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Albert Einstein College of Medicine
OF YESHIVA UNIVERSITY

Graduate Programs in the Biomedical Sciences

2018 Abstract Book

SUMMER UNDERGRADUATE RESEARCH PROGRAM

Albert Einstein College of Medicine
OF YESHIVA UNIVERSITY

Graduate Division of Biomedical Sciences

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2018

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2018 Summer Undergraduate Research Program

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Characterization of Mesenchymal Stem Cells and Their Applications

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Summer Undergraduate Research Program

Introduction

Mesenchymal stem cells (MSCs) are pluripotent cells that have the potential to differentiate into chondrocytes, hepatocytes, neural cells, endothelial cells, osteocytes, adipocytes and myocytes. MSCs are useful due to their plasticity and ability to differentiate into many different types of cells. MSCs exhibit regenerative and immunosuppressant properties, primarily via paracrine mechanisms. Exosomes are small extracellular vesicles that carry proteins, growth factors, cytokines, DNA and RNA strands (miRNA, nucleic acids as well as lipid second messengers. Recent studies suggest that they can generate an in vivo immune response and can also act as extracellular messengers. The characterization of exosomes would allow us to understand stem cell function and communication. This project focused on the isolation and characterization of adipose derived MSCs and their applications.

Methods

Flow Cytometry, Immunostaining, RNA Isolation, qRT-PCR and a functional assay was performed on cultured ApoE and C57B16 cells.

Results

Flow cytometry and immunostaining successfully characterized the MSCs. The RNA was abundant and had preserved its integrity upon lysis and isolation. qRT-PCR showed the expression of certain genes between ApoE and C57B16 cells.

Discussion

The characterization of MSCs would allow for further studies to be conducted on the characterization of their secreted exosomes. The characterization of exosomes would lead to a better understanding of their behavior upon injection.

Acknowledgements

Faculty of the Radiation Oncology Lab

Identification of Enhancers that Drive Glioma Stem Cell Differentiation

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Glioblastoma multiforme (GBM) is one of the most common and fatal types of gliomas with no effective cure. An estimated 10,000 new cases are reported in the US annually. The median survival time is 12–15 months. Glioma stem cells (GSCs) are a subpopulation of cells in GBM tumors that are resistant to chemo and radiotherapy and can reestablish tumors after removal of original tumor. In this study, we are interested in identifying genetic and epigenetic features unique to GSCs. Enhancers are segments of DNA that activate gene transcription. These gene regulatory elements, scattered in the genome in the magnitude of hundreds of thousands, are critical for activation of gene expression. Enhancers can be identified using epigenomic marks such as histone modification (e.g., histone H3 acetylation at lysine 27 (H3K27Ac)). Transcription of noncoding RNA through enhancer regions (eRNA) can be used to identify an active enhancer. Therefore the presence of H3K27Ac is an indicator for an enhancer and presence of RNA polymerase II suggests the enhancer is active. Identifying enhancers unique to GSCs may provide potential targets for intervention in the treatment of Glioblastoma Multiforme. We hypothesize that when tumor stem cells differentiate, there is a set of active enhancers that become deactivated and a set of inactive ones that become active. Each of these enhancers controls a unique set of genes involved in differentiation.

GSCs were isolated, grown, induced to differentiate in vitro, and analyzed for the presence of enhancers and actively transcribed regions by ChIP-seq, using antibodies against H3K27Ac and RNA polymerase II, respectively. After identifying a set of differentially activated enhancers between GSCs and differentiated GBM cells, we located ~500 potentially regulated genes in each sample. Of these ~100 genes were found to be common between two patient samples for each the differentiated GBM cells and GSCs. The size of the overlap between patient samples is statistically significantly different from a random distribution. Gene set enrichment analysis of all the gene sets did not reveal any statistically significant biological clustering of the genes from each cell type.

Finding a set of genes involved in differentiation of glioblastoma stem cells is the first step towards finding a treatment that stops these cells from differentiating and establishing secondary tumors. Future directions for this research include using additional samples from patients with the same or similar glioblastoma subtype to eliminate differences due to type of GBM. Further analysis of the data as well as exploration of alternative definitions of "relevant" genes will help eliminate noise which could lead to a clear clustering of the genes. These results could then be compared with those of previous studies that used histone methylation as an identifier of enhancers.

Special thanks to Dr. Eduardo Fajardo for mentoring me throughout this project and to Dr. Andras Fiser for his invaluable input as well as the opportunity to be a part of his lab. Thank you to all the members of the Fiser lab for being very welcoming of me.

Interactions Between Antiviral Nucleoside and Endogenous Enzymes

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Viral infections are a major challenge for public health and thus, mechanisms that underlie compounds with antiviral activity are of great interest. The human protein viperin, which is a member of the radical S-adenosyl-L-methionine (SAM) family of enzymes, has been shown to generate antiviral activity when expressed during the early stages of an infection. However, how viperin generated this antiviral activity was unknown until it was recently shown by the Almo lab that viperin is an enzyme that catalyzes the formation of 3′-deoxy-3′,4′-didehydro-CTP (ddhCTP) from cytidine triphosphate (CTP). Furthermore, ddhCTP was demonstrated to act as a chain terminator for viral RNA polymerases involved in the replication of viral genomes. In addition, ddhCTP was shown to have low cytotoxicity meaning it did not negatively affect the host's cellular machinery making it an excellent candidate compound for future antiviral studies. Therefore, the goal of this project is to generate ddhCTP chemo-enzymatically enabling the generation of large quantities of the compound for viral studies and generate standards for the full metabolism profile of ddhC to ddhCTP. We have previously chemically synthesized ddhC, which does not contain any phosphate groups, and added it to media to grow HEK293T and Vero cells. The cells showed they were capable of uptaking and converting ddhC to ddhCTP, meaning host kinases are capable of modifying the prodrug to an active state. Therefore, we have selected the proteins uridine-cytidine kinase 2 (UCK2) and cytidine deaminase (CDA) as a starting point to study the metabolism of ddhC in cells and generating a strategy to synthesize ddhCTP. We used ligation independent cloning (LIC) to generate expression plasmids for expression and purification of UCK2 and CDA. We then developed assays for these enzymes to detect both activity with their natural substrates (uridine or cytidine, or cytidine only) and with ddhC using high performance liquid chromatography-mass spectrometry (HPLC-MS). Our findings show that UCK2, in the presence of ATP, can phosphorylate U and C, as expected, but can also phosphorylate ddhC into ddhCMP. However, CDA cannot catalyze the deamination of ddhC to ddhU. These results are consistent with our *in vivo* feeding studies using ddhC.

The Tight Junction Proteins Occludin, Claudin 4, and Claudin 5 Show Differential Interactions with the Gap Junction Nexus

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Gap and tight junctions are cell-cell connections found in tissues throughout the body. Each functional gap junction is comprised of docked connexin hemichannels that act as a passageway between two cells, enabling direct exchange of ions and small molecules between their cytoplasms. Gap junction complexes are hexameric channels, which are arranged as membrane plaques. Of their many tissue-level functions, gap junctions play a critical role in buffering ions and equilibrating water flux during, and following, CNS injury, spread of cellular apoptosis (Bystander Cell Killing) during cancer treatment, and long-range calcium signaling in mechanosensory cells and astrocytes.

Tight junctions perform unique functions, in which they prevent leakage of transported molecules by sealing the extracellular space between two cells. These junctions also separate apical from basolateral membrane proteins and form selective channels for small cations, anions and water. The major tight junction proteins at the blood-brain-barrier (BBB) are Occludin, Claudin 4 and Claudin 5. Under normal conditions, endothelial cells express both Occludin and Claudin 5, thereby establishing an impermeable BBB. As a consequence of inflammation, Occludin and Claudin 5 are downregulated and the more permeable Claudin 4 is upregulated, so that the permeability of the BBB increases.

We hypothesized that despite the differing functions of gap junctions and tight junctions, they would be partially composed of similar membrane proteins which play roles in endocytosis and plaque stability. We expected to see that Occludin, Claudin 4 and Claudin 5 would localize to Connexin 43 gap junction plaques and show varying mobility within the plaque and outside the plaque similar to the differing mobility of these proteins in the BBB.

To test this hypothesis, Neuro2A neuroblastoma cells that otherwise lack these juncitonal proteins were cultured *in vitro* and co-transfected with cDNA for fluorescently tagged Connexin 43, Occludin, Claudin 4 and Claudin 5 in different combinations. Fluorescence Recovery After Photobleaching (FRAP) was utilized on cultures to measure the mobility or stability of these proteins. A Zeiss 5Live laser scanning microscope with 63X objective was used to image the gap junction plaque and perform FRAP experiments.

We found that Occludin, Claudin 4, and Claudin 5 were all localized to Connexin 43 plaques, suggesting that the accessory protein makeup of gap junctions shows resemblance to that of tight junctions. FRAP experiments illustrated that when comparing rigidity of these proteins they had less of an ability to recover their fluorescence while associated with the plaque. When comparing Claudin 4 and Claudin 5, Claudin 4 recovered faster post-photobleaching within mutated Connexin 43 plaques composed of fluidly arranged channel subcomponents, indicating Claudin 5 is a more stable protein within plaques than Claudin 4. However, Claudin 4 and 5 showed similar mobilities freely dispersed in the membrane, showing Claudin 5 is not inherently less mobile than Claudin 4 outside of junctional complexes. The plaque mobilities are consistent with the roles of Claudin 4 and 5 in the blood brain barrier in health and inflammation.

Special thanks to Preeti Dohare for her help. Supported by the Albert Einstein College of Medicine Undergraduate Research Program.

Abstract – Study of Atg13 role on autophagy during CD4 T cell activation.

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Diversity Student Summer Research Opportunity Program

Macroautophagy is a primarily degradation cellular process focused on recycling cytoplasm, damaged or superfluous proteins and organelles. Its main purpose is to maintain homeostasis within the cell and to respond to stress conditions. In T cells, basal macroautophagy is notably important for survival in the periphery and it is induced upon T cell receptor engagement. Macroautophagy relies on a double membrane vesicle, called autophagosomes, that engulf cellular content and then fuses to the lysosome where it releases its cargo to be degraded. The *Atg* (Autophagy-Related Genes) family is required to initiate and regulate this process. The Atg1/ULK complex acts upstream and initiates autophagosome formation. ATG13 is particularly vital for the ULK1 complex activity. Previous data in the lab indicates that, upon T cell activation, ATG13 expression is increased while ULK1 remains constant. The goal of this project was to determine the role of ATG13 on autophagy induction and T cell functions following activation. For that purpose we used small interfering RNA (siRNA) to knock-down (KD) expression of ATG13 in Th1 cells. We have been able to first confirm that ATG13 expression was induced following T cell activation. Unfortunately our attempts to inhibit the expression of ATG13 with siRNA failed. Additional experiments would be required to determine why and attempt to establish a viable set of conditions for effective knock-down. Nevertheless, the use of another strategy, such as shRNA, needs to be explored in order to inhibit ATG13 expression.

