymes. Depletion of GOT1, which converts aspartate to glutamate in the cytosol, repressed proliferation, whereas depletion of GOT2, which converts glutamate to aspartate in the mitochondria, accelerated proliferation. These results suggest that mitochondrial glutamate is limiting for proliferation. Mechanistically, we determined that NH_3 assimilation, which generates a confined pool of glutamate, stimulates mitochondrial translation. Inhibition of mitochondrial translation with antibiotics abrogates the pro-stimulatory effect of NH_3 on proliferation. Thus, NH_3 assimilation stimulates rapid proliferation in breast cancer cells through elevating the mitochondrial glutamate pool and activating local translation.

Arginine catabolism is a metabolic vulnerability in triple-negative breast cancer

Renee Geck
Alex Toker Lab

Triple-negative breast cancer (TNBC) comprises 15-20% of breast cancer cases, but accounts for a disproportionately high percentage of breast-cancer related deaths. This is partially due to a lack of targeted therapies, as the standard of care treatment for TNBC remains genotoxic chemotherapy. Previous work by our lab and others has identified metabolic vulnerabilities in breast cancer that can be used to improve response to chemotherapy. Here we aimed to identify additional metabolic vulnerabilities that arise following chemotherapy treatment in TNBC. Using mass spectrometry-based metabolomics analyses and 13C6-arginine tracing, we found that several metabolites involved in arginine catabolism and the urea cycle were increased in TNBC cells treated with cisplatin and doxorubicin. The levels of polyamines, creatinine and creatinine, carbamoyl phosphate, and aspartate were increased, along with the incorporation of arginine into glutamate. To investigate the mechanism, we measured expression of several genes in arginine metabolism. Cisplatin and doxorubicin most significantly elevated the expression of ARG2 and GLS2. This increased expression was recapitulated by other DNA-damaging agents but not by paclitaxel, suggesting this is a specific response to DNA damage. Applying transcription factor prediction software to our list of upregulated genes suggested that ATF3 is a strong potential regulator of ARG2, and we identified an ATF3-binding consensus sequence in the ARG2 promoter. ATF3 is known to be increased in response to chemotherapy, and we determined that ATF3 knockdown reduced the ability of cisplatin to elevate ARG2, suggesting that DNA-damaging agents increase ARG2 transcription via ATF3. This increase in ARG2 is involved in the response of TNBC cells to chemotherapy, as ARG2 knockdown significantly decreased the IC50 of cisplatin. These findings indicate that alterations in arginine metabolism are a metabolic vulnerability in TNBC cells treated with genotoxic chemotherapy.

Using proteomics profiling to elucidate the interactions of breast cancer-derived exosomes with the blood brain barrier

Golnaz Morad
Marsha Moses Lab

Breast cancer metastasis to brain is associated with a dismal prognosis commonly attributed to a limited understating of the mechanisms driving this pathological process. Elucidation of the early events leading to brain metastasis is essential to the development of more effective therapeutic and diagnostic approaches. With an interest in the role of breast cancer-derived exosomes in brain metastasis, our group has previously shown that exosomes derived from a brain-seeking variant of the breast cancer cell line MDA-MB-231 (Br-Ex) can facilitate brain metastasis by inducing alterations in the protein expression profile of astrocytes, one of the components of the blood brain barrier (BBB). This observation led us to hypothesize that the interaction(s) between exosomes and astrocytes is more efficient compared to brain endothelial cells (ECs) or pericytes, the two additional major components of the BBB, resulting in more prominent alterations in the protein expression profile of astrocytes. To test this hypothesis, we first quantified and compared the uptake of exosomes by brain ECs, astrocytes, and pericytes in vitro. The uptake of Br-Ex by astrocytes was significantly greater than that of brain ECs ($P<4e-3$) and pericytes ($P<1e-3$). In contrast, exosomes derived from parental or bone-seeking MDA-MB- 231 cells (P-Ex and Bo-Ex, respectively) did not show a preferential uptake by astrocytes. We have also demonstrated the uptake of Br-Ex by astrocytes in vivo. The uptake of exosomes by different cell types predominantly relies on the interaction of exosomal proteins with different receptors on the recipient cells. To determine the exosomal proteins potentially involved in the preferential uptake of Br-Ex by astrocytes, we performed quantitative mass spectrometry on the P-, Bo-, and Br-Ex via Isobaric Tag for Relative and Absolute Quantitation (iTRAQ) analysis. Database searches were performed against human proteins in the SwissProt database and a total of 126 proteins were detected with over 95% confidence. Pairwise comparisons identified a total of 27 and 21 proteins with statistically significant differential expression ($P < 0.05$) in the Br-Ex compared to the P- and Bo-Ex, respectively. Functional enrichment analysis of the detected proteins demonstrated that proteins belonging to the cell migration and focal adhesion categories were over-represented in the Br-Ex. Among these proteins, a number of integrins and annexins were highly enriched in the Br-Ex and can potentially be involved in the preferential uptake of these exosomes by astrocytes.

