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KARIN GRUNEBAUM
cancer research foundation

December 2020 ♦ Volume 17

From the Chair

Dear Friends of the Karin Grunebaum Cancer Research Foundation:
A presidential impeachment, a deadly world-wide pandemic, entire cities and states shut down, extended personal quarantines, massive unemployment, the largest single-day stock market drop in history, one of the busiest hurricane seasons ever, a vitriolic presidential campaign and two ZOOM Trustees' meetings: that pretty well sums up the year 2020 from the Foundation's perspective.

But, as always, the Foundation and its dedicated Trustees soldiered on. In spite of the obstacles listed above, we managed to again fund the two annual Fellowships for junior faculty members at Boston University School of Medicine (BUSM) and Harvard Medical School (HMS), fund the third annual Karin Grunebaum Cancer Research Poster competition at HMS (conducted virtually with ten entrants), and fund summer cancer research internships for two medical students at BUSM..

Given the unique mental stresses on the medical school community caused by the uncertainties associated with working in the Covid-19 environment, the Foundation also contributed funds to the HMS Professional Development program to help ease the pressure on that community.

In addition, pursuant to direction from the Trustees, the Foundation established a social media presence on Twitter so that more people could be exposed to the Foundation and its on-going work. Our handle is @ KgcrfR. Many thanks to Trustees Genevieve Boland and Shawna Wallach for setting up the account.

Trustee Julie Palmer, who is also the Karin Grunebaum Professor in Cancer Research at BUSM, used the professorship's endowment to recruit a world-renowned cancer researcher to her Slone Epidemiology Center. In addition to continuing her own cancer research in a variety of modalities, Dr. Palmer, as Co-Director of the BU-BMC Cancer Center, also promoted cross-disciplinary research among the 200+ Cancer Center members, and facilitated the research careers of junior faculty members. In her words, "In multiple ways and for many years the Karin Grunebaum Cancer Research Foundation has meaningfully advanced cancer research of many BUSM scientists at all career stages."

In spite of the many unique obstacles this year, the Foundation was able to continue fighting the war against cancer on many different fronts and with many different tools. But, we now need your donations more than ever to help us win this critical war.

Thank you for your continuing support in these unprecedented times.

Steven Wallach
Chairperson

Outgoing Fellow

Russell W. Jenkins, M.D. Ph.D

Massachusetts General Hospital Cancer Center; Assistant Professor, Harvard Medical School; Faculty, Center for Melanoma and Center for Cancer Research (MGH); Faculty, Laboratory for Systems Pharmacology (HMS); Termeer Early Career Investigator in Systems Pharmacology

TBK1 As A Novel Target to Reprogram the Tumor Immune Microenvironment

Progress Report:

Background/Rationale: TBK1 is a Ser/Thr kinase involved in innate immune signaling and is an emerging target for anti-cancer therapy (6). Importantly, independent orthogonal data from two different laboratories has also identified TBK1 as a cancer immunotherapy target (7,8). **This project aims to investigate TBK1 as a novel cancer immunotherapy target to overcome resistance to PD-1 blockade.** The proposed aims address key unanswered questions of immediate significance with respect to the pre-clinical and clinical development of TBK1-targeted strategies.

Aim 1: Characterize the tumor-intrinsic effect of TBK1 deletion in murine melanoma cells. The goal of this aim is to define the changes in gene expression patterns and signaling pathways in B16F10 murine melanoma cells lacking *Tbk1* (CRISPR) using bulk RNA-sequencing.

Aim 2: Evaluate the impact of TBK1 deletion on the tumor immune microenvironment. The goal of this aim is to define the impact of deletion of *Tbk1* (CRISPR) on the tumor immune microenvironment alone or in combination with PD-1 blockade.

Results:

Aim 1: Characterize the tumor-intrinsic effect of TBK1 deletion in murine melanoma cells. The goal of this aim was to determine the effect of *Tbk1* deletion on inflammatory signaling following challenges with IFNg and TNFa. To explore the tumor-intrinsic mechanisms of sensitivity to immune attack, we evaluated the sensitivity of control and *Tbk1*-null cells to challenge with exogenous inflammatory cytokines. In vitro treatment with inflammatory cytokines (IFNg +/- TNFa) revealed enhanced sensitivity of Tbk1-null B16 mouse melanoma cells to combined interferon-g (IFNg) and tumor necrosis factor-a (TNF-a) that was not observed with either cytokine alone (Fig. 1a). Dose responses studies indicated a greater contribution of TNFa dosing, but requirement for IFNg even at very low doses (data not shown). Mouse melanoma cells lacking TBK1 also exhibited enhanced upregulation of

Aim 2: Characterize the impact of TBK1 deletion on the tumor immune microenvironment. The goal of this aim was to define the impact of deletion of *Tbk1* (CRISPR) alone or in combination with PD-1 blockade on the tumor-immune microenvironment (TIME). Using flow cytometry we determined that baseline immune contexture varied little between control tumors and *Tbk1*-null tumors. To determine if an immune challenge would yield greater changes in the immune contexture, we elected to characterize the impact of *Tbk1* deletion +/- PD-1 blockade using the resistant B16-GVAX model system, as previously described (7). B16 CRISPR (control sgRNA and *Tbk1* sgRNA) cells were implanted (7) and tumors harvested on Day 12 using established protocols (7). Explanted tumors will be processed for basic immunophenotyping by flow cytometry (9), singlecell RNA-sequencing (CD45+ cells), as previously described (7,10). Preliminary results confirm specific changes in distinct lymphoid and myeloid cell states. Further analysis is ongoing to integrate