Metabotropic glutamate receptor 1 regulates dendritic spines structural plasticity

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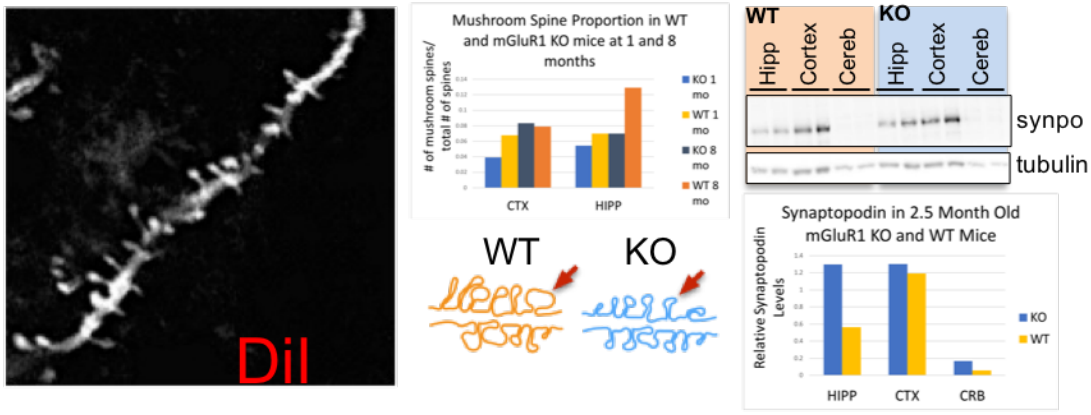
Dendritic spines are small protrusions on dendrites where most glutamatergic excitatory activity occurs. Dysregulation in the development and maintenance of dendritic spines have been linked to multiple neuropsychiatric conditions such as Autism Spectrum Disorders (ASD), schizophrenia, Alzheimer's disease, depression and anxiety. Changes in spine morphology are related to establishment of synaptic connections and synaptic plasticity; mature mushroom spines are linked to increased synaptic strength and long-term potentiation - a cellular process that underlies learning and memory - whereas immature thin spines are linked to weaker connectivity. Mature mushroom spines - which signal stable, fully formed synaptic pathways - develop a specialized organelle called the spine apparatus. The presence of the spine apparatus can be observed through visualization of the protein synaptopodin which is tightly associated with the organelle. There are specific differences in the phenotypes of spines among different disorders and little is currently known about how these differences arise.

In our lab we approached this gap in knowledge by examining the impact of metabotropic glutamate receptor 1 (mGluR1) on dendritic spines structural plasticity. MGluR1 mediates synaptic plasticity in various areas of the brain in mice and patients with mutations that inactivate mGluR1 show severe intellectual disability and motor deficits. We used mGluR1 knockout (KO) and wild type (WT) mice at different ages (one to ten months) to investigate whether mGluR1 contributes to the development of mature mushroom spines.

We used hippocampal and cortical tissue slices incubated with the lipophilic fluorescent dye DiI to fill and visualize dendrites and dendritic spines. I analyzed confocal images at 63x magnification to quantify the presence of mature mushroom spines vs. immature thin spines in WT and mGluR1 KO mice. In parallel I used western blot to quantify the abundance of synaptopodin (synpo) in hippocampal, cortical and cerebellar tissue samples.

I found that mGluR1 KO mice had a lower proportion of mature mushroom spines in the hippocampus compared to WT at both one- and eight-month of age. Interestingly, the relative abundance of synaptopodin was higher in the hippocampus of young (2 ½-month) mGluR1 KO vs. WT mice whereas no discernible difference was observed in the cortex of adult mice (5- and 10-month old). At present, we cannot draw a definitive conclusion about these findings because of the limited sample size. However, there seems to be evidence of a difference in dendritic spine development between mGluR1 KO vs. WT mice.



There is an apparent discrepancy between the reduction in mature mushroom spines and increased abundance of synaptopodin in mGluR1 KO mice. This could be due to the fact that synaptopodin is not always associated with the spine apparatus and its localization may be altered in mGluR1 KO mice. Moreover, increased synaptopodin abundance may also point to potential deregulation of its synthesis and/or degradation in the absence of mGluR1.

Elucidating the mechanisms underlying dendritic spines development through mGluR1 could lead to ideas for new drug therapies in the treatment of neuropsychiatric disorders including ASD and schizophrenia. Since patients with mGluR1 mutations show similar deficits to mGluR1 KO mice such as ataxia and cognitive impairment, these deficits in humans could also be linked to dysfunctions in spine development. Understanding these deficits at the molecular and cellular level in mice will give us the tools to translate these findings to other human conditions.

The Anti-Inflammatory Prodrug Sulfasalazine Alters Gut Microbiome Function

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Short Chain Fatty Acids (SCFAs) are products of the fermentation of dietary fibers by the gut microbiota and are important in maintaining colon health. Monitoring the amounts of SCFAs produced can shed light on the way that the gut microbiota reacts to xenobiotics. Sulfasalazine (SSZ) is an anti-inflammatory prodrug used to treat ulcerative colitis and rheumatoid arthritis that relies on the gut microbiome for activation. Although Sulfasalazine's interaction with the gut is well established, the extent to which the drug alters the gut microbiome remains unclear. The production of SCFAs is a quantitative metric of microbial activity in response to SSZ exposure.

Sulfasalazine is a known antibiotic that interacts with the gut microbiome. Therefore, we hypothesized that over a 48-hour period, there would be fewer SCFAs produced in the samples that received SSZ than in the ones that lacked exposure to the drug. The production of SCFAs was tested in anaerobic fecal slurries in the presence and absence of SSZ. We observed a trend that suggests that the samples that were given SSZ produced fewer SCFAs than the samples that lacked SSZ. This decrease in SCFA demonstrates that microbiome function is altered in the presence of SSZ. The therapeutic function of SSZ relies on activation by colonic bacteria, and is highly variable between individuals. We investigated the role of individual cultures in the activation process. *Escherichia coli* is a member of the gut microbiome and a well-characterized organism to model interaction between individual microbes and SSZ. To measure the rate at which *E. coli* activates SSZ in vitro, we quantified SSZ loss over time with UV-Vis spectrophotometry. We observed that the *E. coli* samples preformed azo reduction and completely metabolized the drug within 54 hours.

Future research will continue to profile the effect of SSZ on SCFA production. We are currently analyzing the contribution of the two derivatives of SSZ to its inhibition of SCFA production and exploring the impact of SSZ on the growth of other isolated members of the gut microbiome, such as Bacillus subtilis. As we continue to test other samples, we hope to increase the sample size to gain a broader understanding of the alterations SSZ causes to the gut microbiome. These experiments bring us closer to building a profile of the gut microbiome's interaction with SSZ, and demonstrate the need for deeper analysis of how SSZ and other azo prodrugs influence microbiome function in individuals.

Acknowledgments
This research was supported by the Einstein Summer Undergraduate Research Program. Thank you to the members of the Kelly Laboratory for their guidance and mentorship this summer, and, specifically, Reese Hitchings for his constant support, encouragement, and enthusiasm throughout my project.

Identification of highly dose dependent PU.1 target loci in neutrophils

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Evidence from human and mice studies have revealed that inhibition of the hematopoietic transcription factor, PU.1, induces acute myeloid leukemia (AML). We previously determined that moderately impaired PU.1 expression induces malignant transformation in aging hematopoietic stem cells (HSCs) driving AML. To determine the underlying molecular mechanisms involving PU.1 impairment leading to leukemogenesis, we aim to conduct chromatin immunoprecipitation with next generation sequencing (ChIP-seq) on purified homogenous cell populations derived from various genetic mouse modles allowing for gradual reduction of PU.1 dosage in myeloid cells. The goal of this project was to develop an optimized PU.1 ChIP protocol.

We tested three cell fixation conditions (1%, 0.75%, and 0.4% formaldehyde), optimized chromatin shearing efficiency, and tested a monoclonal and a polyclonal anti-PU.1 antibody to determine optimal specificity and sensitivity of a standard ChIP protocol. To determine PU.1 binding abilities of the different antibodies, qPCR assessment of PU.1 target loci enrichment with known occupancy (*Myogenin, URE, E2F1, Cybb, GCSFR, and Zbtb11*). Our results illustrate slight epitope masking with the polyclonal antibody and full epitope masking with monoclonal antibody at a high fixation concentration (1% FA). This was overcome by reducing the concentration of the fixative. There was an increased sensitivity with the polyclonal antibody combined with 0.4% formaldehyde cell fixation best conforming to the expected occupancy of the PU.1 target loci. When compared to the reference antibodies, all antibodies functioned similarly to the previously validated antibody (T-21, Santa Cruz). These findings were critical to establish an improved PU.1 ChIP-seq protocol to identify the target PU.1 loci affected and whether they are functionally affected in AML patients.

We are currently working on isolating neutrophils from different mouse strains models (*$PU.1^{+/+}$, $URE^{-/+}$, $PU.1^{-/+,}$ $URE^{-/-}$, and $PU.1^{-/-}$ as a negative control)* , by density gradient centrifugation, to determine PU.1 binding sites that are most sensitive to moderate transcription factor dose changes. Results from our study will ultimately help uncover the molecular mechanism of leukemia predisposition following moderate PU.1 inactivation.

Acknowledgements: Special thanks to Maria Aivalioti, George Georgiev, BARC core, Xenotransplantation and FACS facility, and Albert Einstein College of Medicine Summer Undergraduate Research Program (SURP)

The Effects of SAMHD1 and its Potential to Block HIV-1 Infection

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There are about 37 million people who are living with HIV (human immunodeficiency virus) and there are over one million people who have been diagnosed within the United States. HIV gradually weakens the immune system and can be a fatal disease if untreated. SAMHD1 (sterile alpha motif and histidine-aspartate-domain-containing protein 1) is a viral host restriction factor that controls the intracellular level of (dNTPs) also known as deoxyribonucleoside triphosphates. This enzyme reduces intracellular dNTPs which leads to the degradation of viral genetic information. The antiviral activity of SAMHD1 limits viral replication during the reverse transcription step of the viral life cycle. SAMHD1 is a restriction factor in non-cycling cells. Non-cycling cells include non dividing cells such as T-cells and macrophages. The aim of this study was to determine if SAMHD1 can actually restrict HIV-1 infection by using non-cycling THP-1 cells to infect with an HIV-1 viral pseudotype. The cell line used in these experiments was THP-1 and THP-1 SAMDH1 knockout cells. The cells were infected with serial dilutions of a pseudotyped HIV-1 virus to see the restriction conferred by the presence of SAMHD1. THP-1 SAMHD1 KO cells show more infection compared with the wild type THP-1. Previous scientific discoveries have also indicated that SAMHD1 may be an important factor to counteract HIV-1 infection. Further research concerning SAMHD1 may lead to a possible treatment or cure for the HIV-1 virus.

Manipulation of varying parameters reveal changes in Fidgetin-Like 2 and Microtubules

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The cytoskeleton of eukaryotic cells is made of filamentous proteins, providing mechanical support to the cell. The cytoskeleton contains three components that differ in measure and in protein structure. One of these three parts is microtubules (MTs), which are comprised of tubulin subunits. Their role is to help in cell motility and cell division. At the front edge of moving cells, known as the leading edge, MTs are extremely dynamic, and this encourages cell motility. Fidgetin-Like 2 (FL2) is a human microtubule severing enzyme that cut the MTs at the leading edge of cells to moderate cell movement. When FL2 is knocked down, MTs stay dynamic to have the functionality to enable the cell to move more rapidly. Research has demonstrated that there is a twofold increase in cell motility when FL2 is knocked down. My specific research project aims to qualitatively and quantitatively measure the differences in abundance and localization of FL2 and MTs after changing the cells' conditions. We have manipulated the cell type, the fixation temperature, and MT stability through Taxol treatment. In all three varying conditions, immunofluorescent imaging (IF) was conducted to see the abundance and localization of FL2 and MTs in the cell. To start, we looked at MTs and FL2 in two diverse cell types; Human Bone Osteosarcoma Epithelial Cells (U2OS) and Human Embryonic Kidney Cells 293 (HEK293). In addition to the IF, we conducted a western blot to see the abundance FL2 in both of the cell types. Next, U2OS cells were treated with various concentrations of Taxol, which is a MT stabilizer, attempting to see how the changes in MT stability influenced FL2 localization at the leading edge. Finally, U2OS cells were fixed at three different temperatures: 4°C, 25°C (room temperature) and 37°C (physiological temperature). This was to identify any impact of temperature on FL2 localization and MT abundance.

In conclusion, we found that as microtubules change in the cell, so does FL2 localization, supporting that these two interact with each other. When contrasting U2OS and HEK293 cells, we saw that levels of both FL2 and MTs were significantly increased in HEK293 cells contrasted with U2OS. The cell sizes were standardized to demonstrate a genuine comparison; although, it should be noted that HEK293 cells were larger than U2OS cells. When MT stability was manipulated by treating cells with 0uM, 0.5uM or 5uM of Taxol, the results demonstrated that, with more prominent concentrations of Taxol, more MTs were stabilized, and there was more FL2 at the leading edge. This was unexpected because greater MT stability means less MT dynamics, which has previously been hypothesized to decrease FL2 localization to the leading edge. When the temperature during cell fixation was altered to 4°C, 25°C or 37°C, we saw that, at lower temperatures, there was an increase in the localization of FL2 and abundance of MTs present in the cell. This was surprising, as it was assumed that at physiological temperatures, there would be more FL2 at the leading edge and more MT in the cell; however this condition showed the greatest decline in levels.

