Founded in 1958

KARIN GRUNEBAUM
cancer research foundation

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From the Chair

Dear Friends of the Karin Grunebaum Cancer Research Foundation:

This year marked a mile-stone event for the Foundation, as the second-ever Karin Grunebaum Professor in Cancer Research was named by the Boston University School of Medicine. After a three-year search to fill the vacant position, Julie Palmer, co-director of the BU-BMC Cancer Center, associate director of the Slone Epidemiology Center and professor of medicine and epidemiology at Boston University School of Medicine was awarded the Karin Grunebaum Cancer Research Professorship – a multi-disciplinary position first established by the Foundation in 2002. Please read more about Dr. Palmer later in this newsletter.

The Foundation is honored to have Dr. Palmer join us in our 61st year of fighting cancer through a multi-faceted approach to cancer education and research.

For example, in addition to continuing to award annual Fellowship grants to junior faculty members focused on cancer research at both Harvard Medical School and Boston University School of Medicine, the Foundation continues to fund supplemental cancer research educational projects at both facilities.

This year marked the third annual Karin Grunebaum Foundation-sponsored Cancer Poster Competition at Harvard Medical School – where prospective medical and Ph.D. students design advanced "science-fair" style posters and make presentations describing their research efforts in various fields of anti-cancer endeavor. Of the seventeen competitors who submitted entries, four were awarded cash awards.

At Boston University School of Medicine, the Foundation again funded summer research internships in cancer-related fields to select medical students to help encourage their eventual decision to pursue cancer research as a career.

Additionally, the Foundation provides travel stipends to currently-funded Fellows so that they can attend seminars to exchange information with other researchers in their field of study.

All 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

Meet our new Board Member

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

Julie Palmer, co-director of the BU-BMC Cancer Center, associate director of the Slone Epidemiology Center and professor of medicine and epidemiology at Boston University School of Medicine was awarded the Karin Grunebaum Cancer Research Professorship – a multi-disciplinary position first established by the Foundation in 2002.

Dr. Palmer holds a B.A. from Brown University, an M.P.H. from Boston University and a Sc.D. in epidemiology from Harvard University. She was elected to the Foundation's Board of Trustees in June.

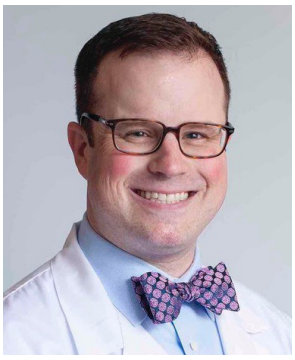
Dr. Palmer has focused much of her career on the etiology of breast cancer, and breast cancer in African American ("AA") women in particular. She is a founder and principal investigator of the Black Women's Health Study, a prospective cohort study of 59,000 AA women who enrolled in 1995 and have been actively followed by biennial questionnaires for more than 20 years. The study includes repositories of saliva, blood and tumor tissue samples from study participants, a tremendous resource for research on modifiable factors and genetic factors related to risk of cancer in AA women. Dr. Palmer is currently developing an improved breast cancer risk prediction model for AA women for use at the primary care level, and is leading an effort to determine the prevalence and penetrance of susceptibility variants of BRCA1, BRCA2 and other cancer predisposition genes in an unselected AA population.

Dr. Palmer serves on the scientific advisory boards of the NIEHS Sister Study and the Dr. Susan Love Research Foundation, and chairs an ad hoc working group of the NIH National Cancer Advisory Board. Dr. Palmer has received numerous awards, including the National Cancer Institute Outstanding Service Award, the Eighth Annual AACR Distinguished Lectureship on the Science of Cancer Health Disparities and the BU School of Public Health Distinguished Alumni Award. Currently she also serves as a Mission Advisor and Komen Scholar for the Susan G. Komen Foundation.

