Founded in 1958

KARIN GRUNEBAUM
cancer research foundation

November, 2018 • Volume 15

From the Chair

Dear Friends of the Karin Grunebaum Cancer Research Foundation –

Diamonds Are Forever, or so numerous DeBeers ads, as well as a song and a book/movie title proclaim. Diamonds have indeed been around a long time and are a valued treasure throughout the world. So, it is with great pleasure (and even a bit of personal amazement) that I can advise you that the Karin Grunebaum Cancer Research Foundation celebrates its ***Diamond (60th) Anniversary*** of promoting cancer research this year. I believe it is also a long-valued treasure.

Given this extended history of philanthropy, allow me to trace with you some of our noteworthy accomplishments during this period, as documented on the Foundation's website (www.grunebaumfaoundation.org):

When the Foundation was first established in the pre-computer days of 1958, it initially created a manual cancer registry at Salem Hospital in Massachusetts so that doctors and staff could review relevant medical information about other patients with similar cancers who had passed through that facility. Later, it was decided to "invest in people" instead of technology. In 1966, under Fritz Grunebaum's (Karin's husband) guidance, and with the help of Harvard Medical School, we started to fund cancer related research by 3rd year medical students at Harvard Medical School. At the beginning, we were only able to fund one researcher annually. But, by 1979, we were able to fund two researchers each year. Next, the Foundation established an annual Distinguished Speaker in Cancer Research Series, which was only discontinued when we started to fund two M.D./Ph.D. cancer researchers at Boston University's School of Medicine every year in addition to the Harvard researchers.

In 2002, the Karin Grunebaum Chair in Cancer Research was established at the Boston University School of Medicine. This Chair, the first of its kind, is intended to be cross-disciplinary, so that cancers of any type can be properly studied and hopefully eradicated.

In 2005 the Foundation welcomed Massachusetts General Hospital to the Karin Grunebaum family of supported institutions – sponsoring clinical 2-year post-graduate surgical research Fellowships at that facility's world-renowned pancreatic cancer laboratory.

In 2006 the Trustees decided that the fight against cancer could be more efficiently waged if the Foundation's funding was channeled to directly help cancer researchers who had already decided that cancer research was to be their life's work. Accordingly, the Foundation decided to annually sponsor projects by junior faculty members at Harvard Medical School and Boston University School of Medicine involved in clinical or translational cancer research.

The Foundation also awards annual cash stipends to allow our sponsored Fellows to attend professional seminars and symposia.

In 2011 the Trustees started funding training programs for potential new cancer researchers at both Harvard Medical School and Boston University School of Medicine. At Harvard Medical School from 2011 - 2016 we annually sponsored the Fall Welcome Event, the Student Data Club and the Seminar Speaker Lunch Series for medical students participating in the Biological and Biomedical Sciences Cancer Biology Area of Concentration. At Boston University, we continue to sponsor the Karin Grunebaum Summer Research Fellowships which provides an opportunity for promising medical and college students to spend a summer working in the laboratory of a leading cancer scientist.

Starting in 2017, we started sponsoring the annual Karin Grunebaum Cancer Research poster competition at Harvard Medical School, with monetary prizes awarded to the winners.

Today, after awarding over 130 Fellowships, we still award annual Fellowships to cancer researchers from Harvard Medical School and Boston University School of Medicine who are working towards a cure for this dreaded disease.

Our Board of Trustees is unique in that it is, and has always been, comprised both of Karin's children and grand-children as well as world-renowned medical educators and cancer researchers, such as the Dean for Graduate Education at Harvard Medical School, the Dean and Provost of Boston University School of Medicine, as well as former Fellows and other medical practioners who have distinguished themselves in the field of cancer research. And, as is our tradition since Fritz Grunebaum established the Foundation in 1958, *the Trustees personally meet with each and every one of these outstanding Fellows to discuss their work and their hopes for the future of cancer eradication.*

All of these opportunities are only available because of your donations. I thank you for your financial support which allows us to provide these unique and diverse programs to so many worthy recipients dedicated to fighting cancer. Please continue supporting our efforts to help eradicate this dreaded disease.

Steven Wallach
Chair

New Board Member

Genevieve Boland, M.D., PhD, FACS

Director, Melanoma Surgery Program, Massachusetts General Hospital
Assistant Professor, Harvard Medical School
Associate Member, Broad Institute of MIT and Harvard

Genevieve M. Boland, M.D., PhD, FACS is Director of the Melanoma Surgery Program and Director of the Surgical Oncology Research Laboratories at the Massachusetts General Hospital. She is an Assistant Professor at Harvard Medical School and an Associate Member of the Broad Institute of MIT and Harvard. Dr. Boland's primary clinical focus is on melanoma and cutaneous oncology. She undertook combined MD/PhD training, completing a PhD in Cell and Tissue Engineering at the National Institutes of Health and graduated cum laude from Thomas Jefferson University as a member of the Alpha Omega Alpha medical honor society. She completed her general surgical training at the Massachusetts General Hospital, followed by combined clinical and research fellowships in Complex General Surgical Oncology at the University of Texas MD Anderson Cancer Center.