The findings presented here are preliminary, based on a small subset of mammalian tissue culture cells. Future research should include these tests being repeated with different cell types to see if these findings are cell-specific. Manipulation of these parameters should be tested in vivo, using a murine model as that would give us the truest result, as mice are good subjects for human research studies. I also plan to repeat these experiments to stain for actin to determine what role actin might play in the interaction between MTs, FL2, and cell movement.

Special thanks to Karishma Smart, David Sharp and the entire Sharp lab for an amazing experience. This research was supported by NIH325065. Thank you to the Summer Undergraduate Research Program (SURP) at Albert Einstein College of Medicine of Yeshiva University for allowing me the opportunity to conduct this research at their institution.

Mapping Synapsin-Cre in Fragile X Rictor Conditional Knockout Mice

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Cre-loxP recombination is a versatile tool that can be used to achieve cell-specific, conditional knockouts (cKO) of specific genes, as well as insertions and translocations at specific sites on the DNA. cKO's are extremely useful for in the investigation of developing neurons, as well as the formation of neuronal circuits in control animals and in disorders such as Fragile X Syndrome (FXS). FXS is the most common cause of heritable intellectual disabilities and prevalent genetic cause of autism. Our laboratory has shown that the mammalian target of rapamycin (mTOR) signaling pathway is dysregulated at synapses of the hippocampus, a brain region central to learning and memory. mTOR assembles to form two independent complexes, mTORC1 and mTORC2, which regulate cellular growth and proliferation in response to external cues. mTORC2 plays a crucial role at the synapse, where it is known to regulate the actin cytoskeleton and spine structure. Because mTORC2 is dysregulated in individuals with FXS, they display abnormal cofilin signaling and dendritic spine abnormalities. The present study sought to determine the efficiency of expression of lentivirus carrying synapsin-Cre and investigating the total area affected by stereotaxic injection synapsin-Cre directly into the somatosensory cortex of living mice. Moreover, we sought to confirm the cKO capacity of synapsin-cre injected/FXS KO animals by comparing heterozygous loxP positive animals to wild type loxP negative animals by means of immunohistochemistry. mTORC2 plays a central role in regulation of the actin cytoskeleton, spine structure and memory. Determining how far synapsin-Cre extends in neuronal cells, can lead to verification that the virus is effective and specifically knocks down the component RICTOR. By targeting RICTOR, the defining component of mTORC2, this construct can reduce mTORC2 specifically and thereby rescue synaptic signaling and spine defects. Our study illustrates that synapsin-Cre extends through the ventral and dorsal areas of the somatosensory cortex. Therefore it can be said that reduction of mTORC2 activity rescues overactivated cofilin signaling via Cre. It is hoped that these preliminary findings will spur future research that will advance diagnosis and treatment of disorders such as autism spectrum disorders.

Isolation of Novel Human Monoclonal Antibodies against Chikungunya Virus

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Chikungunya virus (CHIKV) is the etiological agent of a debilitating arthritic disease. In late 2013, CHIKV's first transmission in the western hemisphere occurred and the virus rapidly spread to more than 40 countries in the Americas, including the United States. A mature CHIKV virion contains 240 copies of the fusion protein E1 and the attachment protein E2 arranged as 80 trimeric spikes of three E2/E1 heterodimers. At present, no vaccine or treatment exists. However, antibodies have shown to be a promising platform for therapy and vaccine design. Here, we utilized single B-cell sorting technology to investigate the humoral immune system.

Previous studies have identified certain domains on the mature E2 glycoprotein as the main antigenic regions. To expand the knowledge of antibody specificity for CHIKV, we used a construct of the immature glycoprotein, p62-E1, to bias our sorting for unrecognized epitopes. Blood samples were collected from Bronx convalescent patients and peripheral mononuclear blood cells (PBMCs) were isolated. Single B-cells were then recovered by fluorescence-activated cell sorting (FACS). Single-cell RT-PCR was conducted and heavy and light chain variable region fragments were amplified via nested PCR. Antibodies were expressed in HEK293FS cells, purified via Protein A chromatography, and characterized by SDS-PAGE with Coomassie Blue staining. We have isolated 108 human CHIKV monoclonal antibodies (mAbs) binding to both E2 and E1 glycoprotein segments. 41 mAbs have demonstrated neutralization activity against the CHIKV-181/25 vaccine strain. 4 antibodies possessed neutralization activity against the authentic BSL-3 virus.

Previous studies have shown that monoclonal antibody combinations of CHIKV mAbs targeting E1 and E2 are synergistic. In order to improve mAb efficacy, we utilized our novel antibodies to engineer bi-specific antibodies (bsAbs) in Single-chain variable fragment (ScFv) formats with a dual affinity for both the E1 and E2 glycoprotein. Parent antibodies were fused with ScFv at either the N-terminus or C-terminus of the heavy or light chain. Future studies include testing the neutralization activity of our bsAbs and investigating all of our human CHIKV mAbs for protection in a mouse model.

Acknowledgments:

This work was supported by the U.S National Institutes of Health grants R01 AI125462 and T32 GM007288. This research experience would not have been possible without the Summer Undergraduate Research Program at Albert Einstein College of Medicine.

Pharmacological Strategies in Selectively Antagonizing IGF-1 and Insulin Receptors

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The insulin pathway is highly conserved throughout nature; insulin-like growth-factor-1 (IGF-1) and insulin are two similar hormones that affect cell signaling and body physiology. Insulin promotes metabolic signaling processes while IGF-1 promotes cell proliferation and growth signaling. These two similar pathways can be triggered in a tissue-specific manner by the affinity of each ligand to their respective receptor. Since insulin and IGF-1 receptors have remarkable sequence homology, for example their tyrosine phosphorylation sits, there has been limited ability to specifically antagonize one or the other pathway without also antagonizing the other. The effectiveness of two newly-developed selectively antagonizing inhibitors, S961, a small peptide inhibitor of insulin receptors, and AMG479, a monoclonal antibody paired to IGF-1 receptors, is assessed in this project using the insulin and IGF-1 receptors in these cells. Using these antagonists to inhibit receptors for both insulin and IGF-1 in two different cell lines, one from mouse hypothalamus tissue (mHypoA-NPY/GFP) and one from human neuroblastoma cells (SH-SY5Y), will allow us to begin to see the downstream inhibiting effects of specific inhibitors on two closely-related metabolic pathways. To this end, cells were separated into seven treatment groups, treated with ligands and antibodies, lysed for total protein to be isolated, quantified, and subjected to Western blotting for p-Akt, total Akt, pErk, total Erk, as well as total Ins-R and IGF-1R levels. The levels of these proteins diagnosed the degree of usage of the insulin and IGF-1 pathways. We found IGF-1 to be a stronger activator of Akt and Erk signaling than insulin, and this signaling to be blocked effectively by AMG479. Likewise, insulin led to a more modest, but significant increase in downstream signaling in SH-SY5Y cells only, and this signal was diminished by S961. Therefore, based on human and mouse *in vitro* data, both S961 and AMG479 appear to be effective in decreasing protein synthesis downstream of the activation of their respective receptors. The next step in diagnosing the *in vitro* utility of these antagonists is to model their activity in mice and rats, as differing levels of receptors and other confounding biological effects may play a role in drug effectiveness. Further work should be performed assessing the level of specificity of these antagonists to their respective receptors.

Acknowledgements: I would like to thank Gabriela Quipildor and Ardijana Novaj for sharing their wide range of skills with me every day and for contributing their valuable time to this project. Thanks to Derek Huffman, my mentor, for his efforts in teaching me and for providing me with the resources and knowledge necessary to complete this project. Special thanks to the Einstein SURP program for creating an exciting and hospitable environment for undergraduate growth.

Activation of perineuronal net-expressing neurons in paternal behavior

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Parental care involves behaviors that ensure the protection and fitness of offspring. In addition to humans, many other animals display parental behaviors including mice in which parental mice retrieve pups to the nest and crouch over the pups in the nest. Whereas virgin females tend to be parental similarly to fathers, virgin males tend be more unresponsive or aggressive toward pups. Some males and females display infanticidal behavior in which they attack pups for reproductive advantage and preservation of resources respectively. Virgin male mice display higher instances of infanticide. Studying the neuronal circuitry governing this social behavior in male virgin mice can give insight into understanding the mechanisms behind the mating-induced switch in behavior towards parental care. However, the plasticity and neurochemical mechanisms behind paternal and alloparental behavior remains largely unknown. The presence of PNNs mediate synaptic activity and plasticity, contribute to critical period closure, and affect learning and memory. We hypothesize that differences in levels of PNN surrounded neurons between virgin and mated males may trigger the mating-induced switch in paternal behavior. Here we compared the number of perineuronal net (PNN)-expressing neurons present and active in previously determined regions of the aggression pathway including the hippocampus, thalamus, hypothalamus, medial preoptic area, and amygdala among infanticidal males, spontaneously parental males, and fathers using the PNN immunohistochemical marker, *Wisteria floribunda* agglutinin (WFA), and activity marker, c-FOS. Greater activity in PNN-expressing neurons in the hippocampal and thalamic regions of fathers and greater levels of WFA intensity in the hypothalamic and amygdala regions of attacking and spontaneously parental mice respectively suggest that enhanced temporally specific plasticity may be a mediating factor responsible for the difference in mating influenced male adult-infant interactions.

Acknowledgements:

We would like to thank Kostantin Dobrenis for assistance setting up the imaging on the Zeiss Axioscan, Jessica Osterhout for advice regarding C-fos antibody staining, and the Summer Undergraduate Research Program at Albert Einstein College of Medicine for the opportunity and support. Funding was provided by startup funds and grant R00HD085188 to A.E.A.

"Par1a Deletion Decreases Tubulointerstitial Fibrosis in Mice"

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Chronic Kidney Disease (CKD) affects 1 in 7 people in the United States. CKD increases both health complication and mortality rates. CKD progression to end stage kidney disease results from tubulointerstitial fibrosis (TIF). One of the regulators of fibrotic progression is the Notch signaling pathway. Notch is an important cell-cell signaling pathway that is required for glomerular and proximal tubular kidney development. However, Notch activation in adult kidneys induces tubulointerstitial fibrosis in mice. Notch inhibition is protective against folic acid induced renal fibrosis. Notch ligands include Jagged (Jag1) and Delta (DII4). These ligands bind Notch receptors, leading to Notch cleavage. The intercellular domain of Notch, (NICD) translocates to the nucleus acts as an activator for the transcription of the effectors of Hes and Hey. Partitioning defective (Par1) is a serine-threonine kinase. Dual deletion of Par1 paralogues 1a and 1b decreased Notch signaling in developing mouse kidneys.

We hypothesized: Par 1a deletion would inhibit Notch activation and protect against folic acid induced fibrosis. To test this, Par1a knockout and wild type mice were given folic acid injection (250 mg/kg dissolved in 300 mM $NaHCO_3$). We used Sirius Red stain to observe and quantify the extent of fibrosis in the proximal tubules. To test whether Par1 is expressed in signal sending or receiving cells, we used immunofluorescence staining to evaluate the localization of Par 1a and Jag1 in folic acid treated kidneys. To develop a system to test mechanisms of Notch-Par1 interaction in vitro, primary proximal tubular epithelial cell cultures were generated from mice with inducible Par1 deletion. To test if this culture system can be used to examine Par1-Notch interactions, we performed a Western Blot, to look at the expression of Notch signaling components.

Sirius Red staining showed increased fibrosis in wild type mice than in mutant mice: the percent area of fibrosis was 12 +/- 6.8% in folic acid treated *Par1a* +/+ vs. 3 +/- 2.2% in *Par 1a* -/- kidneys. Immunofluorescence revealed increased expression of Par1a and Jag1 following folic acid injection. Par1a and Jag1 were co-expressed in renal tubules. The Western Blot revealed that Jag1, N2ICD, Hes 1, and Hey L were expressed in primary proximal tubular cell culture.