Incoming 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

Research Overview

Despite the unprecedented success of immune checkpoint blockade (ICB) in melanoma and other cancers, tackling innate (primary) resistance remains a major challenge and robust biomarkers to guide treatment are lacking. Clinical trials are already underway evaluating novel immune modulatory agents in combination with anti-PD-1/PD-L1 therapies in an effort to overcome innate resistance. Despite increasing reports of 'rational' combination strategies, these therapies remain "one size fits all", due the lack of robust biomarkers to guide clinical decision-making. Given the recent failures of several initially high-profile phase III combination immunotherapy trials coupled with the ever-increasing number of novel therapies and combination trials, there is an unmet need for novel approaches, tools, techniques, and methods for pre-clinical and clinical use to better understand mechanisms of response and resistance to immune checkpoint inhibitors and next-generation anti-tumor immune modulatory drugs. A major focus of my lab's research is the protein, TANK-binding kinase 1 (TBK1). TBK1 is a Ser/Thr kinase involved in innate immune signaling and is an emerging target for anti-cancer therapy. Importantly, independent orthogonal data from two different laboratories has also identified TBK1 as a cancer immunotherapy target. **This project aims to investigate TBK1 as a novel cancer immunotherapy target to overcome resistance to PD-1 blockade by reprogramming the tumor microenvironment.**

Specific Aims:

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 B16.F10 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.

Personal Comment: I would like to express my sincerest thanks for the honor of being selected as the recipient of the 2019-2020 Karin Grunebaum Cancer Research Foundation Faculty Research Fellowship at Harvard Medical School. The support provided to my lab by the Karin Grunebaum Foundation will enable several key studies that we expect will remove barriers to advancing our research another step closer to directly impacting the lives of patients. On behalf of our research team, our clinical team, and our patients, I would like to thank the foundation and each and every donor for your support.

Incoming Fellow

Dafne Cardamone, PhD

2019-20 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 about 90% of lung cancers. Despite significant progress over the last 20 years in developing therapeutic treatments, NSCLC remains the major cause of cancer-related deaths in the world. Although the pathogenesis of lung cancer is still unclear, we know that NSCLC progression requires both rapid changes in cellular metabolism and the activation of the immune system to allow balance between energy production and tumor growth. However, how the cells acquire metabolic advantages during cancer progression and the role played by the cancer immune response during tumor growth remains not completely understood.

Playing a crucial role in the connection between cancer cell metabolism and tumor inflammation is played by the mitochondria, the mitochondria are the "power house" of the cells and are central to immunity. The major focus of my research is to investigate the role of mitochondria in the activation and regulation of the inflammatory microenvironment of tumors. In particular, we are investigating the correlation between mitochondrial dysfunction and mitochondrial DNA (mtDNA) instability in the regulation of the immune response and the metabolic reprogramming during cellular stress in lung cancer initiation and progression.

Recent Progress

Thanks to the generous support of the Karin Grunebaum Cancer Research Foundation, we have recently characterized a novel mitochondrial protein, with Poly-(ADP-ribose) polymerase-like (PARP) enzymatic activity, which we have connected to the regulation of metabolic adaptation and inflammation during NSCLC initiation and progression. In particular, we have shown that this new enzyme is not only required for mtDNA maintenance and regulation of the immune response but also important in the regulation of cell metabolism. We are now working to characterize of the molecular mechanism underling the activity of this new enzyme in order to identify potential new targets for lung cancer therapeutic interventions.

Summer Projects BUSM

Arthi Palani

2019-20 Karin Grunebaum Faculty Research Fellow, Boston University School of Medicine

Breast cancer has always been a disease that I've wanted to contribute to because of the impact it has had on my family. My project last summer focused on Boston Medical Center breast cancer patients and their outcomes over the last 10 years. We looked at the impact of patient demographics, treatment type/length, and disease comorbidities on the incidence of breast cancer recurrence. I hope that the results and analysis from this project will help identify breast cancer disparities, guide further research, and improve the delivery of breast cancer care and treatment

Nicolette Jabbour,

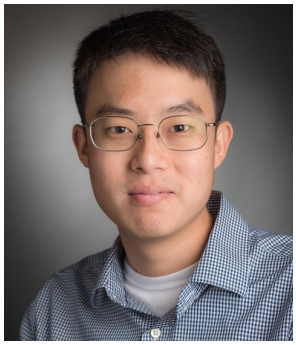
2019-20 Karin Grunebaum Faculty Research Fellow, Boston University School of Medicine

My research last summer focused on vocal cord cancer recurrence; we took a close look at factors that may put patients at an increased risk for cancer recurrence following surgery. This project allowed me to learn both about research process and the invaluable role it plays within medicine in improving patient care and outcomes. Before this project I never saw research as part of my career, but now I can't imagine a career without it.