Dr. Boland is an active participant in many societies including the American College of Surgeons (ACS), the Society of Surgical Oncology (SSO), the Association for Women Surgeons (AWS), the Association for Academic Surgery (AAS), the Society of University Surgeons (SUS), the American Association of Cancer Research (AACR), and the American Society for Clinical Oncology (ASCO). She is a member of the SSO Melanoma Disease Site Working Group and serves on the Editorial Board of *Annals of Surgical Oncology* (Melanoma Section). She participates in the AWS Grants and Fellowships Committee and is the Secretary of the MA Chapter of the AWS. Dr. Boland works with the AAS as a part of the Academic Advancement Committee and functions as a Women in Cancer Research (WICR) mentor for AACR. She is on the Cancer.net advisory committee for ASCO and is on the Board of Trustees for the Karin Grunebaum Cancer Research Foundation.

Dr. Boland has been the recipient of multiple research awards including the American Surgical Association Foundation Fellowship, the Society of Surgical Oncology Clinical Investigator Award, the Association of Women Surgeons Research Fellowship, the Harvard Catalyst Medical Research Investigator Training Award, and the Karin Grunebaum Research Fellowship. Her work has been published in C*ell, Nature, Nature Medicine, Cancer Discovery, Clinical Cancer Research, Cancer, Journal of Clinical Investigation*, and *Annals of Surgical Oncology*. Her research is focused on the characterization of molecular and immunological changes that occur during immunotherapy and the identification of circulating biomarkers of cancer

Follow the Fellows

Joshua Campbell, Ph.D.

Assistant Professor, Division of Computational Biomedicine, Department of Medicine, Member of the BU-BMC Cancer Center
Affiliate member, Broad Institute of MIT and Harvard

Research Overview

I would first like to take this opportunity to thank the Grunebaum Family for supporting the work in my lab. We focus on the development and application of computational approaches to high-throughput genomic technologies in order to advance the understanding of molecular events responsible for cancer pathogenesis. Specifically, we use these technologies and algorithms to better understand the molecular underpinnings of health disparities in prostate cancer as well as development of precancerous lesions in the lung.

Prostate cancer disparities.

Despite gradual declines in mortality related to cancer in the United States, disparities by race have persisted. Prostate cancer is emblematic of this challenge in men with African ancestry have a higher overall incidence, earlier age of onset, increased proportion of clinically advanced disease, and increased mortality from prostate cancer compared to European Americans. Differences in prostate cancer biology in men with African ancestry may contribute to the observed differences in outcome. We are profiling cell free DNA in the blood to identify mutations in men with African ancestry that may cause resistance to androgen-directed therapies. We are also performing single-cell sequencing of prostate biopsies to reveal biological insights into the role of the immune system in aggressive prostate cancer in this population. These studies are supported by funds from the Department of Defense (DoD) Health Disparities Research Award and the National Cancer Institute (NCI) for Feasibility and Planning Studies for Development of Specialized Programs of Research Excellence (SPOREs) to Investigate Cancer Health Disparities (P20) program.

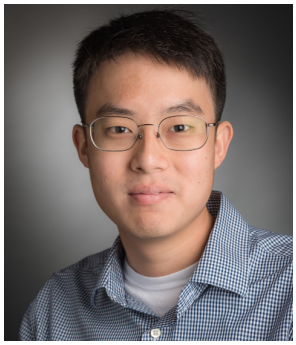
Lung premalignancy.

Approximately 30% of all lung cancers are classified as squamous cell lung cancer, a subtype of non-small cell lung cancer. It is typically found in the main airways of the lungs and is often preceded by the development of abnormal regions called premalignant lesions. However, not all premalignant lesions progress to lung squamous cell carcinoma and many will regress without clinical intervention. We are developing a Pre-Cancer Genomic Atlas by performing various types of molecular and histological profiling of these lesions as they progress towards invasive tumors. Identifying the mutations in the tumor genome and immunological changes in the tumor microenvironment that lead to progression will allow us to develop biomarkers for early detection and therapeutic strategies for disease interception. These studies are supported by funds from Stand Up to Cancer (SU2C), the NCI Pre-cancer Atlas (PCA) initiative, and Johnson and Johnson.

Follow the Fellows

Justin Kim, Ph.D.