These data suggest that Par1a deletion is protective against renal fibrosis and that Par1a regulates Jag1 mediated Notch activation. Ongoing studies are examining the effect of Par1a deletion on Notch signaling activation following folic acid treatment in kidneys and TGF-β in cells. Future studies will further examine the mechanisms of Par1-Notch interactions and the effects of Par 1 deletion on unilateral ureteral obstruction induced renal fibrosis.

This research was supported by the Albert Einstein College of Medicine Office of Diversity Enhancement, the Office of Graduate Studies, and the National Institute of Health Grant R03 DK105242.

Engineering probiotic *Escherichia coli* Nissle 1917 capable of constitutive anti-inflammatory tryptophan metabolite production

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Indole derivative-producing commensal gut bacteria have been shown to have probiotic effects on intestinal inflammation in IBD models through the Pregnane X Receptor (PXR) pathway. PXR stimulation by indole derivatives regulates transcription of intestinal TNF-α and junctional protein-encoding mRNAs, improving gut barrier function to reduce inflammation. Although many bacteria are able to generate indole and tryptophan through metabolic biosynthesis, the ability to produce reductive tryptophan derivatives such as indole-3-propionic acid (IPA, a known PXR ligand) is limited to relatively few species in phylum Firmicutes. We have sought to develop and clone the phenyllactate dehydratase gene cluster from *Clostridium sporogenes* into the commonly used probiotic strain *Escherichia coli Nissle 1917* (EcN) to study the effects on intestinal inflammation. This was accomplished by PCR amplification of the *C. sporogenes* phenyllactate dehydratase gene cluster, overlap extension PCR to incorporate sequence homology to the EcN genome, and electroporation into EcN expressing λ recombinase via the pSIM5 plasmid to insert the gene cluster into the non-essential aconitate dehydratase gene (*acnA*). This recombinant form of EcN will be analyzed for presence of IPA by mass spectrometry and will be administered by gavage to colitis model mice to analyze intestinal inflammation. Introduction of IPA production into *E. coli Nissle 1917* (the active component of Mutaflor®) could improve the probiotic effects associated with the bacterium by adding onto its direct inhibition of other pathogenic microbes, bacterial-epithelial crosstalk, and Immune-modulatory effects. This experiment will give us further insight into microbial mechanisms of action in improving the gut-epithelial barrier and may result in a strain of bacteria with improved clinical use for IBD and related pathology.

The Use of DREADDs to Test the Necessity of Glutamatergic Projections from the Basolateral Amygdala to the Nucleus Accumbens for Cued Approach Learning

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Learning to respond to cues that predict reward is crucial for survival. It is thought that drug addiction follows the same neural pathways that contribute to this behavior. Previous studies in our lab have shown that neurons change their firing rate in the nucleus accumbens core (NAc) as animals learn to respond to cues that predict reward. As animals learn the operant conditioning task, the degree to which the behavior can be predicted through observations of neuron firing increases. Additionally, we have been able to silence NMDA receptors in the NAc. This was accomplished by using a NMDA antagonist AP5. It profoundly impaired acquisition of a cued approach response. We were able to conclude that NMDA receptor activation in the NAc is necessary for this cue-evoked excitation to emerge. However, the neural mechanisms contributing to this plasticity are unclear. The NAc receives glutamatergic projections from the basolateral amygdala (BLA), prefrontal cortex, hippocampus and thalamus, any or all of which may be necessary for plasticity within the NAc. We tested the hypothesis that the glutamatergic projection from the BLA to the NAc contributes to learning cued approach.

A virus containing Gi-coupled hM4D DREADD (Designer Receptor Exclusively Activated by Designer Drug) was injected into the BLA and cannulae were implanted into the NAc. This allows expression of DREADD in the BLA neuron terminals in the NAc. Injection of the DREADD agonist Clozapine-N-Oxide (CNO) into the NAc should therefore inhibit BLA terminals and, if the BLA is necessary for plasticity within the NAc, prevent learning the cued approach task. A second group of rats had cannulae implanted into the NAc but there was no virus injected into the BLA. The rats were allowed to recover for 6 weeks while the expression of the DREADD was allowed to take place. After 6 weeks, the animals were trained on the task for 7 days. During training, CNO or saline was microinjected bilaterally in the NAc. We predicted that learning of the cued approach task would be impaired in the rats that had the implanted virus and that received CNO due to the DREADD/CNO interaction compared with the two controls. This result would show that the basolateral amygdala projections to the NAc play an important role in the plasticity within the NAc and the underlying cued approach behavior. Future projects could test other glutamatergic projections to the NAc and their contributions to cued approach behavior.

Acknowledgments: This research is supported by NIH grants R01DA019473, R01DA038412 to SMN, a NIDA Summer Research Intern Award to AF and F31DA042619 to CR.
I would like to thank the Nicola lab for their continuous guidance and support.

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Phenotypic and genetic characterization of somatosensory dendrite defects in *dz224* mutants

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The nervous system develops due to the influence of various regulators, many of which are unknown. Further knowledge of dendrite development is important to understand abnormal dendritic patterning such as those found in models of Autism and Alzheimer's disease. The PVD somatosensory neuron in *C. elegans* responds to painful stimuli such as extreme temperatures and mechanical force. The PVD dendrites gradually develop during larval development by successive branching into primary, secondary, tertiary, and quaternary structures that resemble menorahs. The FLP neuron is similar to PVD but located in the head region.

Our research aims to determine the genes required for the correct development of the PVD dendrite in order to further understand the process of dendrite formation. An unbiased genetic screen was performed on *C. elegans* to induce mutations. Our experiments are focused on a strain containing the mutation *dz224*, which display claw shaped PVD dendrites and a decreased number of quaternary branches. In addition, animals containing *dz224* often present migration defects of the neuron PQR, which are typically located in the tail.

Through single nucleotide polymorphism (SNP) mapping and deficiency mapping we previously narrowed down the region of candidates to a 0.5 Mbp region on chromosome IV. To further refine the mutation, we introduced sections of genomes contained in fosmids to attempt rescuing the defective phenotype. We created transgenic animals harboring overlapping pools of 10 sections that collectively covered the entire genomic region of candidates. We then observed the PVD dendrite morphology of transgenic animals and compared them to mutant *dz224* and wild-type dendritic structure to look for rescue of PVD dendrite morphology. Furthermore, we counted PQR tail neurons to determine if the fosmid rescued the mutation. Due to the prevalence of neuronal defects, we formed a cross that allowed us to visualize FLP in animals with the *dz224* mutation.

Imaging the PVD of the transgenic animals showed that two of the fosmids rescue the mutant phenotype. Also, there was a statistically significant rescue of PQR neuron migration defects between the transgenic and non-transgenic animals containing the two fosmids. Additionally, we found the FLP phenotype is altered in *dz224* mutants. In the future, we will inject the two fosmids separately to further refine the region where the mutation is located. After, we will test individual genes in the area for rescue. Once the gene is determined, where and when this gene is required in the animals for proper development of the PVD neuron will require further research.

Acknowledgements:
Many thanks to members of the Buelow lab (Leo Tang, Nelson Ramirez, Meera Trivedi, Kristian Saied, Christopher Salazar, Garrett Lee, and Maisha Rahman) for their assistance. This work is supported by the Croucher Foundation (LT), the NIH (HB), the Irma T. Hirschl/Weill-Caulier Trust (HB), and the Albert Einstein Summer Undergraduate Program.

Manganese-Induced Neurotoxicity: *C. Elegans* as a model to study Parkinsonism and other RAGE-Related Neurodegenerative Pathologies

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Parkinson's disease is the second most common neurological disease behind Alzheimer's, affecting close to 10 million people worldwide. Characterized by tremors, rigidity, slowness of movement, and impaired balance, it is linked with oxidative stress and inflammatory responses that ultimately results in neuronal cell death. Advanced glycation end products (AGE) are non-enzymatically glycated proteins whose accumulation in the body, either by consumption or endogenous formation through Maillard's reaction, is linked to oxidative stress and inflammation. RAGE, the multi-ligand AGE receptors, are considered a major mediator in AGE pathogenicity, and thus RAGE expression can be targeted as both an indicator and a therapeutic tool in understanding AGE-related diseases. The vast majority of Parkinson's cases are idiopathic, originating from a combination of non-genetic environmental factors. Manganese (Mn) is an essential heavy metal whose nutritional and metabolic function is paralleled by it role as a neurotoxicant upon chronic exposure via occupational, environmental or nutritional sources. This project investigates the impact of Mn exposure on neurodegeneration, RAGE activation, and the onset and exacerbation of RAGE-related behavioral impairments and pathologies. We hypothesize that due to the respective contributions of Mn and RAGE on oxidative stress and inflammation-related neuronal death, the combination of RAGE expression and Mn exposure will exacerbate toxic effects in *Caennohabditis elegans*.

Using a newly created transgenic RAGE-expressing *C. Elegans* strain, we conducted lethality assays to determine the relevant dose exposure for RAGE compared to N2 worms. Worms were treated at the L1 stage with eight concentrations of Mn solution ranging from 0-100mM and plated in triplicate on NGM OP50 seeded plates with 30-50 nematodes per plate. After 48 hours of exposure, the survival rate of worms was calculated and a lethality curve was generated through Prism. The LD_{50} established for the N2 worms was 52.46 mM. However, due to key developmental and functional differences in the RAGE worm, current exposure protocol must be modified for RAGE to ensure that worms are synchronously exposed at the desired stage of L1. Modifications should include picking off incidentally plated eggs with a sterilized platinum pick or plating worms on FUDR plates which will prevent eggs from hatching. Future studies should investigate the expression of known molecules in the Mn-induced oxidative stress pathway in the presence of RAGE activation, exposing RAGE worms at different development stages like L4 and adult, and performing neurotransmitter-specific behavioral assays, targeting dopamine, serotonin, acetylcholine, glutamate, and GABA. Overall, the novel RAGE-expressing *C. Elegans* model can be instrumental in understanding the impact of Mn exposure on RAGE expression with the goal of developing effective therapeutic strategies for Parkinson's disease and other neuropathies.

Supported by NIH ES R01ES07331; Caenorhabditis Genetic Center (CGC, MN,USA); Albert Einstein College of Medicine Student Undergraduate Research Program.

I want to thank Dr. Michael Aschner for the incredible opportunity to work on this project and his support and guidance. A special thank you to Dr. Adi Pinkas for his constant mentorship and inspiring love of teaching, and to Omamuyovwi Ijomone and Mahfuzur Miah for their enthusiastic and unwavering support, guidance and friendship. Also, thank you to the entire Aschner lab and Einstein community for their passionate commitment to science and for creating a wonderful work environment, and to the Student Undergraduate Research Program for providing so many students with such a valuable research experience.

Cell Competition: Organ Size, Lifespan and Tumor Suppression

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Abstract

Organ size and cell homeostasis can be regulated through different mechanisms. It is thought that organ size and homeostasis is regulated through a process called cell competition. It is the phenomenon in which normal WT cells recognize mutant cells, thus signaling a pathway for the destruction of these cells. Cell competition in the Drosophila melanogaster is regulated through the process of apoptosis – programmed cell death. In the Drosophila, cells with mutations in ribosomal protein genes (e.g. *RpS18* +/-) are eliminated by this competitive apoptosis. It has been previously hypothesized that cell competition suppresses the variation in wing size, based on studies in which all cell death was blocked not just competitive cell death. The Baker Lab has however isolated the missense $RpS12^{G97D}$ mutation in the RpS12 gene thought to have a role in labeling mutant cells and signaling cell competition. This was used to test the previous hypothesis. Because cell competition removes abnormal cells, it might play a role in tumor suppression. The Baker lab has shown that this $RpS12^{G97D}$ decreases Drosophila lifespan from >80 days to <80 days – which could be due to tumor development. Understanding of the mechanisms involved in cell competition, it's promotion and inhibition, will further the discovery of pathways to assist in the promotion of healthy organ development and suppression of mutant cell overgrowth, ultimately resulting in improved human aging and/or expansion of human health span.