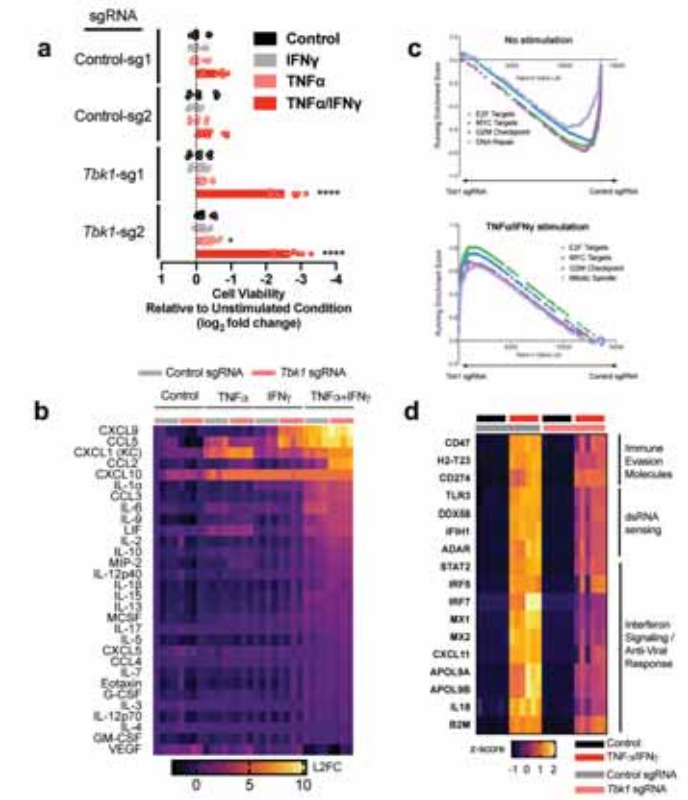

Fig. 1 | Tbk1 deletion enhances response to PD-1 blockade. a, effect of IFNγ (40 ng/mL) +/- TNFα (160 ng/mL) on cell viability (24h) by Cell Titer Glo (n=9, *p<.05 by ANOVA); normalized luminescence shown. **b**, effect of IFNγ (100 ng/mL) +/- TNFα (10 ng/mL) on secreted cytokines (48h) using MAGPIX 32-plex murine panel (n=4). **c-d,** RNA-sequencing results with gene set enrichment analysis (GSEA) (c) and differential gene expression (DGE) (d) for B16 ctl or *Tbk1* sgRNA cells +/- TNFα/IFNγ. (n=3 per condition).

these findings with the results of the data in Aim 1 to better understanding the link between inflammatory cell death and evolution of the tumor immune microenvironment.

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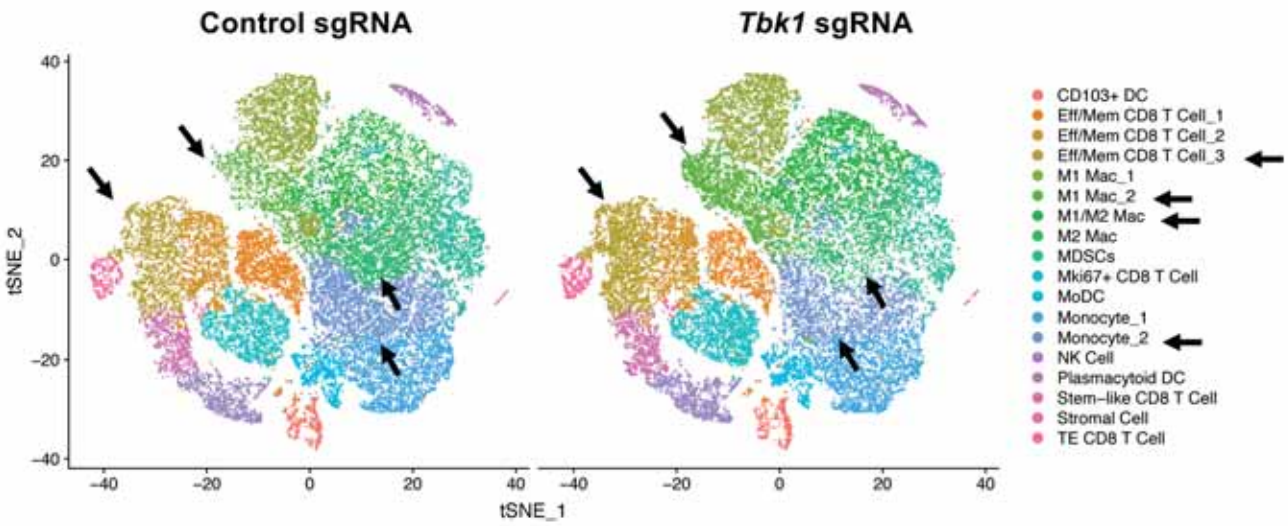

Fig. 2 | Single-cell RNA-sequencing of Control and *Tbk1*-null murine tumors. >50,000 CD45+ immune cells from C57/B6 mice implanted with 5 x 10^5 B16F10 ctl or *Tbk1* sgRNA cells followed by GVAX treatment and PD-1 blockade, as described in Manguso et al. *Nature* 2017 (n=4 mice each)

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Outgoing Fellow

Maria Cardamone, PhD

Karin Grunebaum Faculty Research Fellow, Boston University School of Medicine

Research Overview

Non-Small-Cell Lung Cancer (NSCLC) is the most common type of lung cancer, accounting for approximately 90% of all lung cancers. Despite significant advances in therapeutic treatments over the past 20 years, NSCLC is still the #1 cause of cancer-related deaths worldwide.

Although the pathogenesis of lung carcinogenesis remains unclear, we know that NSCLC plasticity requires both rapid changes in cellular metabolism and activation of the immune system in order to allow balance between energy production and tumor growth. Currently, scientists do not fully understand 1) how cells acquire metabolic advantages during cancer progression and 2) the role played by the immune response during tumor growth. We do know that the mitochondria, as the power house of the cells and at the center of the immune response, play a crucial role in the connection between cancer cell metabolism and tumor inflammation.

The major focus of my research is the role of mitochondria in the activation and regulation of the inflammatory microenvironment of tumors. In particular, my lab is investigating the correlation between mitochondrial dysfunction and mitochondrial DNA (mtDNA) instability in the regulation of the immune response and metabolic reprogramming in lung cancer initiation and progression.