Assistant Professor of Biological Chemistry and Molecular Pharmacology, Harvard Medical School Department of Cancer Biology, Dana-Farber Cancer Institute



Research Overview

During tumorigenesis, the rapid growth of cancer cells outpaces vascularization, depriving tumors of both nutrient and oxygen supply. Non-uniform blood flow and diminished oxygen diffusion rates resulting from malformed and disorganized vasculature frequently lead to tumors that display regions of acute and chronic hypoxia featuring oxygen tensions less than 1%. Regions of radiobiological hypoxia are notoriously resistant to radiotherapy, which is dependent on the radiosensitizing properties of oxygen, as well as to chemotherapy, a majority of which targets rapidly dividing cells. Hypoxic cells, in general, are very slowly cycling or not cycling at all. Additionally, activation of the transcription factor hypoxia-inducible factor 1 (HIF1) at these low oxygen tensions promotes a more aggressive spread of the disease by effecting significant changes to various facets of cancer progression including cellular proliferation and survival, metabolism, angio- and vasculogenesis, cell infiltration, invasiveness, genomic instability, and metastasis. Hypoxia is a prominent feature of the tumor microenvironment in solid tumors of nearly all cancer types, including those of the cervix, breast, pancreas, kidneys, and head and neck. In cancer, the onset of hypoxia is a highly negative prognostic factor for which there are few effective treatment options.

Over the past several decades, hypoxia-activated prodrugs (HAPs) have been explored both at the bench and in the clinic. Nine compounds have gone through or are undergoing various stages of clinical trials. However, recent high-profile failures of tirapazamine and TH-302 to meet survival endpoints in phase III trials have forced the field to reassess its approach to HAP development. These failures demonstrated the imperative of developing diagnostics for predicting sensitivity of individual tumors to the HAPs. It is evident that there exists a remarkable diversity in the severity of tumor hypoxia across cancer patient populations, and it is widely believed that the inclusion of patients unlikely to benefit from tirapazamine and TH-302 has largely contributed to the failure of the respective phase III clinical trials. In retrospective studies, when patient populations were stratified by positive identification of hypoxic tumor regions using ^{18}F-MISO PET imaging, it was determined that only 1 out of 19 patients experienced local-regional failure upon tirapazamine treatment. In contrast, 8 out of 13 failed in the control arm. Levels of hypoxia vary from 0 to 100 percent in tumors, yet no clinical factors, such as size or stage, or genomic features have been identified that are predictive of this value. Given the interest in properly identifying candidate patient populations for hypoxia-based treatments, new imaging agents for diagnosis as well as for image-guided therapies are of growing importance, especially in this era of personalized medicine.

My laboratory focuses on the development of new prodrugs and imaging agents for the treatment and diagnosis of tumor hypoxia. Recently, we designed and synthesized a new chemical motif which can be activated in an oxygen-dependent and irreversible manner by select hemeproteins within the cell. Key features that distinguish our novel oxygen-responsive agent from all other hypoxia-targeted approaches in the literature and in clinical trials are: 1) our compounds are activated by two-electron reductive processes mediated by hemeproteins that are highly upregulated in cancers and specifically expressed downstream of hypoxia-responsive elements in the genome, 2) they are activated *irreversibly*, rendering them amenable to targeting *acute* hypoxia, a condition in which the oxygen levels fluctuate within the tumor mass, 3) the chemical motif is small and modular enabling its structure to be tuned to control oxygen-sensitivity and enabling its attachment to a variety of therapeutic payloads, and 4) hypoxia-activation results in a mechanistically-linked process involving concomitant drug release and covalent modification of the tumor with imaging agents, providing a unique opportunity for theranostic development. Excitingly, we recently demonstrated that cultured glioblastoma, pancreatic, and cervical cells, among others, are labeled selectively under hypoxic conditions versus normoxic conditions using our compounds. In vivo experiments with BxPC-3 pancreatic adenocarcinoma tumor xenografts in mice confirmed selective labeling of tumor tissue and confirmed co-localization of our probes with current biochemical standards for hypoxia labeling.

Furthermore, we have been able to leverage the versatile hypoxia-sensing chemical motifs in prodrugging applications in which we chemically synthesized prodrugs of potent cytotoxic agents. We have recently demonstrated that several indiscriminately toxic chemical agents can be rendered selective against cancer cells in low oxygen microenvironments through our chemical technology. Many of our compounds can achieve hypoxia-selective drug-release in multiple types of cultured cancer cells with hypoxic to normoxic ratios of greater than 10 to 1.

Research Update

During the past three months, we have, through the generous contribution of the Grunebaum Foundation, been able to translate the chemical synthesis of our lead imaging compounds that demonstrate hypoxia-selective imaging in vivo into a synthesis of their positron emission tomography (PET) active versions. By working with Dana-Farber Cancer Institute's Lurie Family Imaging Center (LFIC), we are now able to produce imaging doses of ^{18}F-labeled PET tracers based on our original compound design. In the upcoming weeks and months, we look forward to performing tumor imaging in vivo in mouse tumor xenograft models with these compounds in the hopes of making significant improvements over current standards established by ^{18}F-MISO. We also look forward to iteratively modifying our compounds to improve their overall pharmacokinetic and pharmacodynamic properties. Ultimately, we would like to combine the imaging properties with the therapeutic properties for use in image-guided drug delivery applications in solid tumors.