Keywords: Drosophila melanogaster, cell competition, apoptosis.

In previous studies, all cell death was blocked including that which may have been caused by environmental factors (UV rays, background radiation, spontaneous DNA damage etc.) Thus, previous effects on wing size variation may have been due to factors outside of cell competition. It is hypothesized then, if we isolate the $RpS12^{G97D}$ mutation that blocks cell competition and compare the wing asymmetry as well as wing size variation to normal WT cells, there should be significant variation.

Upon isolation of the $RpS12^{G97D}$ missense mutation and W1118 (WT) genotyped flies, their wings were dissected, mounted and photographed through a light microscope. The photographs were analyzed using *Photoshop* and *Image J*. Considering the Fluctuating Asymmetry Index, FAI and Ttests conducted between genotypes, it was found that there was no significant variation in wing size and asymmetry of the genotypes in either males or females. Thus, it is concluded that cell competition does not generally reduce the fluctuating asymmetry and size variation. However, it was discovered that in $RpS12^{G97D}$ mutant female flies, there is more variability in size, although not significant, and difference in maximal lifespan. Thus, we need to investigate other mechanisms (e.g. reproductive mechanisms, mating behaviors etc.) in male and female which may account for notable differences in lifespan and size variation.

Acknowledgements

The authors would like to acknowledge the Diversity Student Summer Research Program (DSSROP) for this opportunity. Research conducted in the Baker Lab was financially supported by the National Institute of Health (NIH).

Neural Correlates of Multisensory Speech Integration in Adults With Autism

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Autism spectrum disorder (ASD) is a neurodevelopmental disorder that is characterized by difficulties in the development of social skills, both verbal and non-verbal communication as well as repetitive behaviors. Multisensory integration (MSI), the of information from different sensory inputs, for example audio and visual-articulatory speech information, is known to greatly impact perception. For example, sensory cues such as gestures, speech in noise, and visual articulation contribute to MSI enhancement. However, individuals with ASD are not efficient in processing those sensory cues suggesting that impaired MSI may well contribute to the symptoms of ASD. Indeed, it has been shown that individuals with ASD show behavioral deficits in basic audiovisual MSI, and children with ASD also show deficits in multisensory speech perception. Both basic and speech MSI deficits in autism are thought to recover in adulthood, but underlying neural processing differences may remain.

This study aimed to identify the brain network involved in audiovisual speech integration in high functioning adults with ASD and compare to age and gender matched neurotypical adults.

19 adults with ASD and 22 healthy adults took part in the study. Participants were asked to watch and listen to a video recording of a woman telling the story of “The Lorax” by Dr. Seuss inside a 3 Tesla MRI scanner. The task had four different conditions: audio (A), visual (V), audiovisual synchronous (AV), and audiovisual asynchronous (AVa). AFNI and FSL were used to analyze the MRI data. T-tests were performed to examine functional activation for each condition and to compare AV and AVa conditions within the ASD and healthy control groups.

Results indicate that adults with ASD do show activation patterns similar to healthy adults for auditory, visual and audiovisual speech conditions. Although both groups showed differences between AV and AVa conditions, the ASD group seem to show greater differences.

Future directions will assess differences between groups. The results from this study may provide valuable information for our understanding of how individuals with ASD process AV speech, and even inform on remediation strategies.

Key words: fMRI, multisensory integration, autism, audiovisual, speech

This work is supported by Albert Einstein College of Medicine, Einstein Summer Undergraduate Research Program, and Sheryl and Daniel R. Tishman Cognitive Neurophysiology Laboratory, and the Gantcher and Rubenstein Foundations.

The Effect of an Axl-Activating Antibody Treatment on Inflammation in Spinal Cord and Corpus Callosum during Experimental Autoimmune Encephalomyelitis

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Multiple Sclerosis (MS) is an autoimmune neurodegenerative disease that causes demyelination, inflammation, and lesions in the central nervous system. The Tyro3, Axl and Mer tyrosine kinase (TAM) family of tyrosine kinase receptors and their ligands are all known to regulate the innate immune response. More specifically, the Axl/Gas6 signaling pathway is known to be neuroprotective and important for cell survival and remyelination. Previous studies show that during experimental autoimmune encephalomyelitis (EAE), growth arrest-specific 6 null ($Gas6^{-/-}$) mice have elevated clinical scores, worse recovery, increased expression of pro-inflammatory cytokines, and greater influx of macrophages into the spinal cord. Gas6 is the sole ligand for Axl, and has a very short half-life. As a result of the protective role of Gas6 in the CNS we hypothesized that treatment of wildtype (WT) C57Bl6/J mice with an Axl-activating antibody (Ab) would be protective during MOG-induced EAE. WT mice were treated with an Axl-inducing antibody, isotype specific-IgG or PBS 8 days post MOG-sensitization and the course of EAE was monitored over time. Mice treated with the Axl-activating antibody had reduced clinical scores relative to mice treated with IgG or PBS. qRT-PCR analysis of spinal cords of mice 20 days post MOG-sensitization (chronic EAE) showed no significant differences in mRNA expression between groups. Sandwich ELISA analysis of corpus callosum of the same mice showed a significant increase in total IFNγ and TNFα protein levels in Axl-treated mice relative to the IgG control mice. These results were opposed to our expectations that inflammatory cytokines would be decreased in Axl-treated mice with lower clinical scores. This suggests that IFNγ and TNFα may have protective role in the CNS during EAE that requires Axl activation. Immunohistochemical (IHC) analysis of spinal cord sections of mice during acute EAE showed a significant decrease in axonal swelling and a trend towards an increase in Tregs in Axl-treated mice. Future studies will include analyzing the protein concentration of spinal cord samples by ELISAs and comparing the observed mRNA expression to that of naïve WT mice.

Validation of expression quantitative trait loci in childhood obesity-related asthma

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Asthma and obesity are the two most common diseases among children in the United States. The prevalence rates of asthma and obesity are higher in African-Americans and Hispanics populations than that of non-Hispanic White population. Compared to normal weight children with asthma, obese children with asthma have a higher incidence and severity of their asthma, partly due to decreased responsiveness to inhaled steroids. To reveal the genetic contributions to the difference, we performed expression quantitative trait loci (eQTLs) analysis on the asthmatic children with normal weight or obesity recruited from the Bronx residents. We have identified several eQTLs which associated with the *ORMDL3* gene expression status. *ORMDL3* (ORMDL Sphingolipid Biosynthesis Regulator 3) is a gene which encodes an asthma associated locus that may promote pathological airway remodeling in patients with asthma. The goal is to confirm the genetic variants we identified using microarrays in the eQTL analysis. We designed primers to amplify a region which contain *ORMDL3* variant rs11078927 using polymerase chain reaction (PCR). DNA samples were obtained from CD4 T-cells of the six patients. The amplification was performed with Q5 High-Fidelity DNA Polymerase PCR kit. The PCR products were purified with the column purification kit then submitted for sequencing. We were able to amplify the region with the variant (257 bp) (Figure 1). Our genome-wide assay results showed among six patients in this study, three are major allele carriers and other three are minor allele carriers. The direct sequencing on the PCR product confirmed the predicted genetic variants in these individuals and validate the genome-wide results. We successfully validated an *ORMDL3* variant associated with gene expression in obese asthmatic T helper cells. Samples 28A33, 36A43, and 38A45 were homozygous C/C, as expected from genome wide studies. Samples 68B24, 72B28, and 98B44 were expected to be homozygous T/T, but only sample 72B28 showed the expected result. Both samples 68B24 and 98B44 showed in their chromatograms that they were heterozygous for C/T at the locus.

Acknowledgements: I would like to thank everyone the Greally Lab for cooperation, support and guidance throughout my research.

Isoform-Specific Roles for PI3K in Haptotaxis

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Phosphoinositide 3-kinases (PI3Ks) are lipid kinases that facilitate cell growth, survival, and motility. Activating mutations within the PI3K signaling pathway are linked to oncogenesis and spread of malignant cells. Research into the normal and oncogenic functions of these pathways has identified eight PI3Ks, which perform distinct functions in the cell. Amongst these are the Class IA PI3K isoforms, which are composed of a p85 regulatory subunit paired with one of three catalytic p110 subunits (p110α, p110β, p110δ), and the Class IB PI3K isoforms, which are composed of a p110γ and either a p101 or a p87 regulatory subunit.

Cell growth and motility is also regulated by integrins. These transmembrane proteins mediate specific binding to extracellular matrix (ECM) proteins. Engagement of integrins with their ligands supports cell adhesion, tissue repair, and provides context for outside-in inputs from signaling receptors, such as receptor tyrosine kinases (RTKS) or G-protein-coupled receptors (GPCRs). PI3Ks are activated by both integrins and signaling receptors, and loss of PI3K activity can disrupt integrin signaling pathways and alter cell behavior. One such behavior that requires integrin signaling is haptotaxis.

Haptotaxis is defined as the directional motility of cells along a gradient of adhesion sites. Our lab recently documented that haptotaxis of MDA-MB-231 breast cancer cells was selectively disrupted by inhibition of p110β. However, MDA-MB-231 cells express an activated mutant of KRAS, a member of the Ras GTPase family, which could affect this phenotype. The Ras family of proteins have also been identified as upstream effectors of PI3K signaling, and mutation may disrupt the function of other PI3K isoforms. The goal of this study was to investigate the contribution of the PI3K isoforms in mediating haptotaxis of cells expressing wild type KRAS.

Haptotaxis of NIH 3T3 fibroblasts, a cell line expressing WT KRAS, was measured using a transwell cell migration assay with a fibronectin gradient. Cells were starved overnight and then incubated with inhibitors that selectively target each of the Class I p110 isoforms. The migrated cells were imaged and counted by fluorescence microscopy (20x) after DAPI staining.

Our results were consistent with prior findings that the PI3Kβ isoform is involved in integrin signal mediation, as inhibitors of p110β blocked haptotaxis. Inhibitors of p110α and p110δ had no significant effect on NIH 3T3 haptotaxis. The p110γ inhibitors showed a trend toward inhibition of NIH 3T3 haptotaxis, though further investigation is needed to validate this finding. CRISPR knockouts of p110γ will provide definitive insight into the contribution of p110γ to haptotaxis.

Our data supports the involvement of PI3Kβ in integrin signaling. Further studies will test whether mutation of KRAS affects this process.

Acknowledgments: This research is supported by the NIH GM112524 (JMB and ARB) and the Albert Einstein College of Medicine Student Undergraduate Research Program. Special thank you to the entire Backer and Bresnick labs for their support and guidance on this project.

Determining the Role of the SPCA1 Ca^{2+} pump on Rubella Virus Entry

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Rubella virus (RuV) is an enveloped positive sense single stranded RNA virus in the genus *Rubivirus*, related to the *Alphavirus* genus. Similar to alphaviruses, RuV enters host cells via receptor mediated endocytosis and low pH-triggered membrane fusion, induced by conformational changes in the E1 virus fusion protein. RuV differs from the alphaviruses by lacking a furin-dependent glycoprotein maturation step and by budding from the Golgi, rather than the plasma membrane. Uniquely, the RuV E1 protein contains a bound calcium ion that is required for virus-membrane fusion and infection.

SPCA1 is an ATP-dependent calcium transporter located in the trans-Golgi network and endosomal compartment. It has been identified as a potential target for antiviral therapies against viruses that require furin cleavage of viral glycoproteins for viral maturation, due to furin's calcium requirement. SPCA1 is safe to target for inhibition as it is not required for cell survival. RuV requires calcium for entry and potentially for other steps of the viral life cycle, however it is not known to be dependent on furin cleavage, making the impact of inhibiting this pump during Rubella infection unknown. This study aims to identify if SPCA1 Ca^{2+} transporter could serve as an antiviral target against Rubella infections.

SPCA1 knock-out (KO) cells lines were created by the Rice lab at Rockefeller University by using CRISPR genome editing in HAP1 cells. A reconstituted cell line was generated using an isoform that reestablished calcium transport. To search for defects in viral entry, an infectious center assay compared wild type, KO, and reconstituted cells during primary infection with RuV.