These findings indicate that exosomes derived from brain-seeking breast cancer cells can preferentially interact with astrocytes and these interactions can be driven by exosomal integrins and annexins. (The authors are grateful for the support of the Breast Cancer Research Foundation and the Advanced Medical Research Foundation.)

Targeting the Prolyl Isomerase Pin1 with Covalent Inhibitors

Benika Pinch
Nathanael Gray Lab

Pin1 regulates the function and stability of specific phosphoproteins by catalyzing the cis/trans isomerization of peptidyl-prolyl bonds that follow phosphorylated serine or threonine residues. In triple-negative breast cancer (TNBC), Pin1 mediates oncogenic signaling networks to drive the epithelial-mesenchymal transition (EMT) and cell migration, suggesting that Pin1 inhibition could address the critical need for targeted TNBC therapy. However, despite previous efforts, there are currently no Pin1 inhibitors that can serve as informative cellular probes. Using structure-based design and in silico docking studies, we developed and characterized peptidomimetic inhibitors that form a covalent adduct with a critical cysteine residue, C113, in the Pin1 active site. Through iterative rounds of structure activity relationship (SAR) studies, the lead compounds were optimized to generate a highly potent, cell permeable, and Pin1-selective covalent inhibitor. In parallel to inhibitor development, we assessed the expected phenotype of Pin1 loss by employing a chemical genetic strategy to achieve targeted Pin1 degradation in TNBC MDA-MB-231 cells.

Identifying new therapeutic combinations for NRAS-mutant melanomas

Haley Manchester
Karen Cichowski Lab

Melanoma is a highly aggressive, treatment-refractory skin cancer that accounts for ~10,000 deaths in the U.S. per year. It is defined by mutations in the RAS/RAF/MEK/ERK pathway, which is important for cell proliferation and growth. Though the pathway can be targeted by specific therapies, resistance eventually occurs; therefore, identifying new combination therapies that prevent resistance and/or elicit a more durable response is an urgent clinical need for these patients. To address this, we conducted a genome-scale negative selection screen with CRISPR-Cas9 gene editing technology to identify potential targets that cooperate with the current standard of care for NRAS-mutant melanoma patients. We identified 152 top hits that we then validated with genetic knock-down through pooled siRNA in other melanoma cell lines. Out of the top 25 hits, 3 have inhibitors in preclinical or clinical trials, and we are further investigating the cooperative effect between these inhibitors and the standard of care for NRAS-mutant melanoma.

Chemically Induced Degradation of Anaplastic Lymphoma Kinase (ALK)

Chelsea Powell
Nathanael Gray Lab

We present the development of the first small molecule degraders that can induce anaplastic lymphoma kinase (ALK) degradation, including in non-small-cell lung cancer (NSCLC), anaplastic large-cell lymphoma (ALCL), and neuroblastoma (NB) cell lines. These degraders were developed through conjugation of known pyrimidine-based ALK inhibitors, TAE684 or LDK378, and the cereblon ligand pomalidomide. We demonstrate that in some cell types degrader potency is compromised by expression of drug transporter ABCB1. In addition, proteomic profiling demonstrated that these compounds also promote the degradation of additional kinases including PTK2 (FAK), Aurora A, FER, and RPS6KA1 (RSK1).