Outgoing Fellow

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

The primary objective of the project funded by the Grunebaum Foundation was to develop new imaging and therapeutic agents targeting hypoxia in solid tumors. Tumor hypoxia, which refers to a low oxygen microenvironment in tissues, is a characteristic feature of many advanced solid tumors and is a highly negative prognostic factor for cancer patients. The rapid and disorganized proliferation of cancer cells leaves behind vast regions of tumors that are poorly vascularized and thus both malnourished and poorly oxygenated. This leads to large scale adaptive reprogramming of cancer cells, transforming them into a highly invasive and metastatic state. Unfortunately, poor oxygenation also renders these cells extremely difficult to target through traditional treatment modalities such as radiotherapy or chemotherapy. The former relies on molecular oxygen for efficacy and the latter requires cells to be rapidly dividing. Neither condition is met when hypoxia takes hold, and currently, there are no effective treatment options available to patients. Because of this pressing need, two drug candidates targeting tumor hypoxia made it into phase III clinical trials several years ago. While ultimately unsuccessful, those trials taught the scientific community that patient stratification is critically important when designing these clinical trials. There is extreme heterogeneity in the manifestation and severity of tumor hypoxia across the entire patient population and it is critical that only those presenting with hypoxia be selected for drug treatment. It is with this backdrop that we initiated our program in the development of dual mode compounds that provide hypoxia-dependent imaging and treatment.

Research Update

During the past three months, we have, through the generous contribution of tDuring the first several months of the grant period, we continued the development and refinement of a chemical reaction to rapidly access a novel hypoxia-sensitive chemical motif that we recently invented in our laboratory. This reaction allowed us to chemically synthesize a wide range of hypoxia-responsive triggering agents, which was then used to cage drugs and/or imaging agents. The simplicity and modularity of our system enabled us to rapidly compile a large collection of compounds that contained different drug payloads and imaging modalities as well as compounds that were modified near the hypoxia trigger, allowing us to optimize the hypoxia-selectivity of drug release/imaging and perform structure activity relationship studies. After evaluating each of our compounds in vitro using an in-house microsomal assay, we were able to narrow down the list of compounds to those that were likely to be the most selective in cells. Experiments in cell culture allowed us to further pare down our library and settle upon a handful of lead compounds. We were able to take these compounds and evaluate their selectivity in a wide range of human cancer cell lines including epidermoid, pancreatic, breast, lung, brain, and cervical cancer, among others. Our compounds performed extremely well in many of these cell lines, exceeding hypoxia to normoxia selectivities seen in some phase II hypoxia prodrugs like AQ4N. In fact, in highly aggressive, invasive, and poorly differentiated metastatic triple-negative breast cancer cell lines such as the MDA-MB-231 cell line, our compounds displayed hypoxia to normoxia selectivities of 485:1. In parallel, we also sought to fully understand the mechanism of action of our compounds. We learned that our compounds are exclusively activated by iron co-factors and inert to all other mono- and di-cationic metal ions tested. Consequently, select hemeproteins activate our compounds, and our microsomal assays identified specific isoforms of cytochrome P450s that appear to be major players in prodrug activation. We were also able to elucidate the chemical mechanism by which the imaging agents site-specifically localize to proteins in hypoxic regions. Finally, oxygen dependence studies revealed that our probes are most active in radiobiological hypoxia at <0.1% partial pressures of oxygen but can be activated up to 1%, providing more coverage for mild to moderately hypoxic tumors than nitroimidazole-based probes.