Based on the relative infection seen in the SPCA1 knock out and the reconstituted cell lines, there may be a defect in entry for RuV. Additional testing is required to be certain that there is a significant defect in the knock-out cell lines. Since the reconstituted cell lines do not show a complete rescue that brings infection levels back to wild-type, it is difficult to determine if the decrease is due to loss of the SPCA1 Ca^{2+} pump, or caused by other factors not directly related to the pump. Assays focused on measuring viral growth will determine if there is a defect in exit. To further understand the role of SPCA1 during the viral lifecycle, additional experiments to locate where calcium brought in by the pump interacts with viral components can be done. By using calcimycin - an ionophore driving cellular entry of calcium - in KO cells during different time points during infection, it might be possible to specify when calcium is needed for successful RuV infection.

Special thanks to the Rice lab at Rockefeller University for developing and providing the HAP1 cell lines. This work was supported by the Albert Einstein College of Medicine Summer Undergraduate Research Program and University of Arizona NIH MARC Training Grant T34 GM008718.

ABSTRACT

Title- Different Modes of Regulation in AGRP mRNA Isoforms
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Obesity is considered to be a worldwide epidemic, having more than doubled since 1980, according to the World Health Organization. Nationally, nearly 38% of adults are obese. The fundamental cause of obesity is an energy imbalance between calories consumed and calories expended. Recent advances have identified neural circuits that control energy balance including food intake. One critical component is the appetite inducing Agouti related protein (AGRP) neurotransmitter. AGRP neurons located in the hypothalamus produce neuropeptide specific Agouti related proteins. These peptides stimulate feeding by inhibiting the activities of the central melanocortin receptors (MC3-R and MC4-R). Agouti related protein is encoded by the AGRP gene. Overexpression of the AGRP gene is known to cause obesity. Many in vivo experiments have shown that AGRP is upregulated by fasting or negative energy balance. However, there are few studies on regulation of AGRP transcription. In mice the AGRP gene is located on chromosome 8 and consists of 3 transcripts (AGRP-A, AGRP-B, & AGRP-C). Each transcript shares the same three coding exons but differ in their 5' untranslated regions. Based on the information about the AGRP transcripts from public databases, we undertook experiments to determine the expression of these transcripts in the mouse hypothalamus. Additionally, we sought to determine whether the three AGRP transcripts are differentially regulated by energy balance. We hypothesized that since each transcript contains different regulatory regions then they would be potentially regulated in different ways. A total of 10 mice were used in this experiment (5 male and 5 female); 6 (3 male and 3 female) were used for the experimental group, and 4 (2 male and 2 female) were the control group. The experimental group was fasted overnight while the control were free feeding. The hypothalamus was dissected, and homogenized in trizol to extract RNA. The RNA samples were used to synthesize cDNA. Lastly, we used real time PCR (qPCR) to quantify the three AGRP RNA isoforms. Amplification for each isoform was done with an isoform specific primer paired with a coding sequence primer. AGRP isoform A and AGRP isoform B was found in significantly greater amounts in fasting mice than in fed mice (approximately 2-fold increase, $p<0.5$). AGRP isoform C was found approximately in equal amounts in the fasting and fed groups. Our results indicate that the AGRP gene has 3 mRNA isoforms in the mouse hypothalamus. Additionally, two of the three tested AGRP RNA isoforms, AGRP A, and AGRP B are nutritionally regulated. This indicates that AGRP A and AGRP B are likely to be the isoforms that reflect the published reports of nutritional regulation of the AGRP gene. The differential regulation of the three AGRP isoforms strongly suggest that the regulatory elements for the three isoforms share similarities (hypothalamic expression) but differ in responses to energy balance. I would like to give a special thank you to my mentor Dr. Chua, Jr., Shun-Mei Liu, and Albert Einstein College of Medicine Summer Undergraduate Research Program for providing this wonderful opportunity. I would also like to thank NIH, BUILD, and the ASCEND Center for Biomedical Research at Morgan State University.

Immunological Correlates of Protection from Clinical Malaria are not Attributable to Sickle Cell Status

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Malaria poses a major global health burden with an estimated 216 million cases in 2016 (WHO). As the efficacy of drug-based therapy lessens, development of a robust vaccine is critical and relies on determining the immunological mechanisms associated with protection. All arms of the immune system are necessary for protection against malaria. Sickle cell trait, a polymorphism in the β-subunit of the haemoglobin gene, also grants protection to those heterozygous for the allele. In order to specifically study immunological correlates of protection, an 18-month longitudinal study was conducted in Malawi comparing the immune responses of patients with low frequency of malaria episodes to those with high frequency of episodes. Herein, the lab identified an expansion of $CXCR3^{lo}PD\text{-}1^{+}CXCR5^{+}$ memory $CD4^{+}$ T ($CXCR3^{lo}$ T_m) cells, and a heightened functional capacity of antibodies in the protected cohort relative to the susceptible through time-of-flight-cytometry and antibody dependent opsonization assays, respectively. To determine if sickle cell status contributed to protection, we determined the frequency of sickle cell allele (HbS) in our cohort. Owing to the loss of a *Mnl1* enzyme cut site in the HbS allele, we screened patients by restriction fragment length polymorphism on a PCR amplified haemoglobin region from their DNA. Of 69 patients analyzed, 2 were found to be HbS heterozygotes. After data re-analysis, we show that previously defined immunological correlates of protection are not explainable by sickle cell status. This result further validates the immunological mediators of protection identified, and will therefore aid the development of a new and effective vaccine.

Using a Computational Model of Co-transcriptional Splicing to Understand Alternative Splicing Outcomes

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Co-transcriptional, alternative splicing allows for multiple proteins to be coded from a single gene, greatly increasing the diversity of the proteome. Regulation of alternative splicing plays a crucial role in many biological processes and is often dysregulated in cancers. Cassette exon splicing, a specific type of alternative splicing, leads to the complete inclusion or exclusion of a specific exon. Alternative splicing outcomes are affected by multiple variables. Splicing rates play a leading role in determining the likelihood that a cassette exon will be included or excluded. However, RNA Pol II elongation rates have also been recently shown to affect alternative splicing outcomes. Under different elongation rates the transcribing polymerase will spend different amounts of time inside the window of opportunity, the region between the first and second 3' splice sites of the alternative event. It is during this time that an inclusion event is given a "head start" that can affect the overall proportion of transcript that will contain the cassette exon. If the window of opportunity is longer the alternative exon is more likely to be included, at a faster elongation rate the alternative exon is more likely to be excluded. Because splicing is co-transcriptional, at faster splicing rates the alternative exon is more likely to be included.

Our lab has developed an assay called SKaTER-seq (Splicing Kinetics and Transcript Elongation Rates through sequencing) which uses a computational pipeline to analyze nascent RNA-seq data and determine splicing and elongation rates genome-wide in mammalian cells. At the heart of the analysis pipeline is state model of transcription and co-transcriptional splicing which can simulate splicing outcomes under a set of defined rates. We used the SKaTER simulator to calculate the Percent Spliced In (PSI) value for a specific gene given splicing rates, elongation rate, initiation rates, termination rate, as well as the structure of the gene. Using this pipeline, we have explored the parameters that determine if an alternative splicing event will be regulated by elongation. These studies will allow us to predict the conditions that render an alternative splicing event elongation regulatable. The dysregulation of elongation-regulated splicing in cancers highlights the importance of these mechanistic studies for human health.

SURP Abstract

Design and Synthesis of a Transition-State Analog Inhibitor of Human Phenylethanolamine-*N*-Methyltransferase (hPNMT)

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Phenylethanolamine *N*-methyltransferase (PNMT) catalyzes the *S*-adenosyl-L-methionine (SAM)-dependent conversion of norepinephrine to epinephrine. PNMT is responsible for the regulation of autonomic functions such as blood pressure and respiration, as well as the secretion of hormones from the pituitary. PNMT is suggested to play an important role in hypertension, myocardial infraction, Alzheimer's and Parkinson's disease. To combat these problems, we are working towards synthesizing a transition state (TS) analog inhibitor that aims to inhibit the activity of PNMT and consequently limit the catalysis of norepinephrine to epinephrine. TS analogs can be used as inhibitors in enzyme-catalyzed reactions by binding the active site of an enzyme tighter than its natural substrate and thus restricting catalysis. Compounds that are structurally similar to the natural substrates of PNMT, such as SAM and norepinephrine, may act as inhibitors. None of the inhibitors that have been developed for hPNMT are TS analogs. We recently solved the transition state structure of PNMT and elected to synthesize an inhibitor that benefits from structural and electronic similarities to the transition state. Our inhibitor design also incorporates groups designed to enhance potency from electron withdrawing groups, specifically the chlorination of the aromatic ring. Inhibitor synthesis proposes three main building blocks: fragment A is derived from aspartic acid, fragment B is derived from 7,8-dichloroisoquinoline, and fragment C is derived from adenosine. This study focuses on the synthesis of fragment B. We are moving towards completion of this fragment. Following the coupling of fragment B to fragments A and C, the inhibitor will be tested against hPNMT to determine its potential as an inhibitor. If effective, the inhibitor-target interactions will be established by x-ray crystallography, optimized, and tested in biological models of the target disorders.

ZnFX Oncogene's C-terminus Regulates Cell Proliferation and Genomic Binding Dynamics in Breast Cancer Cells

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ZnFX (Zinc Finger Protein) is an oncogenic transcription factor that induces Epithelial Mesenchymal Transition, increased invasive properties and resistance to chemotherapy. ZnFX is known to repress or activate genes involved in survival pathway and cell proliferation. We aimed to investigate the effect of ZnFX's C-terminus on cell proliferation and genomic DNA binding. ZnFX was knocked-out in MDA-MB231 (breast cancer cells) cells to yield Cl73 cells (Cl73-ΔZnFX). The Cl73-ΔZnFX cells were stably transfected with ZnFX WT, ZnFX Δ1014-1048 or ZnFX Δ747-1048 isoforms. We found that knocking-out ZnFX reduces proliferation of MDA-MB231 cells. Re-expression of ZnFX WT or ZnFX Δ1014-1048 enhances proliferation of Cl73-ΔZnFX cells, while ZnFX Δ747-1048 did not have an impact on Cl73 proliferation. Thus, we deduced that the absence of ZnFX or the 301 amino-acid truncation in its C-terminus decreased proliferation of MDA-MB231. This suggests that the ZnFX's c-terminus may positively regulate cell proliferation. To better determine how truncation of ZnFX affects its genomic binding dynamics, we performed Single Particle Tracking Microscopy. We found that the ZnFX Δ747-1048 isoform resides on chromatin for significantly shorter time periods relative to ZnFX WT and the ZnFX Δ1014-1048 isoform. We thus deduced that the 301 amino-acid (aa) sequence (possessing the transcriptional repressor domain) lacking in the Δ747-1048 isoform is crucial for genomic binding. These preliminary data suggest that deregulated ZnFX's DNA-binding behavior, driven by its 301 aa C-terminus sequence, is paired with defect in stimulating cell proliferation.

Acknowledgements

Thanks to my mentors Professor Pascale Cohen and Dr. Robert Coleman for the wealth of knowledge transmitted to me, and for training me amazingly well. Special thanks to Professor Cohen for the patience and guidance. Also, thanks to Charles Kenworthy for his kindness and help. Thank you to the Fulbright program (PC) and SURP at Einstein for allowing it all to happen.

Isoforms-specific effects of host factor INI1/SMARCB1 on the Production of HIV-1

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To tackle the threat posed by the HIV epidemic researchers developed antiviral drugs capable of blocking the catalytic site of integrase. However, the discovery of new interactions between integrase (IN) and cellular proteins has made developing allosteric inhibitors that target IN-host factor interactions a possibility (Malliot et al., 2013). INI1, a protein found in the SWI/SNF chromatin remodeling complex, is one of the host factors that directly binds to IN and affects multiple stages of HIV-1 replication (Malliot el., 2013, and LaPorte et al., 2016). INI1 exists in the cell in two isoforms, a and b, whose key difference is a nine amino acid deletion in INI1b caused by alternative splicing (Pyeaon, Price, and Part, 2015). Both of these isoforms harbor the IN-binding conserved Rpt1 domain. While INI1 has been shown to be important in HIV-1's lifecycle, most studies have been done using INI1a isoform. If INI1's interaction with integrase becomes a drug target, then an understanding of all isoforms of INI1 will be essential.