POLD2 Promotes Error-Prone Repair and Chromosomal Translocations

Jacob Layer
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Chromosomal translocations are recurrent chromosomal rearrangements that can initiate carcinogenesis. While translocation formation is influenced by proximity of partner chromosomes, frequency of double-strand breaks (DSBs), and specific repair factors, mechanisms coordinating translocation formation remain unknown.

We identified POLD2, a subunit of DNA polymerase δ (Pol δ), as a translocation-promoting factor using a medium-throughput shRNA screen (Day et. al. Nat. Comm. 2017). Upon validation in two independent assays, a 50% reduction in translocation frequency was detected in POLD2 depleted cells, which was rescued by POLD2 lentiviral overexpression. These effects were not due to cell cycle arrest, changes in gRNA cutting efficiency, or altered replication stress levels.

To determine how POLD2 affects repair junctions, amplicon resequencing was used to quantify mutations induced by CRISPR/Cas9 at the *CD4* locus. POLD2 was found to suppress accurate non-homologous end joining (NHEJ) and promote the use of longer microhomologies during repair. These data suggest POLD2 modulates NHEJ, either by influencing its precision or by promoting microhomology-mediated end joining (MMEJ), a mutagenic form of NHEJ frequently associated with chromosomal rearrangements.

Because MMEJ and Homologous Recombination (HR) are both initiated by end-resection, we suspected POLD2 might promote HR as well. We observed a 60% decrease in HR in two independent HR fluorescent reporters and a decrease in mutagenic NHEJ in another fluorescent reporter. These results support our sequencing data and imply POLD2 participates in end-resection at DSBs.

POLD2 also interacts with DNA Polymerase ζ (Pol ζ). To determine if Pol δ or Pol ζ promote translocations, translocation frequency was measured in POLD1 (Pol δ catalytic subunit) and REV3L (Pol ζ catalytic subunit) deficient backgrounds. Translocation frequency in POLD1 and REV3L deficient backgrounds decreased by 50%, corroborating the POLD2 data and suggesting Pol δ and Pol ζ cooperate during translocation formation.

These data suggest POLD2 promotes translocations and inaccurate repair, and may play a role in end-resection. We are testing this hypothesis by monitoring RPA deposition at DSBs and sequencing *CD4* repair products in POLD1 exonuclease deficient cells. Together, these data highlight a previously unappreciated role for Pol δ during DSB repair in human cells.

Loss of heterozygosity of essential genes represents a novel class of cancer vulnerabilities

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Despite progress in precision cancer drug discovery, few highly selective therapies exist in the clinic, creating the need for additional therapeutic targets. We have shown that copy number alterations (CNAs) in essential genes represent novel non-driver gene vulnerabilities in cancer. Here we interrogate loss of heterozygosity (LOH) of single nucleotide polymorphisms (SNPs) located in essential genes as a novel class of candidate therapeutic targets. We hypothesized that monoallelic inactivation of the single allele retained in tumors can selectively kill cancer cells, while somatic cells, which retain both alleles, will tolerate allele-specific knockout. We identified a list of over 5000 common missense SNPs in at least 1500 essential genes that undergo LOH in cancer and performed proof-of-concept allele-specific gene inactivation in two essential genes (PRIM1 and EXOSC8) using CRISPR-Cas9. We assessed the fidelity of allele-specific gene disruption and its cellular effects on gene expression, cell growth, and cell death in LOH and non-LOH genetic contexts. We determined that allele-specific knockout of PRIM1 and EXOSC8 selectively targets cells harboring only the single targeted allele of that gene. In cells retaining only the sensitive allele, we observed decreased target gene expression and cell viability that did not occur in cells retaining the resistant allele. We conclude that allele-selective inactivation of essential genes in regions of LOH (such as PRIM1 and EXOSC8) represents a novel candidate therapeutic strategy in cancer. The corresponding class of novel non-driver cancer vulnerabilities may provide a rich source of targets for future precision therapeutic development using gene editing, RNAi, or small-molecule approaches.