Lab News

Broadly speaking, my lab is interested in developing new chemical tools for studying and manipulating protein-protein interactions relevant to cancer. Based on key insights derived from our work described above, we were able to develop a new project involving a novel genetically encoded method for protein surface functionalization. We have recently been awarded the NIH Director's New Innovator Award for our work in this area, demonstrating that funding by the Grunebaum Foundation for early career investigators has the potential to have broad impact in getting a young research lab off the ground, even beyond the project being funded. Our lab is now funded by the William F. Milton Fund, the NIH, the Claudia Adams Barr Program, and the Ellison Foundation in addition to the Grunebaum Foundation. We thank the Grunebaum Foundation for being our first funding source and opening up many more opportunities.

New Lab Members

Gang Shan, PhD: Postdoc, Dhanushka Munkanatta Godage, PhD: Postdoc.

Summer Research Report

Developing a miRNA signature to differentiate metastatic head and neck cancer from primary lung squamous cell carcinoma

Sainath Asokan, Boston University School of Medicine

Background: Head and neck small cell carcinoma (HNSCC) is a morbid cancerous disease that can present as metastatic tumors to distant locations, most frequently occurring in the lung. Conversely, early stage primary lung squamous cell carcinoma (LSCC) is a cancer that originates from within the lung itself. While the overall prognosis of HNSCC patients is very poor with a 5-year survival rate of ~35% after undergoing surgery, stage I and II LSCC patients treated with curative surgical resection have a 5-year survival rate of over 80%. The curative therapies provided to both groups of patients is vastly different; HNSCC patients with new, squamous lung lesions should undergo non-anatomical resection (removal of less than an anatomic portion) of the lung whereas the standard of care for LSCC patients is an anatomical resection (i.e. pulmonary segmentectomy, lobectomy, and pneumonectomy). As a result, differentiating between primary LSCC and pulmonary metastases of HNSCC has significant implications for educating clinical decisions in order to prevent the administration of inappropriate therapies and lower risks of disease reoccurrence and mortality. Previous studies (in particular, Guerts et al. and Lal et al.) have attempted to achieve this differentiation via the use of immunohistochemistry and mRNA profiling, but have clearly encountered pitfalls: namely contamination of tumor signatures with non-tumor tissue and a lack of proper methods to evaluate biomarker accuracy. We hope to avoid these pitfalls by using not only microdissection to extract pure tumor cell populations but also miRNA for greater stability and tissue specificity for cells of a common lineage.

Hypothesis or research question: We hypothesize that miRNA expression signatures will be able to differentiate between primary HNSCC and LSCC and therefore also discriminate metastatic HNSCC from primary LSCC in patients with a history of HNSCC.

Specific Aim(s): Aim 1: Build miRNA biomarker models differentiating primary HNSCC and LSCC in microdissected tumor samples from approximately 150 patients that are analyzed by RNA sequencing. **Aim 2:** Test these models using miRNA expression data from TCGA databases of primary LSCC and primary HNSCC. **Aim 3:** Use qRT-PCR for a more robust and clinically applicable analysis of miRNA expression in the selected models. **Aim 4:** Validate models in an independent set of tumor samples.

Methods, Data Collection, Statistical Analysis: We will be conducting a retrospective cohort study of patients with HNSCC and primary LSCC that were diagnosed and treated at Boston Medical Center between 2003 and 2017. The Department of Otolaryngology will be providing clinical data on HNSCC patients of all stages who have undergone biopsy or resection prior to chemotherapy or radiation. The Department of Pathology will be providing sectioned formalin-fixed paraffin-embedded (FFPE) tumor tissue blocks of these selected patients (approx. 75-100 each) of both diagnoses. H&E stained slides will also be provided to confirm the presence of tumor within the sections. Total RNA isolation will be conducted on the FFPE tissue blocks and RNA sequencing (miRNA-Seq) will follow in order to characterize the tumors and form RNA libraries that can then be amplified via qPCR. miRNA counts will be tabulated after the RNA sequences are aligned to the human genome; the tumor location and known clinical diagnosis will serve as the predictor and the outcome will be the miRNA expression. The LSCC and HNSCC samples will be split into training and control sets by random selection; this random selection and subsequent analyses will be repeated 500 times. A ROC analysis will be performed to evaluate performance of the selected miRNA sets in differentiating HNSCC from LSCC using the 500 randomly selected control sets. The signature we develop will be validated and evaluated via The Cancer Genome Atlas (TCGA) datasets for primary LSCC and HNSCC. The performance of the best-miRNAs will then be further evaluated in independent samples of HNSCC and LSCC using qPCR; this accuracy will be defined as the proportion of cases correctly classified. Overall survival in the future will also be used as the major endpoint to assess classification accuracy.

Anticipated Outcomes We expect to create a miRNA biomarker that will accurately differentiate metastatic HNSCC from primary LSCC and will be validated via qPCR cross-platform validation as well as external validation in the independent HNSCC and LSCC sample cohort. This will provide us future opportunities to further validate our miRNA signature against overall survival in a cohort of patients with HNSC and LSCC to ultimately create a clinically meaningful diagnostic test. In future studies, we also hope to focus exclusively on patients with lung lesions and a history of HNSCC to assess and optimize the positive predictive value of the potential biomarker we create via this project.