Recent Progress

We previously discovered that NEURL4 is the first mitochondrial protein with Poly-(ADP-ribose) polymerase-like (PARP) enzymatic activity required for the regulation of different mitochondrial proteins, such as K-RAS, which are important in the onset of NSCLC.

Thanks to the generous contribution of the Karin Grunebaum Cancer Research Foundation, in the last year we have connected NEURL4 to the regulation of metabolic adaptation and inflammation during NSCLC initiation and progression. In particular, we have generated a NEURL4 knockout in the H1395 NSCLC cell line to shown that this new enzyme is not only required for mtDNA maintenance and regulation of immune response (manuscript in preparation) but also important in the regulation of cell metabolism via K-RAS modulation.

Our new data indicate that NEURL4 is a novel tumor suppressor. We are now in the process of generating a new mouse model lacking NEURL4 in the lungs. We will test in vivo the ability of NEURL4 to work as a tumor suppressor and will characterize its molecular mechanism of action. Our goal is to identify new potential targets for lung cancer therapeutic interventions.

From Boston University Medical School

Julie Palmer, M.P.H., Sc.D
Karin Grunebaum Professor in Cancer Research, Boston University School of Medicine;
Director, Slone Epidemiology Center;
Co-Director, Boston University-Boston Medical Center Cancer Center

Dear Trustees:

As my first year as the Grunebaum Professor in Cancer Research comes to a close, I would like to express my appreciation for the foundation's deep commitment to cancer research at the Boston University School of Medicine. This support has had a tremendous impact, both for recruitment of new faculty, for early-stage faculty and students conducting cancer research, and for my own research, for which I am very thankful.

Funds from the endowed professorship enabled me to recruit a top entry-level cancer epidemiologist, Jessica Petrick, PhD, MPH, for a faculty position as Assistant Professor at the Slone Epidemiology Center, in the Section of Hematology/Oncology, Department of Medicine, Boston University School of Medicine. For her postdoctoral work, Dr. Petrick worked in Dr. Katherine McGlynn's lab at the National Cancer Institute Division of Cancer Epidemiology and Genetics. She was highly productive in that period, publishing 18 first-author manuscripts and making significant scientific contributions to 23 other papers. During the first year of her postdoc, she was awarded a prestigious Sallie Rosen Kaplan Cancer Research Training Fellowship, which provides additional mentoring, networking, seminars, and workshops to ensure fellows successfully transition to independent research careers. She won two NIH awards for research excellence, as well as awards from the American Association for Cancer Research and the Society for Epidemiology Research. I believe that Dr. Petrick has great promise to develop into an independent NIH-funded investigator and will be a future leader in cancer epidemiology.

Since joining the Slone Epidemiology Center in August 2019, Dr. Petrick has been exceptionally productive. She has 13 new peer-reviewed manuscripts published or in press and is first or last author on nine of them. She has also published two invited editorials and one invited review during this time. In March 2020, Dr. Petrick was one of three BUSM junior faculty to receive an American Cancer Society American Cancer Society Pilot Program Award. Under this pilot award of $30,000, she will conduct research on Helicobacter Pylori in tumor tissue of colorectal cancer patients. Dr. Petrick has grant applications under review at the NCI (R21) and the Colorectal Cancer Foundation. Her recruitment would not have been possible without funds from the Grunebaum Professorship.

Support from the Professorship has also allowed me to spend time on unfunded research projects. Prominent among these is the CARRIERS Study, a large collaborative research project designed to produce definitive data on the relationship of both well-known breast cancer genes, such as BRCA1/BRCA2, and 20+ less well-known genes to lifetime risk of developing breast cancer. When the CARRIERS grant was funded by NIH several years ago, the plan was to focus on White populations only due to the small number of available study participants from other racial/ethnic groups. I approached the principal investigator of CARRIERS with the idea of contributing samples from 4,200 African American women from the BU Black Women's Health Study (BWHS), in order to have sufficient numbers for a separate report on Black women. Due to support from the Professorship, I was able to carve out enough time to write a paper on this important and understudied topic. That paper, for which I am first and corresponding author, will shortly be published in the Journal of the National Cancer Institute, with an accompanying editorial. We will share this article as soon as it is published.

As Co-Director of the BU-BMC Cancer Center, I am engaged in promoting cross-disciplinary research among the ~200 Cancer Center members and in facilitating the development of independent research careers by junior faculty. To that end, I recently submitted an application for a renewed American Cancer Society Institutional Research Grant, which supports pilot grants to junior faculty. The six investigators that received pilot funding in 2018 and 2019 have been quite successful.

Finally, I have continued work on my NIH-funded research projects; a list of publications from the last 12 months is attached. I am currently working on a new R01 grant proposal for research on contralateral breast cancer risk in African American women. Data from the US SEER cancer registries indicate that African American women are more likely than other women to develop a second primary breast cancer in the opposite breast. The reasons for this disparity are unknown. Identification of those reasons may improve our understanding of contralateral breast cancer, leading to reduction of risk in all women.

In multiple ways and for many years, the Karin Grunebaum Cancer Research Foundation has meaningfully advanced cancer research of many BUSM scientists at all career stages. I am honored and grateful to be a beneficiary of the foundation's vision and generosity.

I look forward to seeing you when people are able to safely gather in groups. In the meantime, my best wishes to you and all the trustees.

Sincerely,

Julie R. Palmer, ScD

Due to the ongoing pandemic circumstances the Grunebaum Research Poster Competition was held virtually and we are pleased to report once again that the presentations were outstanding and the competition rigorous.