An Integrated Genome-wide CRISPRa Approach to Functionalize lncRNAs in Drug Resistance

Jonathan Lee
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Resistance to chemotherapy plays a significant role in cancer mortality. To identify genetic units affecting sensitivity to Ara-C, the mainstay of treatment for acute myeloid leukemia (AML), we developed a comprehensive and integrated genome-wide platform based on a dual protein-coding and long noncoding integrated CRISPR-activation screening (DICaS). Putative resistance genes were initially identified using pharmacogenetic data from 760 human pan-cancer cell lines. Subsequently, genome scale functional characterization of both coding and long non-coding RNA (lncRNA) genes by CRISPR activation was performed. For lncRNA functional assessment, we developed a CRISPR activation library to target 14,701 lncRNA genes. Computational and functional analysis identified novel cell-cycle, survival/apoptosis, and cancer signaling genes. Furthermore, transcriptional activation of the GAS6-AS2 lncRNA, identified in our analysis, leads to hyperactivation of the GAS6/TAM pathway, a resistance mechanism in multiple cancers including AML. Thus, DICaS represents a novel and powerful approach to identify integrated protein-coding and long noncoding gene pathways of therapeutic relevance.

Cyclin D-CDK4 relieves cooperative repression of proliferation and cell cycle gene expression by DREAM and RB

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The Retinoblastoma protein (RB) restricts cell cycle gene expression and entry into the cell cycle. The RB-related protein p130 forms the DREAM (DP, RB-like, E2F and MuvB) complex and represses cell cycle dependent gene expression during quiescence. Although both RB and DREAM bind to E2F dependent gene promoters, it remains unclear if they cooperate to restrict cell cycle entry. We demonstrate that Cyclin D-CDK4 phosphorylation of p130 during early G1 disrupts binding to E2F4 but not MuvB. Similar to normal cells, cells lacking p130 had low levels of E2F dependent genes during quiescence and early G1. In contrast, cells lacking both p130 and RB had higher levels of cell cycle genes compared to cells lacking only RB. Furthermore, RB knockout cells had partial resistance to the CDK4 inhibitor palbociclib, while the RB and p130 double knockout cells were significantly more resistant. We observed the cooperative effect of DREAM and RB on cell cycle gene expression and sensitivity to CDK4 inhibition in normal cells and palbociclib-sensitive cancer cell lines. These data support a model where DREAM and RB cooperate to repress cell cycle gene expression during G0 and early G1, with RB playing a dominant role during mid to late G1.

Targeting the PIP4K2 lipid kinase family in cancer using novel covalent inhibitors

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The phosphatidylinositol 5-phosphate 4-kinases, PIP4K2α, β and γ been shown to be crucial in driving a number of cancers, for instance cancers harboring *TP53*deletions or loss-of-function p53 mutations, acute myeloid and lymphoblastic leukemia, breast cancer, as well as involved in other disease pathologies such as proliferative vitreoretinopathy, diabetes, and neurodegenerative disorders. However, the druggability of these lipid kinases is severely understudied due to a lack of potent and specific small molecules available. Here we present the discovery and characterization of a novel pan-PIP4K2 inhibitor, THZ-P1-2, that covalently targets cysteine residues on a disordered loop region in PIP4K2a/b/g. Cancer cell line profiling demonstrates AML and ALL cell lines to be sensitive to THZ-P1-2 covalent targeting, consistent with PIP4K2's purported role in leukemogenesis. THZ-P1-2 causes lysosomal disruption, defects in the autophagosome clearance, and an increase in TFEB nuclear localization and TFEB-related gene expression, halting autophagy and phenocopying the effects of genetic deletion of PIP4K2. These studies provide evidence that irreversible inhibition of PIP4K2 by THZ-P1-2 compromises autophagy, an essential alternative energy source during periods of metabolic stress which cancer cells depend on to maintain cellular homeostasis and prolonged cell viability. In conclusion, the development of PIP4K2 tool compounds can be used to investigate PIP4K2 as a novel therapeutic target in cancer metabolism, and further inform drug discovery campaigns for these lipid kinases in the context of cancer and potentially other autophagy-dependent diseases.