The impact of locoregional treatment on survival in patients with metastatic breast cancer: a National Cancer Database analysis. (Presented at the 2018 San Antonio Breast Cancer Symposium)

Daniel Huang, Boston University School of Medicine

Purpose and Objectives: Although systemic therapy is the standard treatment for metastatic breast cancer, the value of locoregional treatment (LRT) of the primary tumor and its impact on survival is controversial. This study evaluates survival outcomes in patients with metastatic breast cancer after receiving LRT (surgery and/or radiation therapy) of the primary tumor.

Materials and Methods: The National Cancer Database (NCDB) was queried for 16,128 qualifying cases of stage IV breast cancer (M1, 2004-2013) who received systemic therapy with or without local therapy. Treatment modality was divided into surgery, radiation therapy (RT), surgery with RT (Sx+RT), and no LRT. Median survival and three-year actuarial survival rates (OS) were analyzed for each treatment group. On multivariate analyses, adjusted hazard ratios (HR) with 95% confidence interval were computed using Cox regression modeling to adjust for patient characteristics, year of diagnosis, clinical T and N staging, and facility type. Additionally, survival outcomes for each treatment group were analyzed by metastasis groups (bone, visceral, multiple).

Results: A temporal trend of each treatment modality used in years 2004 – 2013 illustrated that the relative use of LRT decreased from 47.2% to 36.2% (p for trend = 0.041). Overall, the median follow-up was 28.3 months and median survival for all patients was 37.2 months. With 9,761 deaths reported, the estimated 3-year survival rate for all patients was 51.3%. The Sx+RT group (n = 2,166) had the highest 3-year survival rate of 69.4%, followed by the surgery group (n = 4,293) with 57.6%, no LRT group (n = 8,955) with 44.3%, and RT group (n = 714) with 41.5% ($p < 0.0001$). On multivariate analysis, a decreased hazard of death (adjusted HR, 95% CI) was noted in radiation patients compared to no LRT group but without statistical significance (0.91, 0.81-1.0, $p = 0.057$). Patients receiving surgery (0.68, 0.65-0.71, $p < 0.0001$) and Sx+RT group (0.46, 0.43-0.49, $p < 0.0001$) reported statistically significant improved survival compared to the no LRT group.

Additionally, later year of diagnosis, low Charlson-Deyo score, high income, private insurance, white race, age 18 - <50, low T and N stage, ductal histology, positive ER/PR/HER2 status, bone only metastasis, and academic facility type were considered favorable factors for OS. When stratified by metastasis type, patients with bone metastasis had the longest 3-year survival rates (74.4% for Sx+RT, 69.4% for surgery, 53.8% for no LRT, 49.3% for RT, $p < 0.0001$) whereas patients with multiple metastases had the worst outcomes (56.0% for Sx+RT, 43.5% for surgery, 37.9% for no LRT, 34.4% for RT, $p = 0.003$).

Conclusion: Patients with metastatic breast cancer have a large range of survival rates. Locoregional treatment, especially surgery followed by RT, in addition to systemic therapy was associated with improved survival in metastatic breast cancer patients. When survival rates for each treatment modality was stratified by metastasis location, the most favorable survival was observed for the surgery with follow-up radiation group, which is consistent with the overall analysis.

Follow the Fellows

Mikel Garcia-Marcos, Ph. D.

Assistant Professor, Department of Biochemistry, Boston University School of Medicine

I have been a Karin Grunebaum Cancer Research Fellow for the last two years (2016-2017, 2017-2018), which has had a catalyst effect in my career development and in my research program. Among other things, during this time I have been promoted to Associate Professor, secured additional funding from the NIH, enrolled in the Editorial Board of scientific journals and in NIH study sections, and published a string of important discoveries. Below I provide an overview, some background and the latest advances in our research, which include the development of drug prototypes and tools to understand and combat cancer metastasis.

Overview

My laboratory is broadly interested in elucidating the molecular mechanisms by which cells respond to external signals and how dysregulation of these mechanisms leads to cancer progression. The ultimate goal is to harness this knowledge to develop novel therapeutic approaches. We are specifically interested in a group of proteins called heterotrimeric G proteins, which work as molecular switches that relay extracellular signals to the interior of cells. From the traditional standpoint in the scientific community, receptor proteins bind chemical signals in the exterior of the cell and, as a consequence, induce the activation of G proteins in the interior of the cell, which then induce changes in cell behavior. Our research has challenged this "classical" view of G protein activation because we have identified a new group of G protein activators that are not receptors but exist in the interior of the cell. Importantly, these non-receptor activators "rewire" signal transduction in cancer and our work to date suggests that they could be vulnerability of metastatic cancers, the cause of the vast majority of cancer-related deaths.