These basic investigations were essential as we moved into the in vivo stage of our studies. One of our goals for the grant period was to develop [18F]-PET probes for imaging hypoxia. We initiated those studies by incorporating a chemical handle onto a PET probe analog so that we could first analyze its efficacy through biochemical means. The results we obtained exceeded our expectations. For example, immunofluorescent images of tumor tissue slices from a BxPC-3 human pancreatic cancer xenograft in a mouse that had been treated with one of our lead compounds and pimonidazole, the current gold standard biochemical probe for hypoxia, clearly demonstrated co-localization of these two markers around a region of tissue necrosis. Our compound, in particular, was found to label more strongly at more severe levels of hypoxia, consistent with our hypothesis. This was further corroborated by its ability to label closer to the necrotic region than hypoxia markers such as CAIX and HIF1. Given these highly encouraging results, we designed and validated a synthetic route to access [18F]-PET active compounds. Working with the Dana-Farber Lurie Family Imaging Center, we generated hot radiochemical probes with which we performed in vivo pharmacokinetic/pharmacodynamic (PK/PD) studies. These studies revealed that the fluorinated probe, unlike its counterpart used for biochemical studies, was metabolically labile; fortunately, the mode of metabolism appeared unrelated to the key pharmacophore function so a second-generation [18F]-PET probe was designed, synthesized, and is currently undergoing in vivo PK/PD studies. Excitingly, we have also been able to synthesize probes with near-IR fluorophores so tumor hypoxia can also be visualized through fluorescence. Preliminary data indicate that our current probes possess adequate pharmacokinetic properties with excellent uptake and localization to hypoxic regions. Full body scans are scheduled and a manuscript will be submitted pending the results.

New Lab Members

Dahye Kang, PhD: Postdoc; Yingtang Ning, PhD: Postdoc;
Samuel Perry, PhD: Postdoc; James Siriwongsup: Graduate Student;
Aimee Son, PhD: Postdoc.

BUSM Summer Project 2019

Nicolette Jabbour

Wound Healing After Transoral CO2 Laser Surgery for Early Glottic Carcinoma

Background

Early carcinoma of the vocal cords is often treated by surgical excision with excellent cure rates. The carbon dioxide (CO2) laser is a commonly-used tool for this purpose because of its precise cutting and hemostatic effects. The surgery involves cutting into the vocal folds and invariably and necessarily wounds them. Healing occurs then by secondary intention. Patients have shown a varied healing response after laser surgery with different healing times and healing without or without granulation tissue. (Granulation tissue is vascularized tissue that can form during the healing process due to inflammation.)

All patients seen with glottic carcinoma undergo routine in-office laryngeal visualization with flexible laryngoscopy during the post-operative period. This is the primary way to assess for cancer recurrence. Granulation tissue can appear very similar to carcinoma and its presence may encourage a biopsy to see if there is persistent cancer. Additionally, wounds that may heal slower Nicolette Jabbour than expected can prompt concern for cancer. However, potentially unnecessary biopsy/surgery poses a risk to patients. This project will retrospectively look at the wounds created from CO2 laser surgery for early carcinoma. The goal will be to identify a timeline of healing and investigate patient/surgical characteristics associated with development of granulation tissue.

There is one paper that has looked at wound healing prospectively after CO2 laser surgery, but does not take into account wound characteristics, such as anterior commissure inclusion, length of vocal cords, inclusion of muscle and ligament, that this project will take into account. It does not control for inter-rater reliability, and also rates wounds on a different scale. There is a paper on a completely different laser, a KTP laser, that has entirely different effects and outcomes (which was written by my PI). It is important to fill this gap because it is important for doctors to know when they should decide to put a patient under anesthesia again to biopsy, or if this would be an unnecessary risk to the patient and should wait longer for the would heal.

Hypothesis or research question

What is the wound healing timeline after transoral CO2 laser surgery for early glottis carcinoma, the factors influence healing and the clinical significance of persistent granulation tissue? I hypothesize that larger tumors and larger and more extensive wounds will take longer to heal and have more granulation tissue.

Specific Aim(s)

1. To establish a timeline for wound healing after transoral CO2 laser surgery for early glottic carcinoma.
2. To provide insight on when to biopsy when cancer recurrence is suspected.
3. To determine and analyze the patient and surgical characteristics that influence healing.
4. To determine the clinical significance of persistent granulation tissue.