Saturation mutagenesis of *TP53*

Andrew Giacomelli
Bill Hahn Lab

Most TP53 mutations found in human tumors are missense mutations. Mutant p53 protein is often highly expressed in tumor cells and some variants display dominant-negative or gain-of-function activities in model systems. However, it remains unclear why some TP53 mutations are observed more frequently than others in sporadic human tumors. Through interrogation of large-scale loss-of-function screens conducted in hundreds of human cancer cell lines and saturation mutagenesis screens carried out in an isogenic pair of TP53-wild-type and -null cell lines, we found that the selective advantage associated with TP53 mutation results from loss-of-function and dominant-negative activity. By integrating our screening data with the COSMIC mutational signatures database, we developed a statistical model that describes the TP53 mutational spectrum as a function of the baseline probability of acquiring each mutation and the cellular fitness advantage conferred by attenuation of p53 activity. These observations suggest that mutational processes combine with phenotypic selection to dictate the landscape of recurrent TP53 alterations seen in human cancer.

Covalent USP7 inhibitors for wild-type p53 malignancies

Nathan Schauer
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Deubiquitinating enzymes (DUBs) counteract E3 ubiquitin ligases by removing ubiquitin tags from protein substrates. K48- and K11-polyubiquitin chains target proteins for proteasomal degradation, and alteration of the ubiquitin-proteasome system (UPS) has proven clinically useful, either through direct inhibition of the proteasome (bortezomib) or by targeted recruitment of E3 ligases to specific substrates (lenalidomide). Another UPS targeting strategy is direct inhibition of deubiquitinating enzymes, DUBs, which remove polyubiquitin marks and prevent target degradation. Therefore, DUB inhibition should increase the proteasomal trafficking of native DUB substrates. DUBs are nominally "druggable" enzymes that contain a solvent-accessible binding pocket, but this binding pocket is both very large (accommodating ubiquitin, an 8.6-kDa protein) and highly conserved. It is thus still an open question whether potent and selective substrate-competitive DUB inhibitors can be achieved. Previously, we identified a compound that binds 12 angstroms from the active site cysteine of USP7, the best studied DUB, in a binding site that, surprisingly, is important for ubiquitin binding but not conserved. Here, we show that extending the inhibitor scaffold from this binding site to the active site cysteine results in a covalent inhibitor with dramatic increase in enzymatic and cellular potency while still retaining a high degree of USP7 selectivity across the entire human proteome. USP7 has been hypothesized as a therapeutic target in p53-WT tumors, but previous small molecule studies have shown conflicting effects of USP7 inhibition on p53. Here, we show that our covalent USP7 inhibitors rapidly induce MDM2 degradation and exhibit cell killing in a p53-dependent manner across multiple cell lines. In a panel of > 500 cancer cell lines, response to USP7 loss, MDM2 loss, and TP53 loss all predict response to our inhibitors, implying that the MDM2 / p53 axis is a key therapeutic target for USP7 inhibitors across all cancer types.

MDM2 and MDM4 are Therapeutic Vulnerabilities in Malignant Rhabdoid Tumors

Thomas Howard
William Hahn and Charles Roberts Labs

Malignant rhabdoid tumors (MRT) are highly aggressive pediatric cancers that respond poorly to current therapies. We screened several MRT cell lines each with large-scale RNAi, CRISPR-Cas9, and small-molecule libraries to identify potential drug targets specific for these cancers. We discovered *MDM2* and *MDM4*, the canonical negative regulators of p53, as significant vulnerabilities. Using two compounds currently in clinical development, we show that MRT cells are more sensitive than other p53 wild-type cancer cell lines to MDM2 and dual MDM2/4 inhibition *in vitro*. We show that loss of SMARCB1, a subunit of the SWI/SNF (BAF) complex mutated in nearly all MRT, sensitizes cells to MDM2 and MDM2/4 inhibition by enhancing p53-mediated apoptosis. Both MDM2 and MDM2/4 inhibition slowed MRT xenograft growth *in vivo*, with the former causing marked regression of all xenografts including durable complete responses in 50% of mice. Together, these studies identify a genetic connection between mutations in the SWI/SNF chromatin-remodeling complex and the tumor suppressor gene p53, and provide preclinical evidence to support the targeting of MDM2 and MDM4 in this often-fatal pediatric cancer.