The goal of this project is to determine the effects of over-expression of the two isoforms of INI1 on the production of HIV-1 particles. To accomplish this task, calcium phosphate coprecipitation was used to transfect viral vectors carrying a GFP reporter into 293T cells that would cause the production of psuedotyped HIV-1. Additionally, the plasmids expressing INI1a, INI1b, or a control were co-trasnfected to determine their effects on virus production. Western Blotting and fluorescence imaging was used to confirm successful transfection while a p24 AlphaLisa assay was used to monitor viral protein levels and virus production.

Over-expression of both INI1a and INI1b lead to an increase in the expression of transfected proteins as compared to empty vector control. However, the GFP expression was stronger in cells transfected with INI1a when compared to INI1b. Expression of INI1a lead to an increase in both intracellular as well as particle associated p24 as compared to the empty vector control. Expression of INI1b also increased the intracellular and virion associated p24, but to a lesser extent (~ a log less) compared to that of INI1a. This is the first time we have found differential effects of INI1a and INI1b on particle production. Future experiments to test the effects of INI1 isoforms in cells lacking endogenous INI1a and INI1b will provide additional information on their effects on virus production. Furthermore, work to purify the two isoforms are in progress, which will allow the biochemical characterization of the two proteins. Understanding of isoform-specific functions of INI1 will shed light on the mechanism of INI1-mediated effects on HIV-1 replication, which will be important for future drug development.

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Acknowledgments: This research is supported by the Albert Einstein College of Medicine Student Undergraduate Research Program and NIH grant R01 GM11252 to GVK.

Analysis of Type I IFN Transcriptional Response in the Bone Marrow of Mice undergoing Severe Malaria Using Real-Time Quantitative Reverse Transcription PCR

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Every year, over one million people die from Malaria, affecting mostly children. Malaria is caused by the protozoan parasite, *Plasmodium* with *P. falciparum* being the most common cause of severe malaria. A better understanding of the immune response to this disease at the cellular and molecular level remains essential for rational vaccine design. A previous published study from our lab indicates that type I IFN plays an important role in immune cell activation and inflammatory response to this disease. This was demonstrated using the *Plasmodium yoelii* YM surrogate mouse model that mimics severe human malaria. Compared to wild type mice, mice lacking type I interferon receptor survived this lethal infection. Plasmacytoid dendritic cells (pDCs) were the major producers of type I IFN occurring in the bone marrow (BM) and blood of infected mice via TLR7/MyD88 sensing of the parasite. pDCs were primed by CD169+ macrophages (MPs) via STING mediated sensing. Interestingly, pDCs and CD169+ MPs physically interact in the BM, as visualized by intravital microscopy. The IFNa/b protein levels during Py YM infection were previously measured using a reporter mouse in which IFNb+ cells became YFP+ and by ELISA for IFNb/a. My goal during this internship was to develop a more sensitive method of IFNa/b detection than the one currently used by the lab. We hypothesized that Real-Time Quantitative Reverse Transcription PCR (Real-Time qRT-PCR) would allow us to detect when and which type I IFN transcript will first appear. Thus, I searched for published primers to monitor the different subtypes of type I IFN and precisely localized them on their corresponding mRNA using Snapgene. I next used poly I:C treated BM cells as my positive control of IFNa/b mRNA. As expected, a significant amount of type I IFN b/a was detected in the BM cells treated with 10ug poly I:C for two hours. I then conducted a kinetic of IFNb/a expression in BM cells from *Py YM* infected mice that I normalized to that of of uninfected mice. I found that type I IFN first appeared at 6 hours post infection. Thus, compared to the prior methods used in our lab, Real-Time qRT-PCR allowed us to detect an increase in type I IFN transcription in *Py YM* infected BM cells ~18 hours earlier than with the other approaches.

The Influence of Multitasking on Auditory Working Memory

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Multitasking is common in complex listening environments where attention must be switched rapidly between different auditory streams. Multitasking has been evaluated in numerous studies to examine its impact on task performance. However, limitations reported only provide direct evidence for the active task being performed, and not for unconscious processing of the unattended sounds. To better understand processing of both attended and unattended sounds, we had participants listen to an ambiguous auditory sound sequence that could be interpreted in two ways: as an integrated single stream of sounds or as segregated into two streams of sound. Participants were instructed to press buttons corresponding to patterns they detected (pattern task) for the integrated percept and to focus on only the low tones and respond by a button press when they heard a rarer, louder tone (intensity task) for the segregated percept. Participants were made explicitly aware of only one interpretation of the stimuli at a time and counterbalanced to the task they were exposed to first. We used continuous electroencephalogram (EEG) to measure brain activity in response to the stimuli, which we then converted to time and phase-locked event related potentials (ERPs). Conscious or unconscious processing of a rare sound or pattern (deviant) produces a deviance detection component of ERPs called the mismatch negativity (MMN). Additionally, a P3b target detection ERP was used to analyze responses when the participants actively paid attention to the task. Our results showed that participants were more confident in responding to the targets of the task they were exposed to first, which was indexed by an increased P3b amplitude. However, both interpretations of the task were processed even when only one was in the focus of attention, as shown by the presence of the MMN component. Additionally, there was an effect of task order on MMN and task performance. Exposure to the pattern task first resulted in decreased hit rate and increased MMN amplitude to the pattern deviant when they were performing the second task (intensity task). However, this effect was not observed for the intensity-first task group. There was no decrease in performance in the second task when they performed the pattern task second. This suggests that the group exposed to the pattern first had a preference towards interpreting the pattern task even when attending to the intensity task. Further analysis will need to be conducted to see if these are statistically significant effects and to evaluate how the influence of task order or multitasking is represented in the brain. Better understanding of the neural representation of multitasking allows for a deeper understanding of auditory processing disorders and other attention related disorders.

Acknowledgements: This research was supported by the NIH (Grant # DC004263) and the Albert Einstein College of Medicine Summer Undergraduate Research Program (SURP). Special thanks to Kelin Brace and all other members of the Sussman lab.

Effect of Chemical Inhibitors of Autophagy, Mitochondrial Fission, and Oxidative Stress on Proliferation of NDUFS4 Knockout and Wildtype Vascular Smooth Muscle Cells

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In response to vascular injury or disease, vascular smooth muscle cells (vSMCs) proliferate underneath the endothelium and obstruct vasculature. VSMC accumulation can lead to atherosclerotic plaque formation, resulting in heart attack or stroke. Phenotypic plasticity of vSMCs enables their migration and proliferation, contributing to atherosclerosis. The mechanisms that control vSMC phenotypic modulation are still not completely understood. A recent study shows that FAT1 limits growth of vSMCs by restraining mitochondrial complex I function and respiration and opposes vascular occlusion. To further study the role of complex I in vSMC proliferation, we generated vSMCs with loss of NDUFS4, a subunit of respiratory complex I. NDUFS4 knockout (KO) vSMCs show decreased complex I subunits, complex I and supercomplexes, reduced activity of complex I, and decreased aspartate and growth. How complex I contributes to vSMC proliferation is not fully elucidated. We found that NDUFS4 KO vSMCs have higher levels of PRKN and PINK1, markers of mitophagy, and decreased levels of MFN1 and OPA1, markers of mitochondrial fusion. *We hypothesize that complex I dysfunction increases ROS production, mitochondrial fragmentation, and mitophagy, leading to defective vSMC growth*. This study examines the effect of chemical inhibitors of oxidative stress (N-acetyl L-cysteine (NAC)), mitochondrial fragmentation (mdivi-1), and autophagy (3-Methyladenine (3-MA)) on vSMC growth, looking for suppression of the defective growth observed in NDUFS4 KO vSMCs. **Methods:** Mouse aortic vSMCs, KO and WT for NDUFS4, were treated with NAC, mdivi-1, 3-MA or vehicle. AlamarBlue was used to evaluate cell growth. **Results:** NAC has a dual effect depending on the dose. While low concentrations enhance growth of vSMCs, high concentrations induce cell population decline. Mdivi-1 selectively delays growth of NDUFS4 KO vSMCs at lower concentrations; however, this inhibitor lowers cell number of both genotypes at higher concentrations. 5 mM 3-MA enhances growth of both genotypes and suppresses the difference between WT and KO vSMCs. 10 mM 3-MA seems to have the same effect, but it requires further analysis. **Discussion**: The effect of low concentrations of NAC suggests that oxidative stress opposes growth of vSMCs, while decline in cell number with higher concentrations reflects either the requirement of physiological levels of ROS for cell proliferation or a NAC off-target effect. Our studies with mdivi-1 suggest that NDUFS4 KO vSMCs use mitochondrial fragmentation as a mechanism to maintain proliferation which makes them more susceptible to its inhibition, and that mitochondrial fragmentation is required for growth of vSMCs. The effect of 3-MA suggests that inhibition of autophagy suppresses the defective growth observed in NDUFS4 KO vSMCs and that in general autophagy limits vSMC growth. **Future Work:** Functional evaluation of oxidative stress, autophagy and mitochondrial dynamics in NDUFS4 KO and WT vSMCs in the presence or absence of inhibitors. Further evaluation of these regulatory mechanisms of vSMC phenotype may reveal novel targets to prevent or treat obstructive vascular diseases such as atherosclerosis.

Acknowledgements: Thank you to the members of the Sibinga laboratory, SURP at Albert Einstein College of Medicine, and NIH RO1HL133861.

The Association between Perceived Stress and Chronic Kidney Disease

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Background

Chronic Kidney Disease (CKD) affects approximately 26 million and is associated with increased morbidity and mortality. Patients with CKD are burdened with psychosocial factors, such as higher levels of stress, which may adversely impact their health outcomes. It is widely known that high stress can have negative implications for general health and wellness, but there is a paucity of studies that have characterized the potential effects of perceived stress on the progression of CKD. We examined the association between perceived stress and CKD progression in the Einstein Aging Study. *We hypothesized that higher perceived stress is associated with a higher prevalence of Chronic Kidney Disease.*

Methods

We conducted a retrospective cross-sectional and longitudinal analysis of the Einstein Aging Study (EAS). The EAS, established in 1980, is an ongoing prospective cohort study of elderly patients in Bronx, NY and focuses on the aging brain, specifically the effects of normal and pathological (i.e. dementia disorders) aging. Information on stress measured using a 14-point perceived stress scale (PSS) was available in 440 patients. Comparing the highest quintile of PSS to the lower quintiles and using two cutoffs (< 45 ml/min/ 1.73 m^2 or < 60 ml/min/1.73m2) of estimated glomerular filtration rates (eGFR) to define the primary outcome of CKD progression, we generated logistic and Cox regression models and adjusted for covariates of interest including age, sex, race, and education.

Results

Of the 440 patients included in our analysis, 85 patients fell in highest quintile on the PSS compared to 355 in quintiles 1-4. However, 18% of the participants in the highest quintile of the PSS did not CKD vs 32% in the highest quintile of PSS had an eGFR < 45 ml/min/ 1.73 m^2 at baseline (p= 0.035). In the unadjusted models, perceived stress was associated with increased odds of developing an eGFR's < 45 (OR x CI or P-value) and < 60 ml/min/ 1.73 m^2 (OR x CI or P-value); however, this association was not significant after adjusting for age, sex, and depression.

Conclusions

High levels of perceived stress is common among elderly patients with prevalent CKD. In our study, there was no association between perceived stress and progression of CKD. However, our sample size small with only 85 individuals within our top quintile of perceived stress. Larger studies are needed to better characterize the effect of PS on CKD.

Acknowledgements

A special thank you to the Einstein Aging Study for the use of their data and the Diversity Student Summer Research Opportunity Program for supporting my research endeavors this summer. I would also like to express my gratitude to Dr. Melamed for dedicating her time as a mentor during this summer and my lab partner Matthiew Chen for his aid and support in our data analysis work.

