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KARIN GRUNEBAUM
cancer research foundation

November, 2016 ♦ Volume 13

From the Chair

Dear Friends of the Karin Grunebaum Cancer Research Foundation –

I am pleased to report that our first targeted fund-raising letter from our distinguished Board members to former Fellows who previously received financial support from the Foundation brought in new donors and additional funding capability to the Foundation. Accordingly, in addition to annually funding junior faculty cancer researchers at both Harvard Medical School and Boston University School of Medicine, we were also able to fund two Boston University medical students' summer research programs as well as a new rewarded "poster competition" for cancer biology trainees at Harvard.

The Boston University medical students will research the epigenetic regulation of growth promoting and tumor suppressing genes in ovarian cancer, as well as the impact of socio-economic disparity in cancer treatments. The Foundation underwrites both the researchers and their lab costs.

The Harvard poster competition will be open to graduate students at Harvard Medical School and will be the only Harvard-wide poster competition in cancer biology. Winners of the competition will each receive a $1,000 Karin Grunebaum Professional Development Award.

The Trustees are obviously very pleased to be able to thus expand the Foundation's activities thanks to the generosity of our donors.

Professor Douglas Faller, M.D., Ph.D., the first Boston University Karin Grunebaum Professor in Cancer Research, has relinquished his Chair in order to pursue other cancer-related ventures. We are delighted that he has agreed to remain a Trustee of the Foundation. And, we look forward to welcoming the new Karin Grunebaum Professor in Cancer Research when he or she is appointed by Boston University.

The Trustees were happy to learn that each year there continues to be strong competition from junior faculty members at both supported institutions to secure a Fellowship from the Foundation. We believe that this strong competition is the result of the personal "hands-on" relationship between the Fellows and the Trustees, the caliber of our academic Trustees and the unique travel funds offered to each Fellows in addition to their Fellowship award. Each Fellow is entitled to up to $1,500 reimbursement to attend research-related seminars and symposia. The Fellows invariably report that this was one of the highlights of their Fellowship experience.

Again, 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, and I ask you to 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
Assistant in Surgery, Massachusetts General Hospital

Research Overview:

Recent breakthroughs in the treatment of melanoma through molecularly targeted therapy and immune checkpoint blockade have shown promising clinical success. Currently, the major obstacles to care are non-responsiveness to treatment and the development of resistance to therapeutic regimens. Genomic, transcriptomic, and immunologic profiling of tumors has provided significant insight into changes in the tumor and microenvironment that occur during systemic therapy. While progress has been made in predicting response and clarifying the mechanisms of resistance through analysis of serial tumor biopsies, there is a critical need to develop real-time, minimally-invasive techniques for monitoring changes in the tumor and microenvironment as a function of treatment status and clinical responsiveness to therapy. In the absence of such knowledge, we will lack the ability to identify clinically meaningful changes longitudinally. Without these tools, we will be unable to realize the full potential of precision medicine initiatives that look for individualized and novel/evolving sites of therapeutic intervention.

My overarching goal of this research program is to identify clinically-meaningful assays to detect melanoma markers in the blood, to develop risk-stratification tools for melanoma patients, and to identify predictive signatures of response/non-response to systemic therapy. The overall objective of the current work is to analyze the nucleic acid content of circulating microvesicles, exosomes, from melanoma patients at clinically defined time points. Our central hypothesis is that exosomes contain RNA that maps to tumoral changes and correlates with clinical outcomes. This hypothesis has been formulated, in large part, on our own preliminary findings that we can isolate tumor driver mutations from circulating exosomes and track these levels during surgical treatment and systemic therapy. The rationale for these studies is that blood-based monitoring of the tumor transcriptome will expand our ability to monitor and track disease progression over time without the need for serial biopsies. In addition to our pilot data, I am the PI of the Melanoma Tumor Repository at Massachusetts General Hospital where we collect serial tumor biopsies and blood on patients with melanoma prior to and after surgical resection and at various time points during treatment with the latest systemic therapies. We have > 360 patients enrolled who we have been tracking longitudinally. Profiling efforts of the tumors are ongoing and will guide correlative analysis of blood-based changes in patients over time.