Treatment of SMARCA4/BRG1 mutant lung cancers with ATR inhibition

Manav Gupta
Carla Kim Lab

The SMARCA4/BRG1 gene encodes the ATP-dependent helicase component of the SWI/SNF complex involved in chromatin modulation and is either mutated or lost in up to 20% of human non-small cell lung cancers (NSCLC). BRG1 is often co-mutated with KRAS and p53, and there are currently no targeted therapies for this genetic subtype of lung cancer. Recently, we demonstrated that genetic or pharmacological inhibition of EZH2 sensitizes BRG1-deficient cell lines when combined with the common chemotherapy etoposide, which inhibits DNA topoisomerase II alpha (TopoIIα). Based on these results, we hypothesized that BRG1 deficiency may lead to defects in DNA damage response (DDR) and subsequently DNA repair. To investigate this hypothesis, we used CRISPR-Cas9 genome editing to generate Brg1 knockout cell lines from tumor cells derived from a $KrasG12^{D}/^{+}$; $p53^{\Delta}/^{\Delta}$ (KP) mouse model of lung adenocarcinoma. Similar approaches were taken to generate isogenic human BRG1 knockout cells in KP mutant human lung cancer cell lines H460 and H2009. Brg1/BRG1 knockout cell lines were up to 4-fold more sensitive to an etoposide dose-response treatment. In order to understand the mechanism of this response, we treated our cells with inhibitors that target key DNA DDR kinases, ATM and ATR. Interestingly, while ATM inhibition resulted in no observable change between Brg1 wildtype and mutant cells, ATR inhibition was significantly effective (> 5-fold) in sensitizing cells with Brg1 loss. We looked at levels of gamma-H2Ax and replication protein A (RPA) in Brg1 wildtype and mutant cells upon ATR inhibition, as markers of replicative stress and DNA damage and found that Brg1 mutant cells had significant increase in accumulation of both gamma-H2Ax foci and RPA after washout of the ATR inhibitor. To validate the direct correlation between Brg1 loss and sensitivity to ATR inhibition, we re-expressed human BRG1 protein in mouse Brg1 knockout cells and observed a significant increase towards resistance to ATR inhibition, similar to mouse Brg1 wild type cells. Response to ATM inhibition was unaffected irrespective of Brg1 status. We next compared tumor formation and histology in KP mice versus KP mice harboring floxed Brg1 (KPB) alleles, and approximately 11-12 weeks post tumor induction, we observed that KPB had a significantly higher tumor burden than KP, implicating Brg1 as a potent tumor suppressor gene in lung cancer. 100% of all KPB mice had high grade 4 metastatic tumors compared to KP mice, where none of the mice showed an observable grade 4 tumor. To further investigate the sensitivity to ATR inhibition, we treated cell lines derived from either KP or KPB tumors with ATR inhibitors, and found that Brg1 mutant tumor cells were up to 3-fold more sensitive to ATR inhibition treatment, further corroborating our findings from in vitro generated Brg1 knockout cells. Taken together, our data strongly suggests that ATR inhibition could be a potentially beneficial strategy to treat lung cancer patients with BRG1 mutations or loss.

The SS18-SSX fusion oncoprotein hijacks BAF complex targeting and function to drive synovial sarcoma

Matthew McBride
Cigall Kadoch Lab

Mammalian SWI/SNF (BAF) complexes are mutated in over 20% of human cancers, with gain- and loss-of- function perturbations each implicated in malignancy. In synovial sarcoma (SS), the hallmark SS18-SSX fusion oncoprotein renders BAF complexes aberrant in two manners: gain of 78 amino acids of SSX to the SS18 subunit, and concomitant loss of BAF47 subunit assembly. Here we demonstrate that SS18-SSX globally hijacks BAF complexes on chromatin to activate the unique SS transcriptional signature found in primary tumors and cell lines. Specifically, SS18-SSX targets BAF complexes from enhancers to broad polycomb domains, at which they oppose PRC2-mediated repression to activate bivalent genes. Reassembly of BAF47 upon suppression of SS18-SSX mediates enhancer activation, but is dispensable for SS cell proliferative arrest. These results establish a global hijacking mechanism for SS18-SSX on chromatin, and define the distinct contributions of two concurrent BAF complex perturbations.