Methods, Data Collection, Statistical Analysis

First, the appropriate patients (early glottic cancer who underwent CO2 laser excision) would be identified from the Data Warehouse. Then the previously obtained laryngeal exams, will be independently assessed by 3 BMC laryngologists to rate the healing status of the vocal cords and presence of granulation. Wounds will be rated, prior to data review as 1) having exudate 2) being inflamed/edematous 3) developing granulation tissue or 4) healed completely Raters will be blinded to the patients' diagnoses and all other patient factors while rating. Chart review will be performed for surgery components (margin status, pathologic diagnosis). The presence of granulation tissue will be correlated to subsequent carcinoma recurrence and then compared to depth and extent of resection of the original wound. A healing timeline will then be developed and clinical significance of granulation tissue evaluated.

To look at the effect of clinical factors and surgical parameters on the healing timeline and development of granulation tissue, a X^2 test will be used. It is also important to consider inter-rater reliability for the laryngologists that rate the healing status. To control for this, Fleiss Kappa statistics will be computed across all four categories for consistency and Spearman's rho analysis for consistency.

The query that has already been submitted to the clinical data ware house is: Nicolette Jabbour I am requesting data from patients who were diagnosed with early stage (T1 or T2) squamous cell carcinoma of the glottis and underwent transoral CO2 laser excision. The data includes MRN, name, age, DOB, gender, diagnosis, date of surgery, date of additional surgery, dates of all clinic appointments, diabetes history, smoking history, radiation history, and ethnicity. Selection criteria includes patients 18 and older who have been diagnosed with early stage squamous cell carcinoma of the glottis (T1 or T2) referred to the BMC Otolaryngology Head and Neck surgery department and who subsequently underwent transoral CO2 laser surgery for treatment from 01/01/2008- 12/31/2018.

Anticipated Outcomes

The proposed study will generate an accurate, and unbiased timeline of wound healing after CO2 laser surgery for early glottic carcinoma. It will give greater insight as to when it is necessary to have patients undergo a biopsy on the healing timeline, when the benefits of biopsy outweigh the risks. The outcomes will demonstrate both patient and surgical characteristics that are associated with a shorter healing timeline and which are associated with a longer healing timeline. Characteristics that are associated with the development of granulation tissue will also be determined.

Timeline of Project

Week 1: Compile the all the data from that has been requested from the BMC Clinical Data warehouse.
Week 2: Gather laryngeal examinations and have BMC laryngologists rate the laryngeal examinations and compile the raters' scores.
Week 3: Conduct a chart review for the surgical components, including margin status and pathologic diagnosis.
Week 4: Continue to conduct chart review for the surgical components, including margin status and pathologic diagnosis.
Week 5: Separate patients into groups based on outcomes, characteristics, and stage of cancer.
Week 6: Run statistical analysis' between groups, as well as statistical analysis for inter-rater reliability.
Week 7: Review the current literature on laryngeal cancer and vocal cord healing and compare analyzed data to this literature.
Week 8: Continue to review the literature, create graphics of relevant results, and begin drafting paper.
Week 9: Continue drafting paper and poster
Week 10: Finalize paper and poster submission..

Karin Grunebaum HMS Poster Winners

Jefte Drijvers
Sharpe Lab

Diet-induced obesity causes metabolic reprogramming and suppresses immunity in the tumor microenvironment

Obesity is a well-known risk factor for many cancers. Various systemic alterations, including nutrient availability and signaling changes, are associated with obesity. However, how these changes impact the anti-cancer immune response is not yet clear. Tumors maintain an immunosuppressive, nutrientpoor microenvironment, and it is unknown whether the systemic metabolic changes associated with obesity are reflected in the tumor microenvironment (TME) locally. We tested the hypothesis that obesity impacts anti-tumor immune function in the TME using the murine obesity model of feeding a high-fat diet (HFD). Our studies show that HFD accelerates tumor growth by inhibiting the anticancer immune response. Moreover, HFD induces different metabolic adaptations in tumor cells and intratumoral immune cells, resulting in an altered nutrient composition in the TME. Genetic manipulations that alter metabolic reprogramming in tumor cells normalize the metabolic milieu in the TME and reduce tumor growth in an immune system-dependent manner. These findings demonstrate how cell-type specific rewiring of metabolism in the TME in response to systemic metabolic changes resulting from diet-induced obesity inhibits the anti-tumor immune response locally. Analysis of publicly available transcriptional data of human cancers from The Human Genome Atlas suggest that similar metabolic reprogramming events correlate with obesity and decreased anti-tumor immune function in human cancers as well. Thus, our studies reveal a novel mechanism linking obesity to cancer through decreased anti-tumor immune function and may inform the development of cancer therapies targeting cancer metabolism and anti-tumor immunity.