Research Update:

Circulating exosomal RNA signatures reflect changes in the melanoma tumor microenvironment.

1. Utilization of circulating microvesicles, exosomes, for clinical monitoring in the perioperative period to detect minimal residual disease and early recurrence.

While recent breakthroughs in the treatment of melanoma through molecularly targeted therapy and immune checkpoint blockade have changed the landscape of care for patients with metastatic melanoma, this has not yet extrapolated to standard use in the adjuvant setting for risk-reduction after complete surgical resection of disease. Melanoma-specific survival varies widely based upon T stage and the presence of nodal or distant metastases. In stage III disease the 5 year survival ranges from 39 to 70% depending upon tumor and nodal characteristics delineated in the AJCC staging system. The first agent focused on checkpoint blockade, ipilimumab, has recently been approved for adjuvant therapy in melanoma, but has not been widely adopted in the adjuvant setting given the high toxicity of the therapy and currently interferon remains the standard of care for adjuvant therapy despite only a modest (10%) risk reduction. Given the difficulty of clinical decision-making in this setting, there is a critical need for a more sensitive means of detecting minimal residual disease and/or early recurrence in high-risk melanoma patients. With a precise risk-stratification tool, clinical decision-making would allow for more aggressive adjuvant therapy for those at the highest risk for recurrence and spare toxicities of therapy for lower risk subgroups.

My group has been able to detect melanoma driver mutations in circulating exosomes and can track these levels before and after definitive surgery. We can demonstrate decrease or loss of these signals after successful surgical resection and persistence of signals in a subset of patients who rapidly recur after surgery. The goal of this research program is to validate the use of exosomal RNA signatures to track melanoma recurrence or persistence in patients who are otherwise clinically and radiographically free of disease.

2. Utilization of circulating exosomal signatures to predict response or non-response to immunotherapy.

My lab has been studying RNA signatures detectable in melanoma patients prior to and during treatment with immunotherapy. We are able to do whole transcriptome analysis of circulating exosomal RNA and have demonstrated 80% concordance between exosomal RNA signatures and that derived from the same patient's paired tumor. We see enrichment of miRNA in exosomes as compared to tumors and are currently profiling both mRNA and miRNA from patients with metastatic melanoma initiating immunotherapy. Preliminary suggests we may be able to stratify responders from non-responders to immunotherapy prior to or early on treatment with immunotherapy.

Publications:

1. Zhang G, Wu LW, Mender I, Barzily M, Hammond MR< Ope O, Randell S, Xiao M, Tian T, Krepler C, Brafford P, Sproesser K, Murugan S, Somasundaram R, Gaman B, Wubbenhorst B, Desrichard A, Woo J, Zhang J, Liu JY, Qin L, Frederick DT, Xu W, Karakousis GC, Xu X, Schuchter LM, Gangadhar TC, Watson IR, Kwong LN, Amaravadi RK, Lu Y, Chan TA, Boland GM, Wei Z, Nathanson K, Mills GB, Flaherty KT, Herlyn M, Shay JW. Therapeutic Targeting of Telomerase Prolongs Control of Therapy-Resistant Melanomas. Cancer Cell. Submitted.

2. Lu H, Liu S, Zhang G, Zhu Y, Frederick DT, Hu Y, Zeng J, Wu L, Zhang J, Xu W, Krepler C, Sproesser K, Xiao M, Jianglan L, Song CD, Liu JY, Karakousis GC, Schuchter LM, Lu Y, Mills G, Boland GM, Sullivan RJ, Wei Z, Field J, Amaravadi RK, Cong Y, Chernoff J, Flaherty KT, Herlyn M, Xu X, Guo W. PAK Signaling Drives Acquired Resistance to MAPK Inhibitors in BRAF-mutant Melanomas. Nature. Submitted.

3. 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.

4. 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, BolandGM, Wong K, Barbie DA. Ex Vivo Profiling of PD-1 Blockade Using Organotypic Tumors Spheroids. Nature. Submitted.

