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KARIN GRUNEBAUM
cancer research foundation

October, 2017 ♦ Volume 14

From the Chair

Dear Friends of the Karin Grunebaum Cancer Research Foundation –

I am pleased to report that the first annual KGCRF poster competition was held at Harvard Medical School on May 19, 2017 and was an enormous success. In addition to the contestants, medical school faculty and judges, members of the Grunebaum family and several medical professional Trustee were also able to attend. Thirty-two students entered the competition. Each of the students prepared a poster for presentation to their faculty and student colleagues, and the event was extremely well attended. A distinguished panel of judges (one of whom was Trustee Ed Harlow) chose four winners of the competition -- Molly DeCristo, Josh Pan, Whitney Silkworth, and Jessalyn Ubellacker. The winners each received a $1,000 Karin Grunebaum Professional Development Award to be used for their professional development. The winners also had the opportunity to present their work to the full body of Trustees at the KGCRF Board meeting in June.

The Foundation's Fellowships continue to be a much sought-after grant. For example, the competition for the 2017-2018 KGCRF Faculty Research Fellowship was the strongest we have ever seen at Harvard Medical School. Ten applications were received and the members of the selection committee rated three to five applications in the very top category. The Fellowship was awarded to Sloan Devlin, Ph.D.

At Boston University School of Medicine, the competition for the Fellowship has also continued to be very competitive. In fact, the work performed by current Fellow Mikel Garcia-Marcos, Ph. D. was deemed to be so impressive, that the Trustees were asked by BUSM if the governing documents regarding the award of Fellowships could be revised to allow a second year follow-on Fellowship for a Fellow entering a sixth year of faculty membership. The Trustees agreed to the requested amendment, and Dr. Marcos-Garcia was awarded a second year follow-on Fellowship. During his current Fellowship year Dr. Garcia-Marcos received a travel stipend from the Foundation to present an oral report at the Experimental Biology symposium in Chicago entitled *"The Interaction of GIV with Galpha-I Is a Druggable Protein-Protein Interaction."*

The Foundation has also continued to sponsor summer cancer research projects by medical students at BUSM. This year we sponsored Faisal Al Bahrani in his study of *Reducing Socioeconomic Disparity in Skull Base Tumors* and Meghan Leary who researched *Epigenetic Regulation of Growth Promoting and Tumor Suppressing Genes in Ovarian Cancer*. Both students were invited to attend the annual Fellows' presentation of research to the KGCRF Trustees at their June meeting.

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
Chairperson

grunebaumfoundation.org

Follow the Fellows

Genevieve M. Boland, M.D., Ph. D.

Assistant Professor, Harvard Medical School
Surgical Director, MGH Melanoma & Pigmented Lesion Clinic
Director, MGH Surgical Oncology Research Laboratories, Massachusetts General Hospital

Research Summary:

Immune checkpoints are necessary for self-tolerance, but are misappropriated by tumors for immune evasion. Checkpoint blocking antibodies can reactivate these anti-tumor responses, and immune checkpoint blockade in melanoma has shown immense clinical success with response rates of up to 50% with combination immunotherapy and a subset of durable, longterm survivors. Efforts have focused on profiling of patient-derived tumors, but robust predictive signatures of response or resistance are lacking, and there are no techniques for monitoring immune activation longitudinally. Exosomes are circulating microvesicles that contain a subtranscriptome of their cell of origin and are involved in oncogenesis and immune modulation. Exosomes are produced by tumor and immune cells and are mediators of immune responses in cancer. This project utilizes circulating exosomal RNA to capture changes reflective of both the tumor and the immune system, offering an opportunity to analyze systemic changes reflective of immune activation and tumor-specific data in parallel via blood-based assays. We are able to detect melanoma driver mutations in circulating exosomes in an vitro model of melanoma and in patient-derived samples. We have demonstrated a high concordance between tumor and exosomal RNA profiles and can track exosomal RNA levels over time. There is a subset of transcripts identified only in exosomes (not paired tumors) that represent immune-related pathways suggesting that we can monitor changes in both the tumor and immune system simultaneously, which is particularly relevant for immunotherapy patient populations. Whole transcriptome analysis of pretreatment exosomal RNA demonstrates enrichment of antigen processing and presentation pathways, chemokine signaling pathways, and markers of immune activation in responders versus nonresponders to aPD1 immunotherapy.