Manav Gupta
Kim Lab

The mammalian SWI/SNF complex regulates origin firing in lung cancer

The SMARCA4 gene encodes the ATP-dependent helicase component of the SWI/SNF complex, BRG1, is involved in chromatin modulation and either mutated or lost in up to 20% of human nonsmall cell lung cancers. To investigate how loss-of-function mutations in SMARCA4 contributes to lung tumorigenesis, we generated murine and human BRG1 knockout cell lines from tumor cells derived from a KrasG12D/+; p53Î”/Î” (KP) mouse model of lung adenocarcinoma, and KRAS/p53 mutant human lung cancer cell lines H460, H2009, and Calu6. RNA-sequencing of murine Brg1 null cells and human SMARCA4-mutant patient data taken from the TCGA cancer datasets revealed the upregulation of the ATR-mediated response to replication stress and activation of the pre-replicative complex as the top cancer pathways in these cancers. We hypothesized that loss of BRG1 contributed to increased replication stress associated DNA damage and genome instability. BRG1-deficient cells had significant more gamma-H2Ax foci and RPA-bound singlestranded DNA compared to isogenic controls. Western blot analysis confirmed activation of the ATR pathway through increased phospho-CHK1 activity in BRG1-deficient cells. Mechanistically, we observed that loss of BRG1 expression leads to increased number of fired origins of replication as measured by DNA fiber assays. The correlation between replication stress and DNA damage was assessed using comet analysis and we observed increased olive tail moments in BRG1-deficient cells, indicative of more DNA damage and genome instability. We then treated our cells with inhibitors that target key DNA damage response kinases, ATM and ATR. While ATM inhibition resulted in no observable change between BRG1 wildtype and mutant cells, BRG1-deficient cells were more sensitive (3-5 fold) to ATR inhibition compared to isogenic wildtype cells. ATR inhibition was also able to significantly increase the total amount of DNA damage in BRG1-deficient cells, suggesting that loss of BRG1 leads to dependence on the ATR pathway to prevent further genome instability. We re-expressed human BRG1in murine/human Brg1/BRG1 knockout cells and observed a reversal in response to ATR inhibition. We also found that combinatorial treatment with replication stress inducing reagents such as topoisomerase I inhibitor irinotecan or hydroxyurea further sensitized BRG1-deficient cells to ATR inhibition in some models. To address the role of the SWI/SNF complex in origin firing and DNA replication, we examined levels of the early DNA origin licensing proteins and found that loss of BRG1 expression strongly correlated with increased CDC6 presence across all our models of BRG1 loss. To further study the role of Brg1 as a tumor suppressor gene in the lung, we compared KP mice versus KP mice harboring floxed Brg1 (KPB) alleles and found that KPB mice had a significantly higher number of tumor lesions and highergrade tumors after 13-15 weeks of tumor induction. Interestingly, there was a significant correlation in loss of Brg1 and presence of key immune evasion ligand Pd-l1 by immunohistochemistry in Brg1 null tumors. Subcutaneous injections of Brg1 null murine isogenic lines into flanks of immunocompetent mice further showed the increase in Pd-l1 expression only in tumors derived from Brg1 null cells. Taken together, our data suggests that BRG1 or the SW/SNF complex may have a role in regulating DNA replication in lung cancer cells, and it does so by mediating CDC6 expression and controlling origin firing.