New Lab Members:

Roman Alpatov, PhD: Postdoc, Tabea Moll, MS: Technician, Ali Rabi, MD, PhD: Resident

Plans for Travel:

Abstracts on this work have been submitted to the Society of Surgical Oncology and the Association of Academic Surgery meetings in February and March. I plan to use the Grunebaum funds to support the presentation of our work at these meetings.

Follow the Fellows

Mikel Garcia-Marcos, Ph. D.

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

Overview

My laboratory is broadly interested in the elucidating the molecular mechanisms by which cells respond to external signals and how dysregulation of these mechanisms lead 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 and also 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 solid tumors 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. One consequence of this rewiring is G protein signaling hyper-activation by GIV. Another one is that G protein (hyper)activation occurs in response to receptors different from those that normally activate G proteins. Many of these "alternative" receptors are known to be upregulated in metastasis and to contribute to tumor cells invasiveness. 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.

Active projects

We are currently interested in to identifying small molecule inhibitors that disrupt the interaction between GIV and G proteins and to explore their use as anti-metastatic agents. For this, we use high-throughput techniques to screen 200,000 small molecules for their ability to inhibit the GIV-G protein interaction and dampen tumor cell invasion. In parallel, we are using 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 fir in a lock-and-key fashion), which will be useful to guide our efforts in finding chemical molecules that disrupt it. In addition, we are expanding our research to investigate if other proteins similar to GIV in their molecular function as non-receptor G protein activators are also involved in cancer. In fact, we have already shown that this is the case for DAPLE, another G protein activator of the same class, but several other candidates are under investigation. For DAPLE, we are currently characterizing its normal function, which appears to be important for embryonic development. We expect that the insights gained from studying how DAPLE functions during embryogenesis will become informative about its role in cancer. Such parallelisms between the functions in embryogenesis and cancer have proven to be a recurrent theme for many other proteins.

Research in the Garcia-Marcos laboratory is currently funded by two NIH grants, a research scholar award from the American Cancer Society and the Hartwell Foundation, in addition to the Karin Grunebaum Foundation. Dr. Garcia-Marcos has also been funded by the Elsa U. Pardee Foundation in the recent past and is a member of the Tumor Biochemistry and Endocrinology Study Section of the American Cancer Society.

RECENT PUBLICATIONS

1. Marivin A., Leyme A., Parag-Sharma K., DiGiacomo V., Cheung A.Y., Nguyen L.T., Dominguez I. **Garcia-Marcos M**. Dominant negative Gα subunits as a novel mechanism of trimeric G protein signaling dysregulation in human disease. *Science Signaling.* 2016. Apr 12;9(423):ra37. PMID: 27072656.

2. Leyme A, Marivin A, **Garcia-Marcos M**. GIV/Girdin Creates a Positive Feedback Loop that Potentiates Outside-in Integrin Signaling in Cancer Cells. Journal of Biological Chemistry. 2016 Apr 8;291(15):8269-82. PMID: 26887938

3. Leyme A, Marivin A, Perez-Gutierrez L, Nguyen LT, **Garcia-Marcos M**. Integrins activate trimeric G proteins via the nonreceptor protein GIV/Girdin. Journal of Cell Biology. 2015 Sep 28;210(7):1165-84. PMID: 26391662.

4. Aznar N, Midde KK, Dunkel Y, Lopez-Sanchez I, Pavlova Y, Marivin A, Barbazán J, Murray F, Nitsche U, Janssen KP, Willert K, Goel A, Abal M, **Garcia-Marcos M***, Ghosh P*. Daple is a novel non-receptor GEF required for trimeric G protein activation in Wnt signaling. Elife. 2015 Jun 30;4:e07091. PMID: 26126266

5. **Garcia-Marcos M**., Ghosh P., Farquhar M.G. GIV/ Girdin transmits signals from multiple receptors by triggering trimeric G protein activation. Journal of Biological Chemistry. 2015. Mar 13;290(11):6697-704. REVIEW. PMID: 25605737.