Background

The first identified member of this new class of non-receptor G protein activators is called GIV or Girdin. We and others have observed that it is present at abnormally high levels in carcinomas of patients with metastasis. Importantly, its expression in tumors also predicts high risk of developing metastasis in patients in which it has not occurred yet. It has been subsequently established that GIV promotes metastasis and invasion in experimental models of cancer and that it does so by activating G proteins. Essentially, when GIV expression is elevated in metastatic cancer cells it rewires G protein signaling, making it hyperactive and favoring metastasis. These findings make GIV, and its function of activating G proteins, an attractive target to develop inhibitors of metastasis. This is very relevant because metastasis is the cause of more than 90% cancer-related deaths and the therapeutic strategies to block metastatic progression are very limited.

Recent Progress

In the past years, we have published work showing important mechanistic information of how GIV activates G proteins in cancer cells in response to external stumuli (see 1 below). In addition, we used a combination of biophysical, computational and biochemical tools to know how GIV and G proteins assemble physically at the atomic level (i.e., how they fit in a lock-and-key fashion) (see 2 below), which is the first step toward the rational design of chemical compounds that inhibit this interaction and the subsequent pro-metastatic activity. A challenge for developing the drugs we are seeking is that protein-protein interactions, like the one formed between GIV and G proteins, are traditionally considered as "poorly druggable". This means that it has to be demonstrated in a case-by-case basis that one of such protein-protein interactions can actually be disrupted by small chemical compounds. By taking advantage of the information on the physical interaction between GIV and G proteins that we published earlier this year (see 2), we investigated if the GIV-G protein interaction could be disrupted by small molecules, i.e., we tested whether it was "druggable". Our recently published data shows that this is the case (see 3), as we can selectively inhibit the binding of GIV to G proteins with a drug. Unfortunately, the molecules that we identified as inhibitors of the GIV-G protein interface are not suitable for use in cells or patients. However, the results provide the proof-of-principle that lends confidence that the large screen (200,000 chemical compounds) we are currently conducting will yield promising drug prototypes. Our preliminary data indicates that we have identified new inhibitors of the GIV-G protein interface that block cancer cell migration, a prerequisite for cancer metastasis. Our next step is to fully characterize these new compounds and optimize them based on the detailed atomic-resolution information we have generated. This project has been recently funded by the NIH for the next 4 years.

Along with the efforts described above we have generated a suite of techniques to study our target of interest (see 4 and 5 below), and novel tools to clarify how it operates in cancer metastasis. More specifically, we have been able to rationally engineer an artificial protein that blocks the activity of GIV in cancer cells (see 6 below). Although this synthetic protein cannot be used for therapeutics in humans, it is a very powerful tool that will allow the elucidation of how GIV causes metastasis and the beneficial consequences of inhibiting its activity. This is highly complementary to our drug discovery efforts. Our recent work has captured the interest of others in the field, which has resulted in an invitation to submit a review on our recent work to Biochemistry, as part of an special issue to highlight important work done by junior investigators.

Related Activities

The Karin Grunebaum Cancer Research Foundation funded my attendance to the Experimental Biology 2017 meeting in Chicago and the Experimental Biology 2018 meeting in San Diego. In both occasions, I or a member of my laboratory has presented our work in progress in selected oral presentations. Recently I became a standing member of the Molecular and Integrative Signal Transduction (MIST) Study Section of the NIH. I have also served in the Tumor Biochemistry and Endocrinology Study Section of the American Cancer Society for the last few years, and recently I have been appointed as its Chair. I have also become a member of the Editorial Board of the Journal of Biological Chemistry and of Scientific Reportsogical Chemistry. In 2017, I was promoted to Associate Professor (Department of Biochemistry).