There are multiple novel components to our approach to blood-based monitoring in immunotherapy patients with melanoma. We are the first to demonstrate the utility of exosomes in monitoring both tumor-derived and immune-derived changes in cancer. Currently, there are no robust and reproducible markers to predict response to immunotherapy from tumor-based assays, likely due to an underrepresentation of immune-derived signals in these samples and the heterogeneity of response to immunotherapy across tumors. In contrast to existing techniques, our exosomal platform allows access to information from all tumors simultaneously, as well as information representative of the immune system allowing a more broad and representative assessment of overall clinical response to therapy. Our approach also allows ongoing monitoring of immune activation over time. Our goal is to fill the unmet need to identify predictive markers of response and tools for longitudinal assessment of patients treated with immunotherapy.

New Committee Service:

- MGH Resident Research Advisory Committee
- Association of Women Surgeons: MA Chapter: Secretary; Grants and Fellowship Committee; 2018 Conference Planning Committee
- American Association of Cancer Research; Women in Cancer Research: Mentor and Discussion Facilitator (Annual Meeting 2017)

New Positions:

- Surgical Director, MGH Melanoma & Pigmented Lesion Clinic
- Director, MGH Surgical Oncology Laboratories
- Affiliate Scientist, Broad Institute of Harvard/MIT

Publications:

1. Khosravi H, Akabane AL, Alloo A, Nazarian RM, **Boland GM**. Metastatic Melanoma with Spontaneous Complete Regression of a Thick Primary Lesion: a Case Report. Journal of the American Academy of Dermatology Case Reports. 2016 Dec 3.
2. Raigani S, Cohen S, **Boland GM**. The Role of Surgery for Melanoma in an Era of Effective Systemic Therapy. Current Oncology Reports. 2017 March 19.
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4. Larimer B, Wehrenber-Klee E, Dubois F, Mehta A, Kalomeris T, Flaherty K, **Boland GM**, Mahmood U. Granzyme B PET Imaging as a Predictive Biomarker of Immunotherapy Response. Cancer Discovery. May 2017.
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6. Sade-Feldman M, Jiao J, Rooney MS, Chen JH, Barzily-Rokni M, Stemmer-Rachamimov A, Eliane JP, Hammond MR, Vitzthum H, Blackmon S, Frederick DT, Shukla S, Yizhak K, Getz G, Duncan LM, **Boland GM**, Lawrence DP, Landau DA, Flaherty KT, Sullivan RJ, Hacohen N. Resistance to Checkpoint Blockade Therapy Through Inactivation of Beta-2-Microglobulin. Nature Medicine. Submitted.
7. Jenkins RW, Aref AR, Lizotte PH, Paweletz C, Zhou CW, Bowden M, Liu H, Walker W, Palakurthi S, Ivanova E, Bittinger M, Vitzthum H, Kim JW, Barzily-Rokni M, Smart A, Miao D, Canadas I, Thai TC, Jones RE, Keogh L, Hammond M, Hanna G, Huang W, Hoang M, Piris A, Cameron L, Thakuria M, LeBoeuf NR, Rabinowits G, Gunda V, Parangi S, Cleary JM, Miller BC, Haining WN, Yoon C, Van Allen EM, Freeman GJ, Lorch J, Ott, PA, Hodi S, Flaherty KT, Kamm RD, **Boland GM**, Wong K, Barbie DA. Ex Vivo Profiling of PD-1 Blockade Using Organotypic Tumors Spheroids. Nature. Submitted.
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Follow the Fellows

Lab Members:

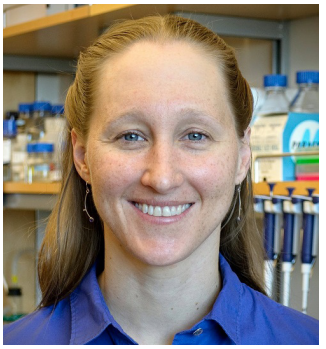
William Michaud, PhD: Staff Scientist
Roman Alpatov, PhD: Postdoc
Tabea Moll, MS: Technician
Rumya Raghavan, BS: Technician
Max Goder-Reisel, BS: Technician
Sonia Cohen, MD, PhD: Resident
Ali Rabi, MD, PhD: Resident
Gyulnara Kusamova, MD: Resident
Michelle Kim, BS: Medical Student

Applications Pending:

This work is the basis for several applications that are pending or under review including an NIH R21, DoD Translational Team Science Award, NIH P01, NIH R01, and Melanoma Research Foundation Young Investigator Award.

Current Funding:

2015 – 2017	NIH 1R01CA193970-01A1 (PI: Sullivan; Boland Investigator) Promoting Bim-Driven Apoptosis
2015 – 2017	CMeRIT Research Investigator Training Program (PI: Boland)
2016 – 2018	American Surgical Association Foundation Fellowship (PI: Boland)
2016 – 2017	Karin Grunebaum Cancer Research Foundation Faculty Fellowship (PI: Boland)
2016 – 2018	NIH Loan Repayment Plan (PI: Boland)
2017 – 2019	Society of Surgical Oncology Clinical Investigator Award (PI: Boland)
2017 – 2018	American Cancer Society Institutional Research Grant (PI: Boland)
2017 – 2019	Melanoma Research Alliance (PI: Mahmood: Boland Investigator)



Sloan Devlin, Ph.D.

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

Lab Overview

The human microbiome plays 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 ways in which the bacterial guests interact with and affect the human host at a molecular level are poorly understood. When asking the question, "what important functions do bacteria perform in vivo that are likely to affect the host?", one striking answer is that human-associated bacteria produce small molecule metabolites, some of which accumulate in the body to levels higher than that of a typical drug. This "molecule making" is therefore one of the most concrete effects that bacteria have on the host. We want to know: what small molecules do bacteria produce? Which bacteria are performing these transformations, and using what genes? How do these molecules affect bacteria-host and bacteria-bacteria interactions? Ultimately, how can we use this knowledge to help patients? We are leveraging our expertise in synthetic organic chemistry, 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. The long-term goal of my laboratory is to understand and control the chemistry of human-associated bacteria in order to uncover how the bacterial guests affect the human host in states of both health and disease.

Background

One of the main axes through which the bacterial guest and human host communicate is via the host immune system. In a positive sense, commensal strains play an important role in the development and modulation of the immune system. In a negative sense, growing evidence suggests that gut dysbiosis can promote inflammatory conditions that promote tumor initiation and progression. We are committed to 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.

Active projects

We are currently investigating the biosynthesis of deoxycholic acid (DCA), a secondary bile acid that promotes carcinogenesis in animal studies and whose levels in blood, bile, and feces are correlated with increased risk of colon cancer in humans. It is known that the consumption of a high-fat diet results in both increased amounts of DCA-producing bacteria in the large intestine and higher DCA fecal concentrations, suggesting a link between diet and cancer mediated by the gut microbiota. Despite the putative role of DCA as an obesity-associated carcinogen, only a small number of bacterial producers have been identified. These strains represent only ~0.0001% of all bacterial cells in the human gut. Our goal is to identify novel producers of this compound as well as the genes responsible for its synthesis. By performing bioinformatic analyses on strains of bacteria whose levels increase significantly during high-fat diet intake, we have identified putative DCA-producing gene clusters in common gut strains. We are currently using chemical and genetic tools to elucidate the function of these clusters in vivo and in vitro as well as investigating the hypothesis that DCA is produced through collaborative metabolism, a process in which multiple different species participate in the biosynthesis of the final product. By identifying novel bile acid-metabolizing organisms and the genes responsible for this activity, we will expand our currented limited ability to use metagenomic data to determine the bile acid metabolizing potential of gut communities in healthy and diseased people. Furthermore, elucidating the functions of genes responsible for bile acid transformations will facilitate the discovery of other bile acid-metabolizing genes and clusters. The identification of novel DCA-producing strains will facilitate the development of therapeutic strategies for colon and liver cancers in which DCA producers are eliminated from the microbiota.