Lisa Situ | Targeting mitochondrial dynamics in melanoma
Nouran Abdelfattah | Breaking tolerance with engineered T-cell receptors for adoptive cell therapies
Sam Barritt | Investigating Dependencies on Coenzyme A Metabolism in Cancer
Priscilla Cheung | Therapeutic reprogramming of the intestinal stem cell state via Hippo signaling
Camille Cushman | Characterization of viral-mediated epigenetic dysregulation in Merkel cell carcinoma
Alissandra Hillis | Identifying synthetic lethal combinations with PI3K/AKT inhibitors in TNBC
Patrick Loi | Investigating EZH2 as an oncogene and therapeutic target in colorectal cancers
Theresa Oei | Understanding Polycomb Phase Separation in Neuroblastoma Epigenetics
Kristin Qian | Elucidating the differential roles of human ATP-dependent chromatin remodeling complexes
Bing Shui | KRas activation induces global down regulation of miRNA function in colorectal cancer

First Place Winner:

Lisa Situ | G2

Biological and Biomedical Sciences
Cichowski lab

Targeting mitochondrial dynamics in melanoma

Although 15-30% of melanomas harbor activating mutations in NRAS, there are currently no approved targeted therapies for this subtype of melanoma. We are interested in developing a novel combination therapy that can improve the clinical efficacy of MEK inhibitors in these tumors. We previously performed a genome-scale CRISPR negative selection screen to identify genes which when suppressed confer sensitization to MEK inhibition. MARCH5 was one of the most significant hits, and our goal is to understand the mechanism by which dual inhibition of MARCH5 and MEK specifically kills NRAS-mutant melanoma cells. MARCH5 is a mitochondrial E3 ubiquitin ligase that has been implicated in the regulation of mitochondrial morphology. We hypothesize that MARCH5 and MEK cooperate to regulate mitochondrial dynamics and that disruption of this axis sensitizes NRAS-mutant melanomas to cell death. While the goal of this research is to advance a potential combination therapy into the clinic for patient benefit, our work will also elucidate novel information about an important but understudied mitochondrial E3 ligase and its interactions with the Ras signaling pathway.

Second Place Winner:

Bing Shui | G4

Biological and Biomedical Sciences
Kevin Haigis lab

KRas activation induces global down regulation of miRNA function in colorectal cancer

KRas is frequently mutated in three of the four deadliest human cancers (PDAC, CRC, NSCLC) and regulates miRNAs. miRNAs are immediately actionable therapeutic targets given the large repertoire of miRNA mimics and inhibitors. Investigations of miRNA targets have been dependent on computational algorithms. My thesis project has used newly developed HEAP-CLIP to map physiologic targets of miRNA in vivo in murine colorectal cancer +/- oncogenic KRas. Our data suggest that the activation of KRas in tumors greatly expands the miRNA target repertoire and increases miRNA targeting intensity across all major miRNA families. However, this global up-regulation of miRNA targeting paradoxically de-suppresses target expressions. The concordant global increase of miRNA targeting and miRNA targets could be attributed to the down-regulation of Ago2 phosphorylation at S829-S835, regulated by CK1. We hypothesize that KRas activation in colon cancer suppresses CK1 family kinases, subsequently decreases phospho-Ago2 (S829-S835). A recent study reported that the loss of these phosphorylations inhibited the dynamic cycling of Ago2, causing increased miRNA binding to mRNA targets with decreased gene suppression. My work will elucidate a novel interaction between KRas signaling and miRNA machinery and potentially reveal therapeutic vulnerabilities of colorectal cancer, which remains elusive to modern therapies.

Third Place Winner:

Kristin Qian | G2

Biological and Biomedical Sciences
Kadoch lab

Elucidating the differential roles of human ATP-dependent chromatin remodeling complexes

ATP-dependent chromatin remodeling complexes (CRCs) play critical roles in the maintenance of tissue- and state-specific chromatin structure and the regulation of gene expression by dynamically positioning nucleosomes along the genome. Importantly, these CRCs are involved in development and differentiation, and mutations in genes encoding CRC protein subunits result in diverse pathologies of cancer and neurodevelopmental disorders. SWI/SNF remodeling complexes, which are mutated in ~20% of cancers, have been extensively studied in our lab. In addition to SWI/SNF, there are three other families of related ATP-dependent CRCs: ISWI, CHD, and INO80. Across these families, there are approximately two dozen CRCs conserved from yeast to human. However, the field lacks an integrative analysis dissecting the roles of these CRCs, and it is unknown how perturbations in each complex family affect the global chromatin landscape. I propose to assess the chromatin localization and remodeling activities of each family of CRCs and their role in regulating accessibility and gene expression at various stages of differentiation and in disease settings driven by CRC mutations. To that end, this study aims to advance our understanding of CRC-directed chromatin changes and to define differential CRC functions in establishing and maintaining chromatin architecture.

Nouran Abdelfattah | G3

Biological and Biomedical Sciences
Elledge lab

Breaking tolerance with engineered T-cell receptors for adoptive cell therapies

Cytotoxic T lymphocytes are potent effector cell s of the adaptive immune system and have the ability to recognize and clear tumors. This function serves as the foundation for promising immunotherapies such as adoptive T cell transfer and immune checkpoint blockade. In fact, the clinical success of adoptive T-cell therapy with T-cells expressing CD19-targeted chimeric antigen receptors (CARs) in treating B-cell malignancies has driven FDA approval of both Kymriah and Yescarta. However, CAR-T therapy has proven difficult to translate into solid tumors due to the low number of targetable cell surface antigens, the immunosuppressive microenvironment and the potential for severe systemic toxicity. Genetically engineered T cells with T-cell receptors (TCRs) can overcome some of these challenges because they enable targeting of intracellular antigens presented on major histocompatibility class I (MHC I) molecules increasing tumor antigen breadth and signaling through the physiological CD3 complex, providing more potent signaling. However, TCR therapeutics themselves face two major challenges: isolating effective TCRs to self-antigens and off-target reactivities. Here, I describe an in vitro-directed evolution approach to create collections of potent and efficient TCRs that target self-antigens for which central tolerance prevented their production. In this approach, I first raise high-avidity T-cells that recognize a related “foreign” peptide (that differs by one amino acid from the self-peptide) and then modulate the fine specificity of the TCRs by mutagenesis directed to the CDR3 region to engineer self-reactive TCRs. This TCR display system should prove to be a useful strategy for the generation of high-affinity tumor-specific TCRs for adoptive cell therapies.