Karin Grunebaum HMS Poster Winners

Adrija Navarro
Toker Lab

Investigating the role of ALG3 in the regulation of N-glycosylation by the PI3K/AKT signaling pathway

The PI3K/AKT signaling pathway, which is frequently dysregulated in cancer, controls key cellular processes such as survival, proliferation, metabolism, and growth. Protein glycosylation, the process by which carbohydrates are added to amino acids, is essential for proper protein folding and is deregulated in cancer. High proliferation rates in cancer require amplified protein folding. The glycosyltransferase ALG3 catalyzes the addition of a mannose to a glycan precursor once it is flipped into the endoplasmic reticulum lumen during glycan production. ALG3 is required for proper glycan formation and is implicated as a putative AKT substrate. ALG3 resides proximal to PIK3CA in the 3q26 amplicon. Consequently, PIK3CA and ALG3 are co-amplified in 89%, 28% and 76% of lung (SCC), breast and ovarian carcinomas, respectively. Notably, we find that in both lung and breast cancer cells, ALG3 is also phosphorylated downstream of PI3K. This represents, to our knowledge, the first identified link between PI3K oncogene signaling and protein glycosylation in the context of cancer. I hypothesize that ALG3 plays a role in the regulation of protein N-glycosylation by PI3K/AKT signaling, and that aberrant PI3K/AKT signaling alters glycosylation, leading to functional consequences in cancer. Specifically, I postulate that cells that harbor PIK3CA amplification and ALG3 up-regulation increase glycosylation and protein folding rates, allowing cells to cope with increased protein translation in response to hyperactive PI3K/AKT signaling. This project will advance our understanding of the regulation of glycosylation metabolism by PI3K/AKT signaling and its role in cancer progression; future studies may determine the extent to which combination therapies targeting the PI3K/AKT pathway and proteinglycosylation are effective.

Alexandra Pourzia
Letai Lab

Cancer cell defects in apoptosis attenuate killing by CAR T cells

CAR T therapy is now an FDA approved treatment for several hematologic malignancies, yet not all patients respond to this treatment. While some resistance mechanisms have been identified, the possibility of cell death pathways modifying response to CAR T therapy remains unexplored. To assess whether cell death pathways in target cancer cells could impact response to CAR T therapy, we utilized a HeLa in vitro model system. HeLa cells with intact (HeLa-19) and deficient Bak/Bax (HeLa-DKO-19) expressing CD19 were co-cultured with CD19 CAR T cells. We observed that Bak/Bax deficiency, which blocks the intrinsic pathway of apoptosis, conferred resistance to CAR T killing. However, this resistance could be overcome at high E:T ratios. To confirm that the intrinsic pathway of apoptosis contributes to CAR T killing, we forced the expression of Bcl-2 and Bcl-XL in HeLa-19 cells, and observed that both of these anti-apoptotic proteins conferred protection from CAR-T killing in a similar manner to Bak/Bax knockout. Additionally, we wanted to assess whether caspases may be required for CAR T killing, given the role of intrinsic apoptosis in our model system. The caspase inhibitor Z-VAD-FMK protected both HeLa-19 and HeLa-DKO-19 cells from CD19 CAR T effector cells in our in vitro model system. Lastly, we wanted to ascertain the precise mechanism by which CAR T cells eliminate target cancer cells. CAR T cells were co-cultured with HeLa-19 and HeLa-DKO-19 targets in the presence of blocking antibodies against Fas ligand and TRAIL, along with 3,4-dichloroisocoumarin, a granzyme inhibitor. We observed that granzyme inhibition, but not death ligand blocking antibodies, provided protection from CAR T cells. Additionally, a soluble factor contributed to CAR T killing, as demonstrated by conditioned media experiments in which target cancer cells were exposed to filtered supernatant from CAR T coculture experiments. In conclusion, intrinsic apoptosis allows for efficient elimination of target cancer cells by CD19 CAR T cells. This process also requires downstream caspases, and seems to be mediated in part by granzymes. This work implies that agents that promote tumor cell intrinsic apoptosis may be candidates for combination treatment with CAR T therapy; and suggests that tumor cells that are resistant to intrinsic or downstream apoptosis may resist CAR T therapy. Future work will explore whether the intrinsic apoptotic pathway also modifies response to CAR T cells in a mouse model, and identify soluble factors that contribute to CAR T killing of target cells.

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.

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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