In other research, we have engineered a commensal gut strain, Bacteroides thetaiotaomicron, to produce ursodeoxycholic acid (UDCA). This compound has been reported to be immunomodulatory, but the mechanism of its effect on the immune system is unknown. We have preliminary evidence to suggest that it increases the levels of anti-inflammatory regulatory T cells in vivo, and we are continuing to investigate its mode of action and host targets. In future work, we are committed to further exploring the intersection between diet, the gut microbiota, inflammation, and cancer initiation and development.

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.

Publications

1. Devlin, A.S., Marcobal, A., Dodd, D., Nayfach, S., Plummer, N., Meyer, T., Pollard, K.S., Sonnenburg, J.L. & Fischbach, M.A. "Modulation of a Circulating Uremic Solute via Rational Genetic Manipulation of the Gut Microbiota." ***Cell Host Microbe.*** 2016, 20, 709.
2. Toma, T., Logan, M.M., Menard, F., Devlin, A.S., Du Bois, J. "Inhibition of Sodium Ion Channel Function with Truncated Forms of Batrachotoxin." ***ACS Chem. Neurosci.*** 2016, 7, 1463.
3. Devlin, A.S. & Fischbach, M.A. "A biosynthetic pathway for a prominent class of microbiota-derived bile acids." ***Nat. Chem. Bio.*** 2015, 11, 685.
4. David, L.A., Maurice, C.M., Carmody, R.N., Gootenberg, D.B., Button, J.E., Wolfe, B.E., Ling, A.V., Devlin, A.S., Varma, Y., Fischbach, M.A., Biddinger, S.B., Dutton, R.J. & Turnbaugh, P.J. "Diet rapidly and reproducibly alters the human gut microbiome." ***Nature*** 2014, 505, 559.
5. Devlin, A.S. & Du Bois, J. "Modular Synthesis of the Pentacyclic Core of Batrachotoxin and Select Batrachotoxin Analogue Designs." ***Chem. Sci.*** 2013, 4, 1053.
6. Wolckenhauer, S.A., Devlin, A.S. & Du Bois, J. "δ-Sultone Formation Through Rh-Catalyzed C–H Insertion." ***Org. Lett.*** 2007, 9, 4363.

Follow the Fellows

Mikel Garcia-Marcos, Ph. D.

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

It was great to receive the news that I had been selected as the Karin Grunebaum Cancer Research Fellow for a second consecutive year (thanks!). The last year has been very productive to us and we have made great progress toward developing a new class of anti-metastatic drugs. Below I provide an overview, some background and the latest development of our research.

Research 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. 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 last year, 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.

Related activities:

During this last year, the Karin Grunebaum Cancer Research Foundation also funded my attendance to the Experimental Biology 2017 meeting in Chicago. In this meeting, one of my postdoctoral fellows, Dr. DiGiacomo, was selected to give an oral presentation in the Cancer Pharmacology session on the topic that was eventually published in Ref. 3 below. Dr. Garcia-Marcos has also become a standing member of the Molecular and Integrative Signal Transduction (MIST) Study Section of the NIH, while he continues to serve as member of the Tumor Biochemistry and Endocrinology Study Section of the American Cancer Society. Dr. Garcia-Marcos has also become a member of the Editorial Board for the Journal of Biological Chemistry, and his Department has proposed him for promotion to the Associate Professor level.

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.

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3. DiGiacomo V, de Opakua AI, Papakonstantinou, MP, Nguyen LT, Merino N, Blanco-Canosa JB, Blanco FJ, **Garcia-Marcos M**. The Gαi-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.

Poster Competition Winners

Molly DeCristo,
McAllister Lab, G4

Abstract:

The cyclin D:CDK4/6 axis is one of the most frequently dysregulated pathways in human cancers, and CDK4/6 inhibitors have shown significant activity against several solid tumors, including breast cancer. We found that the CDK4/6 inhibitor, abemaciclib, caused significant tumor regression in the MMTV-rtTA/tetO-HER2 mouse model of luminal breast cancer. CDK4/6 inhibitors are known to block breast cancer cell proliferation, but do not directly induce tumor cell apoptosis. Therefore, the mechanisms by which CDK4/6 inhibition causes tumor regression are not clear.