6. Coleman BD, Marivin A, Parag-Sharma K, DiGiacomo V, Kim S, Pepper JS, Casler J, Nguyen LT, Koelle MR, Garcia-Marcos M. Evolutionary conservation of a GPCR-independent mechanism of trimeric G protein activation. Molecular Biology and Evolution. 2016 Mar;33(3):820-37. PMID: 26659249

7. Bhandari D, Lopez-Sanchez I, To A, Lo IC, Aznar N, Leyme A, Gupta V, Niesman I, Maddox AL, Garcia-Marcos M, Farquhar MG, Ghosh P. Cyclin-dependent kinase 5 activates guanine nucleotide exchange factor GIV/Girdin to orchestrate migration-proliferation dichotomy. ***Proceeding of the National Academy of Sciences.*** 2015. Sep 1;112(35):E4874-83. PMID: 26286990

Thank You to the Foundation

Neil Ganem, Ph. D.

The Cancer Center, Boston University School of Medicine
Departments of Pharmacology and Medicine, Division of Hematology and Medical Oncology

Dear Members of the Karin Grunebaum Cancer Research Foundation,

I would like to take this opportunity to once again thank the Grunebaum Family for supporting my laboratory over the past 2 years. As I hope you will appreciate from this final progress report, quite a lot has happened during that time!

My laboratory has grown significantly despite the fact that I have now graduated my first Ph.D. student (Elizabeth Shenk), two Masters students (Hatim Mustaly and Jasmine Vakhshoorzadeh), and an undergraduate student (Tenny Mudianto). The lab is now comprised of 10 trainees, which doubles the size of the lab from when the Grunebaum Fellowship was first awarded.

Scientifically, we have published 7 articles in peer-reviewed journals since 2014, and I am excited to report that we have several exciting stories, all supported by Grunebaum funds, very near completion. Funds from the Grunebaum award have also helped to initiate many new scientific projects, and I am happy to report that these projects are now being supported by several additional grants, including a Research Scholar Grant from the American Cancer Society and an R01 from the NIH.

A complete update of current/past laboratory members, publications, and newly acquired research support is summarized below.

Current Laboratory Members

Elizabeth Shenk, Ph.D. (Postdoctoral Fellow), Kristyna Kotynkova, Ph.D. (Postdoctoral Fellow), Amanda Bolgioni, Ph.D. student, Sanghee Lim, M.D./Ph.D. student, Ryan Quinton, M.D./Ph.D. student, Marc Vittoria,, M.D./Ph.D. student, Jasmine Vakhshoorzadeh, Masters student, Hatim Mustaly, Medical Rotation Student, Shiyi Jiang, Undergraduate Student Victoria Kacprzak: Research Technician

Recent Graduates:

Elizabeth Shenk: Elizabeth graduated from the Biomedical Engineering Ph.D. program at the Boston University School of Medicine this spring. Elizabeth trained in my lab from 2013-2016. Her thesis is entitled, *"Mechanisms of Genome Instability in Oncogenic Tetraploid Cells"*. Elizabeth will stay on in my lab as a postdoctoral fellow through the remainder of the summer.

Jasmine Vakhshoorzadeh: Jasmine graduated from the Masters in Medical Science Program this spring. Her thesis is entitled, "*Defining YAP/TAZ-Dependencies in Breast Cancer Cells"*. Jasmine will stay on in the lab until July, at which time she will matriculate at Creighton Medical School as an M.D. student.

Hatim Mustaly: Hatim graduated from the Masters in Medical Science Program in 2015. His thesis is entitled, "*Identification of Small Molecule Modulators of the Hippo Tumor Suppressor Pathway".* After graduating from my lab, Hatim matriculated as an M.D. student at the Boston University School of Medicine. He is conducting a summer research rotation in my lab between his first and second years of medical training.

Tenny Mudianto: Tenny graduated from Boston University this spring. As an undergraduate in my lab from 2014-2016, she was awarded two undergraduate research opportunity fellowships (UROPs). Tenny recently accepted a research position at the Dana-Farber Cancer Institute.

Publications (2014-2016)

Ganem NJ*, Cornils H, Chiu, SY, O'Rourke, KP, Yimlamai D, Thery M, Camargo FD, and Pellman D. 2014. Cytokinesis failure triggers Hippo pathway activation. Cell. 158(4):833-848. *Co-corresponding author.