Sam Barritt | G3

Biological and Biomedical Sciences
Dibble lab

Investigating Dependencies on Coenzyme A Metabolism in Cancer

Highly proliferative cells must meet the metabolic demands of cell growth by promoting the synthesis of macromolecules including lipids, protein, and nucleotides. The accumulation of biomass through central carbon metabolism is dependent on coenzyme A (CoA), a critical cofactor for the TCA cycle, lipid and sterol metabolism, and mitochondrial function. CoA is also necessary for protein acetylation, including that of histones, and redox balance, both of which are important for cancer cell growth. Using stable isotopic labeling and targeted mass spectrometry, we discovered that oncogenic signaling through PI3K stimulates the de novo synthesis of CoA from its precursor vitamin B5. Given the established role of the esterified CoA derivative acetyl-CoA in cell growth and cancer progression, we aim to determine whether cells with oncogenic PI3K signaling are more sensitive to perturbation of CoA metabolism. In addition, we have observed regulation of cell growth and division in response to CoA abundance. Perturbation of CoA metabolism inhibits growth through well-defined tumor suppressor-dependent pathways, suggesting that a similar metabolic vulnerability may exist in tumor suppressor-deficient cancer cells. Overall, we aim to lay the foundation for identifying therapeutic opportunities to target metabolic pathways by investigating the cross-regulation between oncogenic signaling and CoA metabolism.

Priscilla Cheung | G5

Biological and Biomedical Sciences
Camargo lab

Therapeutic reprogramming of the intestinal stem cell state via Hippo signaling

The intestine is intricately regulated by crosstalk between the Hippo and Wnt signaling pathways to control epithelial cell proliferation and differentiation. While the Hippo transcriptional coactivator YAP is considered oncogenic in many tissues, its roles in intestinal homeostasis and colorectal cancer (CRC) remain controversial. Here, we demonstrate that the Hippo kinases LATS1/2 and MST1/2, which inhibit YAP activity, are required for maintaining Wnt signaling and canonical stem cell function. Hippo inhibition induces a distinct epithelial cell state marked by low Wnt signaling, a wound healing response, and transcription factor Klf6 expression. Notably, loss of LATS1/2 or overexpression of YAP is sufficient to reprogram Lgr5+ cancer stem cells to this state and thereby suppress tumor growth in organoids, patient-derived xenografts, and mouse models of primary and metastatic CRC. Finally, we demonstrate that genetic deletion of YAP and its paralog TAZ promotes the growth of these tumors. Collectively, our results establish the role of YAP as a tumor suppressor in the adult colon and implicate Hippo kinases as therapeutic vulnerabilities in colorectal malignancies.

Camille Cushman | G4

Virology
DeCaprio lab

Characterization of viral-mediated epigenetic dysregulation in Merkel cell carcinoma

Merkel cell carcinoma (MCC) is an aggressive neuroendocrine carcinoma of the skin caused by either excessive UV-induced genomic mutations or the integration of the Merkel cell polyomavirus (MCV) genome. Virally-induced MCC is characterized by having a low mutational burden and the primary oncogenic drivers are viral proteins known as small tumor antigen (ST) and large tumor antigen (LT). Previous work has revealed commonalities between virus-positive MCC (VP MCC) and other neuroendocrine carcinomas, including defects in p53 signaling, RB activity, and MYC family hyperactivation. More specifically, our lab determined that ST interacts with the oncogenic transcription factors LMYC/MAX and the Tip60/P400 complex, a large chromatin remodeling complex, to aberrantly activate specific target gene expression. In this study, we aim to further characterize the effect of T antigen expression on the cellular chromatin architecture. Preliminary results suggest that T antigens cause a decrease in chromatin accessibility at genes involved in neuron differentiation, which highlights the role of these viral proteins in promoting a poorly differentiated state. This work serves as a basis towards better understanding the mechanism of epigenetic dysregulation in VP MCC.

Alissandra Hillis | G2

Biological and Biomedical Sciences
Toker lab

Identifying synthetic lethal combinations with PI3K/AKT inhibitors in TNBC

The phosphoinositide-3-kinase (PI3K) pathway is hyperactivated in almost all human cancer types, promoting cell growth and survival. Triple negative breast cancer (TNBC) is a highly heterogeneous disease with poor prognosis and limited targeted therapies. Among the few common genetic features in TNBC is PI3K pathway hyperactivation, which occurs in greater than 50% of TNBC cases. PI3K/AKT inhibitors have been developed to treat PI3K pathway-mutant cancers, but they have been generally ineffective as monotherapies due to the presence of other oncogenic mutations in heterogeneous tumors, the development of acquired resistance, or on-target toxicities. However, in 2019, the PI3K inhibitor, BYL719, was approved, in combination with hormone therapy, for the treatment of estrogen receptor-positive, PIK3CA-mutant breast cancer. This motivates developing additional combination therapies with PI3K/AKT inhibitors in other cancer types. I hypothesize that PI3K/AKT inhibitors can effectively treat PI3K pathway-mutant TNBC, if synthetic lethal drug combinations are identified. With the myriad of potential anti-cancer drug combinations available, it is difficult to predict which combinations will be effective. To identify effective drug combinations, I performed a negative selection CRISPR screen with PI3K/AKT inhibitors in TNBC. This work aims to advance the treatment of TNBC by identifying targetable vulnerabilities in this heterogeneous disease.