Recent Publications

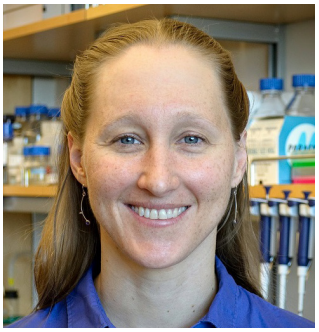
1. Parag-Sharma K, Leyme A, DiGiacomo V, Marivin A, Broselid S, Garcia-Marcos M. Membrane Recruitment of the Non-receptor Protein GIV/Girdin (Galpha-interacting, Vesicle-associated Protein/Girdin) Is Sufficient for Activating Heterotrimeric G Protein Signaling. Journal of Biological Chemistry. 2016. Dec 30;291(53):27098-27111. PMID: 27864364
2. de Opakua AI, Parag-Sharma K, DiGiacomo V, Merino N, Leyme A, Marivin A, Villate M, Nguyen LT, de la Cruz-Morcillo MA, Blanco-Canosa JB, Ramachandran S, Baillie GS, Cerione RA, Blanco FJ, Garcia-Marcos M. Molecular mechanism of G?i activation by non-GPCR proteins with a G?-Binding and Activating motif. Nature Communications. 2017. May 18;8:15163. PMID: 28516903
3. DiGiacomo V, de Opakua AI, Papakonstantinou, MP, Nguyen LT, Merino N, Blanco-Canosa JB, Blanco FJ, Garcia-Marcos M. The G?i-GIV binding interface is a druggable protein-protein interaction. Scientific Reports. 2017. Aug 17;7(1):8575. doi: 10.1038/s41598-017-08829-7. PMID: 28819150
4. Maziarz M, Garcia-Marcos M. Fluorescence polarization assays to measure interactions between G? subunits of heterotrimeric G proteins and regulatory motifs. Methods in Cell Biology. 2017. Sept 142:133-143. PMID: 28964332.
5. Maziarz M, Garcia-Marcos M. Rapid kinetic BRET measurements to monitor G protein activation by GPCR and non-GPCR proteins. Methods in Cell Biology. 2017. Sept 142:145-157. PMID: 28964333.
6. Leyme A, Marivin A, Maziarz M, DiGiacomo V, Papakonstantinou MP, Patel PP, Blanco-Canosa JB, Walawalkar I, Rodriguez-Davila G, Dominguez I, Garcia-Marcos M. Specific inhibition of GPCR-independent signaling by a rationally engineered protein. Proceedings of the National Academy of Sciences. 2017 Nov 28;114(48):E10319-E10328. PMID: 29133411
7. DiGiacomo V, Marivin A, Garcia-Marcos M. When Heterotrimeric G Proteins Are Not Activated by G Protein-Coupled Receptors: Structural Insights and Evolutionary Conservation. Biochemistry. 2018; Jan 23; 57(3):255-257. Review (peer reviewed). PMID: 29035513.

*Selected for Special Issue "Future of Biochemistry""

Follow the Fellows

Sloan Devlin, Ph.D.

Assistant Professor, Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School



Lab Overview

Human-associated bacteria play a vital role in health and disease. Microbial imbalance has been linked to a wide range of disease states, including colon and liver cancers, inflammatory bowel disease, autism, and obesity. However, the molecular mechanisms by which the bacterial guests interact with the human host at a molecular level are poorly understood. One of the most concrete ways that the microbiome affects the host is through the production of small molecule metabolites, some of which accumulate in the body to levels higher than that of a typical drug. I am leveraging my strong training in synthetic organic chemistry as well as expertise in molecular biology, microbiology, analytical chemistry, and gnotobiotic mouse experimental design to 1) elucidate the biosynthetic pathways and biological functions of small molecules produced by the human microbiome and 2) design, synthesize, and utilize small molecules to probe and manipulate human-associated bacteria in vivo.

Progress Update

We are investigating the biosynthesis and biological activities of secondary bile acids, bacterial metabolites that may play crucial roles in both the suppression and development of colon and liver cancers. In particular, the abundant metabolites deoxycholic acid (DCA) and lithocholic acid (LCA) (**Fig 1A**) are obesity-associated carcinogens. Only a small number of bacterial producers have been identified, however, and these strains represent only ~0.0001% of all bacterial cells in the human gut. We hypothesize that there exist as-of-yet unidentified 7α-dehydroxylating bacterial strains that produce DCA and LCA in vivo. Using bioinformatics, we identified strains of the human gut bacterium *Clostridium bolteae* that contain homologs for known 7α-dehydroxylation genes (i.e., the bai operon). Importantly, the abundance of this organism is significantly increased in people eating a high-fat diet. Although we observed conversion of the primary bile acid cholic acid (CA) into DCA by the *C. bolteae* strain 90A5, we have been unable to observe reproducible dehydroxylation from this strain, perhaps due to differences in in vitro culture conditions we have yet to identify. We have observed activity from putative *C. bolteae* 90A5 enzymes that we heterologously expressed and purified in *E. coli*. This activity is weak, however compared to the activity of known 7α-dehydroxylation genes, suggesting that we may not have paired the ideal substrate with each enzyme. Using qRT-PCR, we have determined that all of the putative 7α- dehydroxylation genes in *C. bolteae* 90A5 are induced by bile and that this induction is growth-phase dependent. These results support our hypothesis that these enzymes are bile acid-responsive and suggest that we may not yet have identified the native bile acid substrate necessary for dehydroxylation by this organism.

In order to assay the activity of *C. bolteae* in its the endogenous environment, the mammalian gut, we monocolonized germ-free mice with either a *C. bolteae* strain containing the putative bile acidmetabolizing cluster (90A5) or a strain lacking this cluster (90A9). Consistent with our in vitro results, we observed significantly more deconjugation of primary bile acids in the feces of 90A5- compared to 90A9- colonized mice. Since bile acid deconjugation via a bile salt hydrolase bacterial enzyme is a necessary first step prior to dehydroxylation (Fig 1A), these results are encouraging. We have not yet detected dehydroxylated products in feces, so we are currently analyzing bile acid composition in cecal contents, blood, and liver from these mice. We are also beginning to explore a related hypothesis, that dehydroxylation is a collaborative endeavor carried out by enzymes from multiple gut bacteria. Indeed, we have already identified portions of clusters containing homologs for bai operon genes in the human gut commensals *Eggerthella lenta* and *Blautia producta* as well as the probiotic species *Lactobacillus plantarum*. We will co-culture these species along with common gut bacteria from the phyla *Firmicutes* and *Bacteroidetes* to explore the possibility that groups of bacteria working in tandem can produce secondary bile acids.