- Highlighted in Cell, "Hippo Pathway Key to Ploidy Checkpoint" Minireview,c 158(4):695-696; Nature Reviews Cancer, "Hippo Signaling Arrests Tetraploid Cell Growth", Research Highlight; Science Signaling, " Hippo Arrests Tetraploid Cells", Editor's Choice, Cancer; and Cancer Discovery, "Tetraploidy Activates the Hippo Tumor Suppressor Pathway", Cancer Discovery News.

Lim S, and **Ganem NJ**. 2014. Tetraploidy and tumor development. *Oncotarget*. 5(22):10959-60.

Flynn RL, Cox KE, Jeitany M, Wakimoto H, Bryll AR, **Ganem NJ**, Bersani F, Pineda JR, Suvà ML, Benes C, Haber DA, Boussin FD, Zou L. 2015. Alternative lengthening of telomeres renders cancer cells hypersensitive to ATR inhibitors. Science. 347:273-277.

Mustaly HM and **Ganem NJ**. (2015) Mitosis: Chromosome Segregation and Stability. *eLS. John Wiley & Sons, Ltd*: Chichester. DOI: 10.1002/9780470015902.a0005774.

Russo A, Pacchierotti F, Cimini D, **Ganem NJ**, Genesca A, Natarajan AT, Pavanello S, Valle G, Degrassi F. 2015. Genomic Instability: Crossing pathways at the origin of structural and numerical chromosome changes. *Environ Mol Mutagen.* (7):563-80.

Bolgioni A.F and **Ganem N.J.** 2016. The interplay between centrosomes and the Hippo tumor suppressor pathway. *Chromosome Res.* 24(1):93-104. *(Grunebaum Family cited in acknowledgements)*

Shenk EM, and **Ganem NJ**. 2016. Generation and purification of tetraploid cells. *Methods Mol Biol.* 1413:393-401. *(Grunebaum Family cited in acknowledgements).*

Additional Funding Acquired Since Acceptance of the Grunebaum Award

1) 1R01 GM117150-01, NIH/NIGMS (PI: Ganem) Maintenance of Genome Stability by the Hippo Tumor Suppressor Pathway *Pending, but 7% score is well within funding range*

2) The American Cancer Society Research Scholar Award (PI: Ganem) Mechanistically Defining the Role of STK25 in Mediating Contact Inhibition

3) Searle Scholar Award (PI: Ganem) The Causes and Consequences of Aneuploidy

4) Smith Family Award for Excellence in Biomedical Research (PI: Ganem) Maintenance of Genomec Stability by the Hippo Tumor Suppressor Pathway

5) The Skin Cancer Foundation (PI: Ganem)The Todd Nagel Memorial Award Defining Novel Mechanisms of Genome Instability in Melanoma

6) Melanoma Research Alliance (PI: Ganem)The Jackie King Young Investigator AwardDefining Novel Mechanisms of Genome Instability in Melanoma

7) The Sarcoma Foundation of America (PI: Ganem) The Alex Burdo Research Award Therapeutically Targeting the Hippo Pathway in Osteosarcoma

Thank You to the Foundation

8) Aram V. Chobanian Assistant Professor (PI: Ganem) Boston University School of Medicine Career Development Award

9) The American Cancer Society (PI: Ganem) The BU Clinical and Translational Science Institute Defining Gene Expression Signatures for YAP/TAZ-Dependent Breast Cancers

I look forward to seeing the trustees on October 21st, at the next Grunebaum meeting.

Sincerely,
Neil J. Ganem, Ph.D.

Rachel L. Flynn, Ph.D.