Chromatin remodeler SATB2 reactivates neural crest developmental program in melanoma leading to invasion and drug resistance

Maurizio Fazio
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Epigenetic regulators are emerging as key factors in the pathogenesis of both solid cancers and hematological malignancies. In melanoma, *KDM5B* has been shown to drive a slow cycling drug-resistant population and *ARID2* has been identified among the most recurrently mutated genes in The Cancer Genome Atlas sequencing project. To gain insight into additional epigenetic pathways that might contribute to melanoma progression, we undertook an *in vivo* transgenesis overexpression screen for chromatin factors that accelerate melanoma development in a genetically engineered zebrafish model driven by the most frequent melanoma oncogene *BRAFV600E* and *p53* loss of function.

Out of 83 tested factors, we identified Special AT-rich Binding protein 2 (SATB2) as a novel potent accelerator of melanoma. SATB2 is a chromatin remodeler required for vertebrate neural crest development, whose alterations in humans lead to a neurocristopathy known as SATB2-associated syndrome, which is phenocopied by morpholino knockdown in developing zebrafish embryos. Its overexpression in melanoma, which is observed in ~5% of patients, does not affect cell proliferation but leads to the formation of invadopodia and a significant increase in tumor-propagating and metastatic potential *in vivo*. Chromatin analysis by ChIP-seq and ATAC-seq revealed that SATB2 binding at target loci leads to altered levels of H3K27ac and chromatin accessibility, resulting transcriptionally in the reactivation of an invasive neural crest EMT developmental program. Additionally, we observed a transcriptional switch in SATB2 overexpressing melanoma towards activation of the PDGF-SRC signaling axis and the recently described AXLhighMITF low state. This transcriptional phenotype switch functionally results in resistance to targeted MAPK inhibition in allotransplanted primary tumors and A375 human melanoma cells.

In summary, our work illustrates how epigenetic regulation of transcriptional states affects melanoma progression and informs patient treatment with the current standard of care MAPK inhibitors.

SMARCE1 de novo Missense Mutations in Coffin-Siris Syndrome Perturbs mSWI/SNF Structural Assembly at Neurodevelopmental Genes

Roodolph St. Pierre
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SMARCE1 germline *de novo* missense mutations have been strongly linked to Coffin-Siris syndrome (CSS), a rare multiple-congenital disorder primarily characterized by intellectual disability, microcephaly, cardiac defects and hypo/aplastic nails of the fifth fingers and/or toes. *SMARCE1* is a dedicated core member of the mSWI/SNF complex, a chromatin remodeling entity that regulates accessibility of transcriptional factors to regulatory DNA elements and consequently elicit or repress tissue-specific gene transcription. However, mechanistic understanding linking the CSS *de novo* single-base substitutions to the congenital disorder remains a mystery. Here, we have coupled structure and function to elucidate the biochemical ramifications of all *SMARCE1*-CSS mutations on DNA binding activity, complex assembly on chromatin and resulting epigenetic deregulations. Further, we map the genome-wide occupancy of *SMARCE1* to elucidate the transcriptional consequences of the CSS point mutations in cortical neuron-like cells derived from CRISPR-edited human-induced pluripotent stem cells (hiPSCs). We find that the *SMARCE1*-CSS mutations perturb DNA binding and mSWI/SNF complex assembly on chromatin at Polycomb-repressed neurodevelopmental genes. Strikingly, we observe specific deregulation to transcriptional programs that alters cell cycle exit, cortical neurons differentiation and migration. Our work indicates that *SMARCE1*-CSS mutations impede mSWI/SNF(BAF) opposition to Polycomb at neurodevelopmental genes.