Because DCA and LCA are tumor-promoting metabolites, limiting their production by human gut bacteria may reduce colon and liver cancer incidence in at-risk populations. Moreover, recent research suggests that host-produced primary bile acids foster a tumor-rejecting environment in the liver through the accumulation and activation of natural killer T (NKT) cells,[1] further suggesting that shifting the bile acid pool toward primary bile acids and away from DCA and LCA may limit cancer initiation and progression. Because deconjugation of primary bile acids by a bacterial bile salt hydrolase (BSH) enzyme is necessary before dehydroxylation can occur **(Fig 1A)**, we posit that inhibiting BSH activity will limit the production of toxic DCA and LCA metabolites in vivo. We identified a selective bile salt hydrolase in *Bacteroides thetaiotaomicron (Bt)*, an abundant gut species in people eating a Western diet and generated a genetic mutant that lacks this enzyme. We then monocolonized germ-free mice with wild type Bt or the genetic knock-out strain (BSH KO), fed mice a Western (i.e., high-fat, high sugar) diet for 4 weeks, and monitored bile acid and metabolic changes in the host. We found that BSH KO-colonized mice gained less weight on the Western diet than Bt WT-colonized mice **(Fig 1B)** and also exhibited less liver steatosis. A manuscript describing this work is currently under review at eLife. We are currently designing and synthesizing small molecule inhibitors of bacterial BSH. Two of our compounds display near complete inhibition of BSH activity both against purified enzyme and in growing bacterial culture (Fig 1C). We plan to test these inhibitors first in fully colonized mice to demonstrate reduction of DCA and LCA production, then in mouse models of colon and liver cancers.

Finally, growing evidence suggests that inflammation is involved in the etiology of colorectal cancer. Indeed, patients with inflammatory bowel diseases are at increased risk of developing colon cancer. Recent reports suggest that the pro-inflammatory cytokine IL-17, which is produced by Th17 cells found in the intestinal lamina propria, plays a role in colon inflammation and carcinogenesis.[2] In collaborative work with Prof. Jun Huh's lab at Harvard Medical School, we have discovered that the bacterially produced secondary bile acid isolithocholic acid (isoLCA, **Fig 1A**) limits both the differentiation of naïve T cells into Th17 cells and the IL-17 production of these differentiated cells both in vitro and in vivo in mice (**Fig 1D**). The Devlin lab has, for the first time, identified human gut bacteria that produce isoLCA as well as the enzymes responsible for the biosynthesis of this molecule. We are currently generating isoLCA producer- and non-producer bacterial strain pairs. We will then colonize germ-free and conventional mice with these strains and assay their abilities to limit IL-17 levels and inflammation in vivo. These experiments will lay the groundwork for the rational manipulation of the microbiome in a clinical context to limit inflammation-induced colon cancer initiation and progression.

Publications supported by Grunebaum Foundation

1. Yao, L., Seaton, S. C., Ndousse-Fetter, S., Adhikari, A., DiBenedetto, N., Bry, L., Devlin, A. S. A selective gut bacterial bile salt hydrolase alters host metabolism. *Under review at eLife.*

References

1. Ma, C. *et al.* Gut microbiome-mediated bile acid metabolism regulates liver cancer via NKT cells. *Science* 360, eaan5931 (2018).
2. Kathania, M. *et al.* Itch inhibits IL-17-mediated colon inflammation and tumorigenesis by ROR-γt ubiquitination. *Nat. Immunol.* 17, 997–1004 (2016).

Research in the Devlin laboratory is currently supported by grants from the Harvard Digestive Diseases Center and the Center for Microbiome Informatics and Therapeutics in addition to the Karin Grunebaum Foundation.

Poster Competition Winners

Matthew McBride

Cigall Kadoch Lab

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

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.

Caitlin A. Nichols

Rameen Beroukhim Lab

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

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.

Jessica Spinelli

Marcia Haigis Lab

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

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.

Your Support is Vital to our Mission

The KGCRF relies soley on private donations. In order to continue the fight, we ask for your support and hope that you will give what you can.

Your tax-deductible contribution will directly help fund the cancer research effort, since all our Officers and Trustees are unpaid volunteers, and the Foundation has no paid employees.

I enclose my gift of:

❑ $50 ❑ $100 ❑ $250

❑ Other: $_____

Name: _____

Street Address: _____

City, State, Zip: _____

Country: _____

Telephone: _____

Email: _____

Please return to: KGCRF, 1100 Massachusetts Ave., 5th Floor, Cambridge, MA 02138

You may also donate online at:
grunebaumfoundation.org/html/SupportContributions.asp

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