Boston University School of Medicine, Departments of Pharmacology & Experimental Therapeutics and Medicine, Division Hematology and Medical Oncology

Dear Members of the Karin Grunebaum Cancer Research Foundation,

I cannot thank you enough for the support you have provided my laboratory over the past two years. We are incredibly grateful. There is a steep learning curve in starting your own laboratory and one of the many challenges in this fast paced environment is generating exciting and innovative science on a limited budget. With the support of the Karin Grunebaum Cancer Research Foundation we were able to move our research further, faster. I have listed the accomplishments of the lab over the past two years below. However, as highlights I would like to mention that we have published 2 primary research articles in less than 3 years in high impact journals including Science and Cell Reports. The data generated by these studies led to the award of 3 more grants from the Elsa U. Pardee Foundation, Edward Mallinckrodt Junior Foundation (a first ever for BU), and perhaps most significant an R01 from the NIH/NCI. I don't believe we would have been this successful without the support and enthusiasm from all of you. I am truly excited about the research being generated in the lab and am looking forward to representing the Karin Grunebaum Cancer Research Foundation in future publications.

I have listed the highlights from the past two years below,

Current Lab Members

Emily Mason-Osann, PhD candidate, Jess Floro, PhD candidate, Dr. Ching-Shyi Wu, Postdoctoral Fellow, Victoria Kacprzak, Research Technician

Former Lab Members

Kelli E. Cox, PhD – Kelli was a graduate student in my lab who received her PhD in May of 2016 from Boston University School of Medicine. Kelli was a student in the Program of Biomolecular Pharmacology. For two years, Kelli was supported by the Departmental NIGMS T32-Program in Biomolecular Pharmacology training grant. Her thesis was entitled 'Replication Stress and the Alternative Lengthening of Telomeres Pathway'.

Aneesh Patel – Aneesh was an undergraduate student at Boston University who worked in my lab for a year and a half. During this time Aneesh was supported by a fellowship from the Undergraduate Research Opportunity Program.

Alysia R. Bryll – Alysia was a research technician in my lab for two years. She was an incredibly productive member of the lab and received authorship on our first publication. Alysia left the lab in the summer of 2015 for the University of Massachusetts Medical School where she is enrolled as an MD/PhD candidate.

Recent Grant Awards

1) 1R01CA201446-01A1 NIH/NCI (PI: Flynn) Defining the Molecular Mechanisms of the Alternative Lengthening of Telomeres Pathway

2) Elsa U. Pardee Foundation Grant (PI: Flynn) Targeting the Alternative Lengthening of Telomeres Pathway in Cancer

3) Edward Mallinckrodt Foundation Grant (PI: Flynn) Defining ATR function in the Alternative Lengthening of Telomeres Pathway

Patents

Treating Cancer Using Inhibitors of Ataxia-Telangiectasia Mutated and Rad3-Related (ATR). PCT/US2016/012797.

Recent Publications

Mason-Osann E and Flynn RL. Purification and Quantification of DNA C-Circles. ***JoVE***. 2016. In Press

Cox KE, Marachel A, and Flynn RL. SMARCAL1 resolves replication stress at ALT telomeres. ***Cell Reports.*** 2016 Feb 9;14(5):1032-40.

Flynn R L§, Cox KE, Jeitany M, Wakimoto H, Bryll AR, Ganem NJ, Bersani F, Pineda JR, Suvà ML

Benes CH, Haber DA, Boussin FD, Zou L§. Alternative Lengthening of Telomeres Renders Cancer

Cells Hypersensitive to ATR Inhibitors. ***Science***. 2015 Jan 16;347(6219):273-7. PMCID: PMC4358324.

§Co-Correspond Author

- **Ranked as Highly Cited Paper by Google Scholar**
- **Highlighted in Nature Reviews Drug Discovery:**
  Crunkhorn S. Anticancer agents: An alternative route to targeting telomere elongation. Nat Rev Drug Discov. Nature Publishing Group, a division of Macmillan Publishers Limited. All Rights Reserved.; 2015 Mar;14(3):164–5.