Patrick Loi | G3
Biological and Biomedical Sciences
Cichowski lab

Investigating EZH2 as an oncogene and therapeutic target in colorectal cancers

Polycomb Repressive Complex 2 (PRC2) is a highly conserved developmental regulator that maintains cellular identity by dynamically silencing key genes involved in differentiation. Alterations in PRC2 have been shown to play a driving role in many cancers. EZH2 is the major catalytic methyltransferase of PRC2 and is found to be overexpressed in multiple solid tumors, including prostate, breast, and colorectal cancer. EZH2 expression levels progressively increase in advanced tumors, and has been functionally shown to drive prostate cancer metastasis. Specifically, EZH2 is overexpressed in 78.5% of colorectal cancers (CRC), which makes it an attractive therapeutic target, although its role and targets in CRC is unknown. CRC is the is one of the leading causes of cancer deaths worldwide, and advanced metastatic disease is still incurable. Thus, there is a significant unmet clinical need for treatments for CRC, especially those with activating mutations in KRAS. In developing more effective therapies, we have found that EZH2 inhibitors are frequently effective when combined with agents that target other key oncogenic pathways in a given tumor type by clamping down on crucial oncogenic signals at both the kinase level and the transcriptional level. Specifically, I discovered that a combination of EZH2 and MEK inhibitors cooperate to kill KRAS mutant CRC, which reveals a novel approach for treating this advanced disease.

Theresa Oei | G3
Chemical Biology
Kingston lab

Understanding Polycomb Phase Separation in Neuroblastoma Epigenetics

Polycomb group (PcG) proteins are important epigenetic regulators with significant roles in defining developmental pathways and maintaining cell identity. Polycomb Repressive Complex 1 (PRC1) is involved in the formation of PcG bodies - liquid phase separated droplets that appear as puncta in the nucleus. The role of these condensates in PRC1,Äôs function as a regulator of silent chromatin is unknown. Increasingly, phase separation has been shown to play a role in transcriptional activation and silencing by concentrating and compartmentalizing proteins in molecular condensates within the nucleus. PcG bodies may cluster multiple genomic targets and PcG proteins to create and remember a repressed state. The CBX2 subunit has been shown to drive PRC1 phase separation and it is also an essential regulator in neuroblastomas. My research aims to develop chemical and molecular technologies to explore the role of CBX2 condensates in neuroblastoma development. Overall, this project will reveal the role of phase separation in polycomb epigenetics and provide new insights and therapeutic opportunities for cancer.

COMPETITION JUDGES

Christian Dibble, Ph.D.
Assistant Professor | BIDMC | HMS

Kevin Haigis, Ph.D.
Chief Research Officer | DFCI
Associate Professor | HMS

Nada Kalaany, Ph.D.
Associate Professor | BCH | HMS

Naama Kanarek, Ph.D.
Assistant Professor | BCH | HMS

Andrea McClatchey, Ph.D.
Professor of Pathology | MGH | HMS

Mohammad Rashidian, Ph.D.
Assistant Professor | DFCI | HMS

Incoming Fellow

Nilay Sethi, MD, PhD
Karin Grunebaum Faculty Research Fellow,
Principal Investigator at Dana-Farber Cancer Institute

Background:
The overall objective of our proposal was to (1) define the molecular mechanisms of hyperactive stem cell programs and (2) determine the therapeutic potential of blocking stem cell signaling in colorectal cancer. In order to accomplish this, we proposed to design a new discovery platform that will enable drug screens to identify compounds that block hyperactive stem cell signaling and promote differentiation/death of cancer cells.

Summary of Progress:
To identify drug compounds that block stem cell activity and induce intestinal differentiation, we have generated an endogenous reporter system that reads out stem cell activity (i.e. SOX9) using a GFP knock-in fluorescent probe (Figure 1). We have also tested and successfully validated the stem cell reporter system using a genetic screening format (Figure 2). Furthermore, we have generated the differentiation reporter system (i.e. KRT20) using a Cerulean (blue) knock-in fluorescent probe, which we validated is activated upon inhibition of SOX9 (Figure 3).

Detailed Results:
Development of endogenous stem cell reporter cell line: To establish an endogenous stem cell activity reporter, we knocked-in GFP in-frame at the end of the SOX9 coding region using the combination of CRISPR/Cas9 and template-based homologous recombination (Figure 1A). Compared the parental CRC cell line, the engineered GFP knock-in stem cell reporter line demonstrated high GFP levels due to the elevated SOX9 expression in CRC (Figure 1B). Since SOX9 drives a stem cell transcriptional program, GFP levels in the engineered reporter line will reflect stem cell activity. To validate that GFP levels faithfully reflect SOX9, we stably expressed two different SOX9 shRNAs in the stem cell reporter line, which led to a greater than 50% reduction in GFP (Figure 1C-D). We next performed a CRISPR/Cas9 mini-screen using the stem cell reporter line to test its functionality in a screen context. After introducing a pool of 6 sgRNAs targeting GFP, 6 sgRNAs targeting SOX9, and 58 sgRNAs targeting 10 control genes, we sorted the cells after three days based on GFP expression (Figure 2A). Genomic DNA was extracted and sequenced to evaluate sgRNA representation in the top 20%, bottom 20%, and GFP negative fractions. If the stem cell reporter line is functional, we would expect the GFP and SOX9 targeting sgRNAs to be enriched in the bottom 20% and GFP negative sorted populations. Indeed, only GFP and SOX9 sgRNAs were enriched in these sorted fractions (Figure 2B), validating the stem cell reporter line in the genetic screen format.

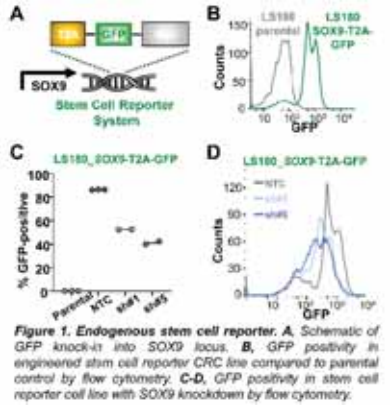

Figure 1. Endogenous stem cell reporter. A, Schematic of GFP knock-in into SOX9 locus. B, GFP positivity in engineered stem cell reporter CRC line compared to parental control by flow cytometry. C-D, GFP positivity in stem cell reporter cell line with SOX9 knockdown by flow cytometry.