Notably, abemaciclib therapy increased total CD3+ T cells in these tumors, while decreasing the immunosuppressive CD4+ regulatory T cell population. Further investigation revealed that CDK4/6 inhibition directly suppresses regulatory T cells by inhibiting their proliferation. This decrease in Tregs is generalizable across tumor types and mouse strains as evidenced by confirmation in the CT-26 colorectal carcinoma and PyMT S2WTP3 mammary carcinoma models. Importantly, antibody-mediated depletion of CD8+ T cells established that response of MMTV-rtTA/tetO-HER2 tumors to abemaciclib is in part dependent on CD8+ T cells.

Finally, given the observed immunomodulatory effects of abemaciclib, we sought to determine if abemaciclib treatment would sensitize MMTV-rtTA/tetO-HER2 tumors to immune checkpoint blockade. Pretreatment with abemaciclib followed by combination abemaciclib and anti-PDL1 significantly enhanced the depth and duration of tumor regression compared to abemaciclib or anti-PDL1 alone. Our studies reveal a novel mechanism by which CDK4/6 inhibitors directly enhance an anti-tumor immune response, and these results provide strong rationale for further investigations into combining CDK4/6 inhibitors with immune checkpoint blockade in breast cancer.

Josh Pan,
Kadoch Lab, G4

Abstract:

Efforts to define protein complexes and their functional networks are critical for systems-level understanding of the pathways involved in human cancer. Current methods to catalog human protein complexes via physical interaction are often unable to resolve functional differences between complex members or infer relationships governed by sub-stoichiometric interactions. While functional wiring maps in yeast have been generated by measuring epistatic interactions between pairs of genes, efforts to scale this concept in individual human cell lines have been met with challenges and have only been able to characterize limited numbers of genes at a time.

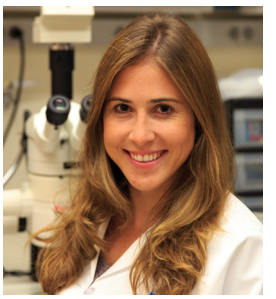
We have developed a scalable approach that can measure functional similarity without the constraints of pairwise genetic interaction experiments. Using data from genomewide RNAi and CRISPR dropout screens performed in hundreds of cancer cell lines, we leveraged the heterogeneity of gene dependencies across cancer types to measure functional similarity between thousands of genes at once, which in turn allowed us to recreate known inter- and intra-complex functional relationships and to uncover tumor suppressive and oncogenic functional modules in cancer-relevant pathways such as proteolysis, metabolism, and transcription.

Applying these approaches to the mammalian SWI/SNF (BAF) chromatin remodeling complex, which is mutated in over 20% of human cancers, revealed three functional modules that arose separately during metazoan evolution, one of which is entirely novel and uncharacterized. We then performed biochemical experiments that fully support three specialized complex configurations, each with distinct size, subunit composition, and function. These data reorganize the BAF complex into previously unrecognized modules that better explain mutational burden in human cancer. Notably, we observe that that all known BAF-driven, highly penetrant rare cancers and neurodevelopmental disorders involve disruption within a single functional module we defined, underscoring the value of evaluating disease genomics through the lens of functional modulari

Whitney Silkworth
Fisher Lab, G5

Abstract:

Though initially promising, advances in targeted and immune therapies for melanoma are met with resistance and an inadequate response rate. Analysis of patient biopsies and cell lines revealed that both acquired and intrinsic resistance to these therapies is associated with an undifferentiated cell state characterized by low levels of MITF, the master regulator of melanocyte development. Using the Project Achilles data set, we identified a genetic dependency in low-MITF melanomas on LSD1, a lysinespecific histone demethylase that has commercially available inhibitors. We validated the LSD1 dependency in multiple melanoma cell lines using both genetic and pharmacologic inhibition. To understand the mechanistic basis of this dependency, computational analysis of publicly available datasets suggests 3 candidate targets of LSD1 in low-MITF melanomas. We have shown that LSD1-mediated cell death in low-MITF melanoma cells is dependent upon the function of 1 of these 3, NDRG1, a suppressor of metastasis, and is mediated by LSD1 to affect the motility and invasiveness in neuroblastoma. Both our in vitro and in vivo results suggest that combination of pharmacologic inhibitors targeting LSD1 and BRAF is synergistic, extending xenograft survival. Pharmacologic inhibition of LSD1 is also synergistic with immune checkpoint blockade, providing complete and durable cures in 25% of the syngeneic xenografts. We will analyze the downstream



Jessalyn Ubellacker
McAllister Lab, G4

Abstract:

The bone-targeting agent zoledronic acid (ZA) increases breast cancer survival in subsets of patients when used as adjuvant therapy, but the underlying reasons for this protective effect are unknown. ZA is known to modulate the activity of osteoclasts and osteoblasts, which participate in hematopoietic stem cell niches. We demonstrate that ZA induces transient changes in numbers of hematopoietic stem cells, myeloid-biased progenitor cells, and lymphoid-biased cells in the marrow, concomitant with its known effects on bone. Importantly, we demonstrate that bone marrow cells from mice treated with a single, clinically relevant dose of ZA inhibit breast tumor outgrowth when admixed with tumor cells in vivo.

Using a preclinical model of bone metastasis, we identified matched human breast cancer cell lines that were sensitive or resistant to ZA treatment and discovered that the tumor-derived cytokine G-CSF mediates resistance to the tumor suppressive effects of ZA. Interestingly, exogenous G-CSF administration was also sufficient to negate the tumor-suppressive effect of ZA. These findings provide novel evidence that hematopoietic cells play a fundamental role in mediating tumor suppressive effects of ZA and that G-CSF negates the anti-tumor activity of ZA.

We are currently analyzing G-CSF levels in blood samples from stage II/III breast cancer patients (n=396) enrolled in a clinical trial for adjuvant ZA treatment. Our results suggest possibilities for capitalizing on the beneficial effects of ZA in reducing breast cancer development and progression in patients.

Summer Research Report

Attributes of Cancer Patients Presenting to Acute Care Surgery at Boston Medical Center and Potential Ways to Inprove Surgical Outcomes

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I had the wonderful opportunity to spend the summer with Dr. Tracey Dechert and her research team analyzing the variables associated with patients who present to the Emergency Department at BMC with previously undiagnosed malignancies that are diagnosed as a result of a Trauma and Acute Care consults.

My research started with a series of literature reviews evaluating what kinds of studies have been done on this cohort of patients, if any, throughout other hospitals in the United States. I was able to find a few articles speaking on the consequences of emergency surgeries vs. non-emergency surgeries as it relates to patients who present with emergent cancer complications. One of the main studies I came across, which matched our aim quite well, was one conducted by the University of Florida College of Medicine, Jacksonville. Although this study did not look into patients specifically being treated by the trauma and acute care department, it looked into the demographics of patients who presented to the Emergency Department of safety net hospitals in Jacksonville, Florida. This was valuable as the patient population closely resembles that of BMC. Interestingly, we found the same pattern of cancer diagnoses between the Jacksonville vs. BMC safety net hospitals – majority of patients being diagnosed in the Emergency Department were being diagnosed with advanced stage cancer (AJCC scores 3>), whereas patients being diagnosed in non-Emergency Department settings were more likely to be diagnosed with early stage cancer (AJCC scores > 2).

Our patient population had been identifies via IRB H-34010, we divided our patients into 2 cohorts: cohort A consisted of patients diagnosed with cancer at the index visit by the Trauma and Acute care Surgery department (i.e.: same day as Emergency department admission)(n=138); cohort B consisted of patients diagnosed with cancer during subsequent visits within one year of Emergency Department admission as a result of findings noted by the Trauma and Acute Care surgery department (n=97). We obtained various demographic variables pertaining to this patient population with the help of the Clinical Data Warehouse. I then went into these patient's medical charts to asses 5-year mortality rates.