Thank you again for all of your support and I look forward to speaking with you soon,

All the best,
Rachel L Flynn

Summer Research Report

Epigenic Regulation of Growth Promoting and Tumor Suppressing Genes in Ovarian Cancer

Meghan Leary

Cancer Center, Department of Medicine

The goals of this research project were to identify genes in ovarian cancer cells that were either overexpressed or underexpressed, and to determine the mechanism which regulates the expression of these genes. I identified these dysregulated genes through literature search. I also looked for genes that were known to be growth promoters (oncogenes) or tumor suppressor genes in ovarian cancer. From these searches, I compiled a list of genes to investigate. For the growth promoting genes, I was interested in whether the protein CTCF was involved in their overexpression. While CTCF normally functions to silence genes, when its binding sites are methylated, it can no longer bind, and expression of that gene will increase. Since genome methylation patterns often differ between normal cells and cancer cells, I wanted to know if this had an effect on CTCF binding.

To determine whether CTCF bound any of the genes of interest, I performed chromatin immunoprecipitation (ChIP) on SKOV3 ovarian cancer cells. This cell line was chosen because it is highly metastatic and resistant to a number of cytotoxic drugs. To perform the ChIP, DNA and protein were crosslinked using formaldehyde. The cells were analyzed, and DNA was sonicated into small fragments so that only the portions bound to CTCF would be collected. CTCF was precipitated using anti-CTCF antibody. Then the proteins and DNA were de-crosslinked, and the DNA was purified. To determine whether CTCF bound any of the genes I had identified, I designed primers for those genes on the list which had CTCF binding sites and CpG islands (frequent sites of DNA methylation). qPCRwas performed using these primers and the ChIP DNA. A number of growth promoting genes, including HER-2, showed little to no CTCF binding in the controls, but following treatment with DNA demethylatingdrugs, increased CTCF binding was observed.

To perform an expression analysis, total RNA was collected from cells that had received the same treatments as the ChIP group. The RNA was reverse transcribed to cDNA. Reverse transcription primers were made for genes which showed differential CTCF binding in the Chip assay, and qPCR was performed. I found that, relative to the control, expression of growth promoting genes decreased following treatment with demethylating agents. This supports the hypothesis that CTCF is involved in silencing growth promoting genes when the binding site is not methylated. Tumor suppressor gene expression was investigated using qPCR. I found that, following treatment with demethylating agents, the expression of tumor suppressor genes such as ARHI and RAR **B** 2 increased.

Overall I feel my project was a success. I accomplished most of what I intended to do in the 8 weeks I had. The only significant setback I had was that I intended to preform these experiments on another cell line, CAOV3, in addition to SKOV3 to compare the results between the two different lineages of ovarian cancer. Unfortunately, the chromatin immunoprcipitation in CAOV3 was not successful, and it would have taken too long to regrow the cells and try again, so I proceeded with only SKOV3.

Reducing Socioeconomic Disparity in Skull Base Tumor Patients: How Patient Services Could be an Important Consideration.

Faisal Al Bahrani

Otolaryngology & Neurological Surgery

My research began initally as a search on previous studies on socioeconomic and racial disparities in health care. This was important for many reasons. First, I was able to identify the extent to which literature exists on the topic. I was also able to identify the various correlations that were identified in individual studies and the realms of healthcare that were involved. After my initial search and after I was able to confirm that my specific research goals were novel with respect to existing literature, I was able to continue with the planning process.

In my literature search I noted that there were multiple ways in which socioeconomic status was marked for a patient. First, some studies used a patient's insurance status as a marker of socioeconomic status. For example, those on Medicaid would be marked as being of low socioeconomic status while those who were privately insured were not. Others used a patient's address as a marker. Some neighborhoods would be listed as indicators of low socioeconomic status. For our project we decided to stick to using insurance status as our primary marker.

In terms of the data analysis, we decided that the use of Chi Squared tests would be ideal given the qualitative nature of our dataset.

Once these things were determined and after I acquired access to EPIC, SCM, and logician, I was able to begin collecting the relevant data for the patients in the skull base surgery database. For each patient, I had to make sure that any instances in which the patient made use of a social or financial service were noted as well as well as any uses of the food pantry. In addition to those data points, the relevant indicators of disease outcomes were also available in the database for use in the analysis.

At this point in the project the data collection is nearly done, but the data anaylsis has yet to be complete. I expect to be fully done with the analysis in time for the abstract submission (August 31st) and for the MSSRP poster presentation.