Development of endogenous differentiation reporter cell line: Using CRISPR/Cas9, we have also successfully established a differentiation reporter line by knocking-in a Cerulean fluorescent probe into the KRT20 genomic locus of CRC cell lines, which is activated upon intestinal differentiation induction (Figure 3A). We have validated that suppression of SOX9 induces reporter activity in these engineered lines (Figure 3B).

Figure 2. Optimization of endogenous stem cell reporter system. A, Schematic of mini-screen using stem cell reporter system. B, Normalized guide abundance from bottom 20% and GFP-negative fraction from flow

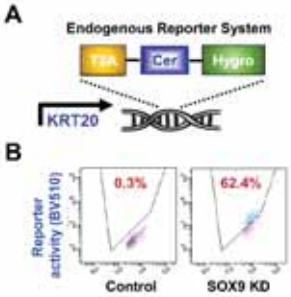

Figure 3. Knock-in intestinal differentiation reporter system. A, Schematic of endogenous reporter system. B, Validation of reporter system using shRNA-mediated SOX9 knockdown (KD)

Moving forward:
We now plan to utilize these systems for genetic and drug screens. We will perform the following screens in the coming 2-3 months:

- A genetic screen of 147 SOX9 co-factors
  - A novel experiment has identified 147 potential co-factors that bind to SOX9 in order to promote stem cell signaling. We will generate a small screening library to identify which of these potential co-factors functions with SOX9 in regulating this important program co-opted by colorectal cancer
- A drug screen of 30 epigenetic inhibitors in collaboration with Jun Qi
  - New data indicates that specific epigenetic regulators bind to SOX9
  - We will screen 30 epigenetic inhibitors at multiple doses across three cell lines
- A drug screen of 5400 compounds
  - These are FDA approved drugs utilized for other conditions
  - The goal is to determine if any of these can be repurposed for the treatment of colorectal cancer based on screening results.

Incoming Fellow

Muzhou Wu, PhD

Karin Grunebaum Faculty Research Fellow,
Assistant Professor of Dermatology, Boston University School of Medicine

Research Overview

Targeted- and immuno-therapies improved management of advanced melanoma; however, relapse usually occurs and is driven by a small subpopulation of 'drug-tolerant' cells, which are called 'minimal residual disease'. Our lab identified the CoREST transcriptional repressive complex as being a critical mediator of tumor cell plasticity and resistance to targeted therapy in melanoma. Notably we developed a novel two-pronged hybrid agent, Corin, that potently and specifically targets CoREST complex. Our studies suggest Corin can resensitize BRAF inhibitor-resistant melanomas to such targeted therapies. RNAseq analysis revealed Corin inhibits critical mediators driving cell plasticity during development of MAPK inhibitor (MAPKi) resistance. Our proposal currently funded by Grunebaum Foundation aims to identify epigenetic/transcriptional vulnerabilities mediated by Corin that are associated with cell plasticity and resensitization in MAPKi-resistant melanoma. Using melanoma clinical specimens, we will identify epigenetic/transcriptional markers associated with therapy resistance/resensitization which can be used to develop an assay on tumor biopsies to predict response to targeted and epigenetic therapies.

Research Update

Corin mediates phenotype switching in melanoma and re-sensitizes BRAF-inhibitor-resistant melanoma cells to BRAF inhibitor therapy. We investigated effects of CoREST inhibition on BRAF-inhibitor-resistant (BRAFi-R) melanoma cell proliferation when combined with BRAFi PLX4032 (Vemurafenib). Corin (1μM) significantly increased anti-proliferative effects of PLX4032 in BRAFi-R cells (**Fig. 1a, b**); with strong synergy identified between PLX4032 and Corin versus the LSD1 inhibitor (Cpd 7) or HDAC1 inhibitor (MS275) alone (Fig. 1c). Interestingly, we observed Corin and combination treatment (Corin+PLX) led to MITF/AXL switching, decreasing MITF in 451Lu-R and AXL in 1205Lu-R. However, Corin does not appear to inhibit MAPK signaling (**Fig. 1e**), suggesting a different mechanism. Further comparison analysis of our RNAseq data with publicly available datasets on proliferative/invasive melanoma cell lines42-44 and patient primary/metastasis biopsies identified a biological similarity to phenotypic switching in Corin-treated cells, as reported by Hoek, Dummer, et al. Corin induced a transcriptional switch between 'proliferative' and 'invasive' signatures in 451Lu-R and 1205Lu-R (Fig. 1d). Corin differentially regulated genes encoding transcription factors driving phenotype switching (AXL, WNT5A, PAX3, TGFb, NFkB, TEAD2, PPARGC1a).

Combination treatment (Corin+ BRAFi) potently inhibited tumor growth in a BRAFi-resistant melanoma xenograft mouse model. A 1205Lu BRAFi-resistant melanoma xenograft mouse model was established to evaluated combination treatment efficacy. BRAFi-R tumors on PLX4032-chow showed progressive tumor growth resembling that of control tumors (**Fig. 2a**). In contrast, Corin significantly inhibited tumor growth, and combination treatment (Corin+PLX4032) chow showed the greatest tumor growth inhibition (**Fig. 2a, b**). Interestingly, qPCR on tumor tissue demonstrated both MITF and AXL expression increased in tumors treated with PLX4032. However, Corin treatment, alone or in combination, abrogated this increase in MITF and AXL (**Fig. 2c**). These data suggest that in BRAFi-R tumors, continuous BRAFi treatment is likely to further induce subpopulations of both MITFhigh and AXLhigh drug-resistant cells, while Corin may target these populations by reversing cell plasticity driven by MITF/AXL.