In addition to this, we also brainstormed ideas as to future directions for this project. I met with various people, and have thus far submitted 2 new IRBs for future directions. The first IRB h-36524 was submitted to expand our data set to include patients that fit our inclusion criteria between January 2015 and May 2017-this is because the original IRB H-34010 only included patients through January 2015. This IRB has been successfully approved, and I am currently working with the Clinical Data Warehouse personnel to gather information on the same demographic variables as we with IRB H-34010. The second IRB H-36601 is currently undergoing analysis for the approval process. This latter IRB looks to create a new patient cohort, which we will call "cohort C", and will compose of patients who were diagnosed with cancer through the "traditional route" (i.e.: annual colonoscopies and screening methods through their primary care physicians). In addition, this IRB also looks to acquire permission to contact all patients that fit our inclusion criteria to assess for Quality of Life, as well as other cariables that may not be present in the patient medical record (i.e.: living status).

Ultimately, our goal with these IRBs are to create a story that can be presented to hospitals and government leaders to bring awareness to the overwhelming incidence of patients being diagnosed with cancer in the Emergency Department, and consequences of such scenarios. This will include presenting information on differences in quality of life, mortality rates, healthcare costs, insurance status, demographic status, and prognosis. Our hope is that this data will not only contribute to the reform of the current healthcare system, but also get healthcare providers and public health officials to think about better ways to address this by analyzing the underlying factors leading to such circumstances.

Immuni-Histochemical Study of LIF Levels and its Relation to Oligodendrocyte Function, White Matter Pathology and Cognitive Impairment in Normal Aging Monkeys.

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Summary: Our laboratory has established that normal aging is associated with cognitive decline and both these factors correlate with increasing white matter pathology. The white matter pathology seen in aging manifests as defects in myelination of tracts located in the corpus callosum, cingulum bundle and other locations. Myelin defects have been studied using electron microscopy. These studies have confirmed that with aging, the types of defects observed are accumulation of cellular debris between the layers of myelin sheaths as well as redundant myelination of axons. Another study conducted with this laboratory determined differential expression of several cell-signaling factors between young and old monkeys. One such cell-signaling factor that was in fact upregulated in older animals as compared to younger animals was the molecule leukemia inhibitory factor (LIF). These studies were completed using RT-PCR. Based upon all this prior work, my project this summer concentrated on staining brain tissue from young and old animals to determine if there is differential levels of LIF present in specifi brain areas that we know suffer from white matter pathology as age increases.

Accomplishments: Over the course of my 7 weeks of work I was able to learn quite a bit and achieve the goals I had set for myself prior to beginning research. I was able to assist with different types of procedures involving the monkeys. I learned how to draw up anesthetic drugs, administer them via intramuscular injection. Additionally, I was taught how to start an intravenous line and administer exosome drugs by IV infusion. Moreover, I was able to assist with stroke surgeries and was taught how to close the dura, skull flap, muscle and skin layers appropriately. In addition to gaining all this procedural knowledge, I conducted my own experiments. I was able to successfully obtain tissue from our frozen tissue storage and work on optimizing two antibodies over the course of the summer. I was simultaneously optimizing a staining protocol for a LIF antibody as well as a LIF receptor antibody. By the end of the summer I had run a larger experiment to see if there is a significant difference in LIF levels between 3 young and 3 old animals. I have yet to complete the analysis of that experiment. I will continue working in the Rosene lab this semester with hopes of finalizing this experiment as well as optimizing and testing out the LIF receptor antibody between young and old animals.

Challenges: The major challenges I faced this summer related to optimizing my immunohistochemistry protocol for both the LIF and LIFR antibodies. I was conducting chromogenic staining and initially my staining had too much background. Additional, the signal that I was getting wasn't specific enough to be considered real staining. After making certain adjustments to my protocol such as filtering the DAB solution I use and changing to a NiDAB solution I was able to achieve clearer results. My PI and other graduate students in the lab were always a source of help and because of their patience and guidance I was able to get my staining to work properly.

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KARIN GRUNEBAUM
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