Plans moving forward

Nonbiased survey of CoREST epigenetic landscape to define specific epigenetic signatures associated with phenotype switching that predict sensitivity to Corin. We will use chromatin-based methods to identify significantly regulated gene sets and regulatory elements within the genome of BRAFi-R melanomas that are specifically re-sensitized to BRAFi in the setting of CoREST inhibition. To identify targets associated with phenotype switching and BRAFi re-sensitization, chromatin immunoprecipitation (ChIP)-seq will be performed for key epigenetic components of the CoREST complex

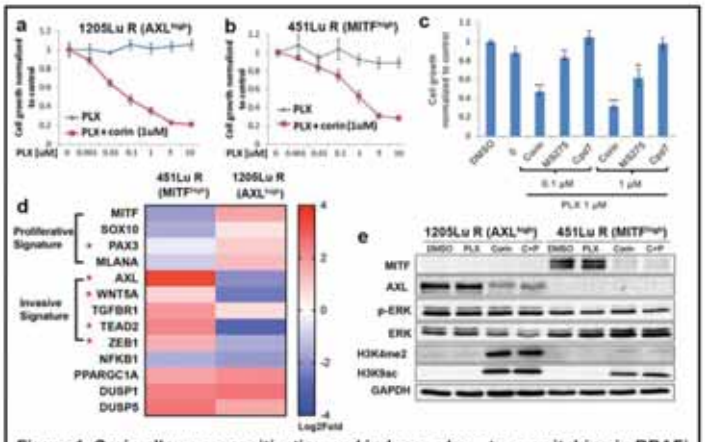

Figure 1. Corin allows resensitization and induces phenotype switching in BRAFi-resistant melanoma cells. a, b), Pico-green proliferation assay demonstrates growth inhibition in response to Corin in AXL^{high}-1205Lu R and MITF^{high} 451Lu R cells. **c),** Proliferation assays of 451Lu-R cells treated with PLX4032 1 μM +/- corin, MS275 or Compound7 (Cpd7) (0.1 μM and 1 μM). **d),** Heatmap from RNA-Seq illustrates differential expression pattern of proliferative vs. invasive signatures, as well as transcriptional regulators driving phenotype switch (depicted by * in red) in melanoma. The Log2 fold change difference is color coded (red, upregulated; blue, downregulated). **e),** Western blot depicting changes in MITF/AXL levels.

(LSD1 and HDAC1) and histone markers impacted by CoREST (H3K9Ac, H3K4Me2) in BRAFi-R tumors after Corin treatment. ChIP-seq data will be integrated with RNAseq data to identify enrichment of sequence motifs for transcriptional factors in Corin-activated/-silenced regions. Furthermore, transposase-accessible chromatin coupled with high-throughput sequencing (ATAC-seq) will be performed to detect changes in chromatin accessibility for binding motifs of various transcription factors in BRAFi-R melanoma cells after Corin treatment.

<u>Identify epigenetic/transcriptional markers associated with resistance/re-sensitization to MAPKi therapy in patient specimens.</u> Markers whose expression confer resistance and are reversed during Corin-induced re-sensitization can potentially predict patient

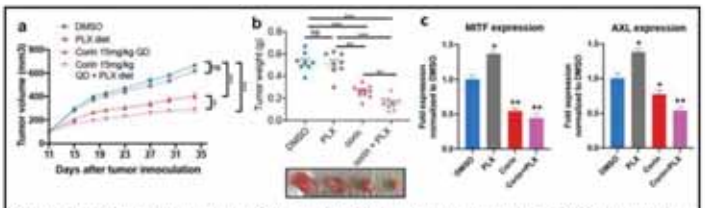

Figure 2. Corin allows resensitization of BRAFi-resistant tumor to BRAFi therapy in a melanoma xenograft. a) tumor volume and **b)** tumor weight plots demonstrate daily IP administration of corin (15 mg kg−1) potently inhibited BRAFi-resistant tumor growth in a melanoma xenograft model (n = 10/group). **c-d**) Corin alone or combination treatment overcame the increase of MITF and AXL in xenograft tumors (*p<0.05. **p <0.01. ***p < 0.001)

response to Corin therapy. The sequencing studies will identify markers for transcriptional regulators that mediate drug-resistant phenotypes, as well as markers associated with distinct drug-tolerant states that show response to Corin, and we will examine the association between these markers and MAPKi-resistance. We will use formalin-fixed paraffin-embedded patient tissues (FFPE) that are patient-matched melanoma tumors before MAPKi therapies (BRAFi, MEKi, or BRAFi+MEKi) at baseline, On-Tx (on treatment), and disease progressive (DP). To evaluate the relevance of the CoREST complex, we will perform IHC staining in clinical melanoma tissue specimens, using antibodies against CoREST components (RCOR1, REST, LSD1, and HDAC1), transcriptional regulators that mediate drug-resistant cell phenotypes and were shown to be reversed by Corin (MITF, AXL, SOX10, PAX3, WNT5A, TEAD2) (**Fig. 1d**). Comparison analysis will be conducted across groups of baseline, On-Tx, and DP specimens, to identify markers associated with MAPKi therapy resistance and potential predictive markers for re-sensitization to Corin.

Recent publication supported by Karin Grunebaum Foundation

Wu M, Hanly A, Gibson F, Kuang K, Kalin J, Nocco S, Collard M, Cole M, Xiao A, Agus F, Labadorf A, Cole PA, Alani RM. The CoREST Repressor Complex Mediates Phenotype Switching and Therapy Resistance in Melanoma. e-published in BioRxiv 2020.09.30.320580 Currently in peer-review Nature Communication

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The Foundation's Mission and its chosen path to Mission Accomplishment.....

Because Karin Grunebaum died at age 39 from an unknown primary site malignancy, the overriding objective of the Karin Grunebaum Cancer Research Foundation is the eradication of all types of cancer. The Foundation's original Declaration of Trust, written in 1958, mandates that the Foundation's funds be exclusively used for "...aiding research in and study of the cause, treatment and cure of cancer."

The Foundation's Trustees firmly believe that the eradication of cancer will only occur through successful research accomplishments which are followed by successful practical/commercial application. Thus, the Foundation has chosen to invest its funds directly in dedicated cancer researchers in hope of helping them achieve significant accomplishments to eliminate all types of carcinomas and thereby eradicate each and every type of cancer.

KARIN GRUNEBAUM
cancer research foundation