Population Coding and Spectrotemporal Tuning in the Owl's Optic Tectum

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Barn owls (*Tyto furcata*) are a model organism to study sound location and provide access to a neural population representing auditory space. They use interaural time differences (ITD) and interaural level differences (ILD) to localize sound. Neurons in the barn owl's optic tectum (OT), a part of the midbrain, which respond to combinations of these binaural cues, form a neural map of auditory space which supports sound-orienting behavior. Understanding population responses in the auditory system involves identifying the frequency components of acoustic signals which evoke spikes. Together, these frequency components and the time between their occurrence in the stimulus and a spike form the spectrotemporal receptive fields (STRFs) of auditory neurons. Using spike times to measure STRFs can elucidate if and how neurons along the sound localization pathway are uniquely tuned to spectrotemporal features. This question holds importance in light of a previous study which showed that neurons within the owl's map of auditory space are selectively tuned to frequencies carrying the most reliable spatial cues (Cazettes et al. 2014). Therefore, in order to achieve a more complete understanding of population responses in the OT, we investigated the neurons' STRFs.

To compute STRFs, we presented dichotic stimuli via headphones to anaesthetized barn owls while recording OT neuron responses with a microelectrode array. We analyzed the recorded spike trains in conjunction with the stimuli using custom written MATLAB scripts. Analysis of the neurons' STRFs required identification of the stimulus features which most frequently precede a spike. Therefore, we captured 20 ms of the stimulus waveform which precedes each spike and band-pass filtered these waveforms into 80 frequency channels. Next, we calculated the power over time within each frequency channel and averaged over all spikes to yield a STRF plot for the neuron. A baseline, produced analogously with randomized spike trains, was subtracted to account for global stimulus features triggering spikes. A peak in the STRF indicates high power within a specific frequency preceding the spikes at a given latency.

To test our method, we first computed STRFs in auditory nerve (AN) neurons, known to have narrow spectrotemporal tuning. STRFs of AN neurons indeed showed a single peak, indicating precise tuning to frequency, and confirmed that our MATLAB code was able to determine a neuron's STRF efficiently. The AN neurons had firing rates of 100-400 spikes/s and STRFs were calculated with 4,000-10,000 spikes. The same analysis of OT neurons showed that STRF peaks in the OT were not as precisely localized in a single spectrotemporal region as in AN. A reason for this difference could be that OT neurons have lower firing rates (20-50 spikes/s) than AN and STRFs were based on a smaller number of spikes (500-1,000 spikes). To verify whether more defined STRFs were achievable in OT, we selected cells showing reproducible responses across trials of identical stimulus (frozen noise). This method yielded more precise STRFs.

Our preliminary results indicate that neurons in the owl's OT are tuned to spectrotemporal features of sound. In addition, we identified response properties that generally lead to well-defined STRFs (low jitter to frozen noise, high number of spikes and/or high spike rates). Future directions in this area may include (a) screening more neurons in the OT with these properties in mind, (b) continuing to compare STRFs across neural populations and across varying stimulus parameters (ITD, ILD, frequency, …), and (c) investigating if OT neurons follow the previously shown relationship between spatial cues and frequency tuning (Cazettes et al. 2014).

Funding: Brain Initiative grant NS104911
References: Cazettes F, Fischer BJ, Peña JL (2014) Spatial cue reliability drives frequency tuning in the barn owl's midbrain. eLife 3:e04854

Glutamatergic projections from the basolateral amygdala to nucleus accumbens are necessary for cued-approach behavior regardless of auditory or visual modality

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Drug-associated stimuli are powerful because they can influence an addict's behavior even after periods of abstinence, contributing to relapse in recovering addicts. Plasticity generated by addictive drugs occurs in the ventral tegmental area and the nucleus accumbens (NAc), where neurons are excited by reward-predictive cues. These excitations in the NAc are dopamine-dependent and critical for driving approach to the reward in response to such cues. Cue-evoked excitations in the NAc are also dependent on the basolateral amygdala (BLA), and inhibition of the BLA through GABA A and B receptor agonists impairs auditory cue-response in animals who have already learned the task. However, it is unknown if inhibition of the BLA causes the same impaired cue-response for visual cues. In this study we investigated whether glutamatergic projections from the BLA to the NAc contribute to cued-approach behavior specifically when animals are presented with an auditory cue versus a visual cue. Rats were first bilaterally implanted with microinjection cannulae in the BLA and then trained on a cued approach task with two modalities (light versus sound). On test days, animals were bilaterally injected with either a GABA A and B receptor agonist (Muscimol/Baclofen cocktail) or a saline vehicle directly into the BLA. Animals injected with the Muscimol/Baclofen cocktail demonstrated impaired cued-approach behavior compared to animals injected with a saline vehicle, as previously observed. The effect was observed in cues of both modalities. Future work on this study should include the addition of animals to the study.

Acknowledgements: Thank you to the Nicola laboratory, specifically Mercedes Vega Villar and Cindy Reyes, for mentoring me on this project. Supported by R01DA019473, R01DA038412 to SMN and F31DA042619 to CMR.

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Development of *Plasmodium falciparum* Equilibrative Nucleoside Transporter 1 (PfENT1) Fusion Proteins to Increase Hydrophilic Surface Area for Protein Crystallization Trials

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Plasmodium falciparum causes the most severe form of malaria infection. The limited treatment options and the rapid spread of resistance to current first line drugs makes it essential to develop new antimalarial medicines. A potential target for novel antimalarial drugs is the main purine import transporter that *P. falciparum* utilizes, the *Plasmodium falciparum* Equilibrative Nucleoside Transporter (PfENT1). This protein is essential because malaria parasites are purine auxotrophic, meaning that they cannot perform *de novo* purine biosynthesis. They rely on PfENT1 to import purines from the host red blood cell. The purines are necessary for RNA and DNA synthesis, and for a variety of metabolic processes during parasite proliferation. The function of PfENT1 is well characterized, but the structure remains elusive/unknown. Our long term goal is to determine the structure of PfENT1 through protein crystallization. Preliminary attempts to crystalize PfENT1 were unsuccessful perhaps because it has very limited extra-membrane hydrophilic surface area. One strategy to crystalize integral membrane proteins has been to increase their hydrophilic surface area by forming fusion constructs with water-soluble proteins. We sought to generate fusion constructs that would add a protein tag, either maltose binding protein (MBP), glutathione-S-transferase (GST), or T4 lysozyme, to the C-terminus of PfENT1. We used PCR to create unique restriction sites on the ends of DNA fragments encoding PfENT1 and the protein tags. After restriction enzyme digestion and gel purification, we attempted to ligate the fragments into the pCM189 plasmid cut with the appropriate restriction enzymes using either a lab-made ligation kit or a Gibson Assembly kit before transformation into *E.coli*. Plasmid DNA from colonies was purified and sequenced to verify the correct alignment of protein and tag in the vector. Once this is achieved, further characterization experiments will take place to confirm that the placement of the tag did not disrupt the function of PfENT1. If the protein and tag are aligned without affecting PfENT1 function, then the PfENT1 fusion protein will be prepared for protein crystallization trials. Supported in part by NIH grant R01-AI116665 and the Einstein SURP Program.

Assessing the effect of AIF1 Gene on Microglia Activity and Synapses

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Microglia are known to function as specialized macrophages in the CNS that remove damaged cells and clear debris. Growing evidence indicates that microglia prune synaptic connections between neurons. The AIF1 (allograft inflammatory factor 1) gene promotes macrophage activation, however, its role in microglial function is poorly understood. The purpose of this study is to assess the contribution of AIF1 to microglial function during hippocampal neuronal development of mice. Our current working hypothesis is that removal of the AIF1 gene will reduce microglia's ability to prune neuronal connections, leading to complex morphology that may alter synaptic function.

To test our hypothesis, we used AIF1 knock out mice and measure microglia activity, neuronal morphology, and synaptic transmission. Using immunohistochemistry, the Iba1 marker revealed that microglia was least active at P15 in knock out mice compared to wild type littermates. At this time point, two photon imaging of CA1 pyramidal neurons demonstrated changes in morphology, specifically dendritic arborization and spine density. There was an unexpected reduction in spine density in the secondary branching. Sholl analysis was also used to examine dendritic arborization. To assess synaptic transmission, we used electrophysiology in acute hippocampal slices and monitored miniature excitatory postsynaptic currents (mEPSCs) from CA1 pyramidal neurons. We observed a slight reduction albeit non-significant in mEPSC frequency and amplitude.

Our results suggest that the absence or dysfunction of the AIF1 gene may reduce microglial activity. Due to the small sample size and lack of statistical significance, we cannot currently conclude that dendritic arborization, synaptic transmission, or spine density are affected by the absence of the AIF1 gene. More research is required in order to determine a role of AIF1 gene in synaptic transmission, dendritic arborization and spine density.

Supported by NIH R01s MH081935 and DA017392 to P.E.C. and the Office of Diversity Enhancement

Disruption of double strand break repair through selinexor combination therapy in high-risk neuroblastoma

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Neuroblastoma is the most common extracranial solid tumor occurring in childhood. Overexpression of XPO1, a protein responsible for the export of over 200 regulatory proteins, has been shown to be associated with poor survival in high-risk neuroblastoma patients. Selinexor, a Selective Inhibitor of Nuclear Export (SINE) drug that inhibits the activity of XPO1, has been shown to have synergistic, anti-tumor effects when treated in combination with cisplatin or doxorubicin, two commonly used chemotherapeutic agents. Our goal was to elucidate a mechanism through which selinexor functions in tandem with these DNA damage inducing agents. We hypothesized that selinexor enhances the effects of cisplatin and doxorubicin by increasing the nuclear localization of factors that disrupt DNA double strand break (DSB) repair.

We treated neuroblastoma cell lines SKNSH, IMR5, and NLF with vehicle control, selinexor, cisplatin or doxorubicin, and combination treatment. For the combination treatment, we treated cells with cisplatin or doxorubicin first, then introduced selinexor six hours after. We then collected whole-cell protein and quantified via western blotting.

Cisplatin and doxorubicin treatments increased the abundance of γH2AX in the SKNSH cell line, suggesting that they induced DSBs. Both treatments also increased the level of phosphorylated checkpoint kinase 1 (Chk1), the activated form of Chk1, in the NLF cell line. Chk1 is phosphorylated through a variety of different DNA damage repair (DDR) pathways, suggesting that the chemotherapeutics induced a DDR response. The control and selinexor treatments did not result in any detected γH2AX or phosphorylated Chk1. These results were expected, as only treatment groups involving cisplatin or doxorubicin induce DNA damage. However, in combination treatment, phosphorylated Chk1 levels were lower than in the cisplatin or doxorubicin treatments. Furthermore, when selinexor was treated in combination with cisplatin, levels of γH2AX and P53, an inhibitor of Chk1, were higher than in cisplatin alone. This result was not seen in the doxorubicin combination.

These findings suggest that cisplatin and doxorubicin treatments induce DSBs and activate Chk1-dependent DDR pathways. Selinexor increased γH2AX and p53 abundance and decreased Chk1 phosphorylation when treated in combination with cisplatin, suggesting a disruption of DSB repair. Because mammalian cells predominately use non-homologous end joining (NHEJ) to repair DSBs, future studies will aim to find which NHEJ proteins associated with Chk1 are also affected by selinexor. Selinexor treatment may disrupt other types of DDR when treated in combination with doxorubicin, and more research is needed on the subject. The preclinical data here support future clinical studies, and we anticipate developing a clinical trial using selinexor with cisplatin and doxorubicin in patients with relapsed and refractory neuroblastoma.

This study was supported by the Albert Einstein College of Medicine Summer Undergraduate Research Program. We also acknowledge the lab of Dr. David Loeb for resources and assistance.

Engineering IgG3C- Bispecific Antibodies Targeting Ebolaviruses

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The Ebolavirus genus includes five virus species, Zaire(EBOV), Bundibugyo(BDBV), Taï Forest(TAFV), Sudan(SUDV), and Reston(RESTV) ebolaviruses. All except RESTV cause fatal hemorrhagic fevers in humans. Currently, there are no Food and Drug Administration approved therapeutics against these viruses. The magnitude of the 2014-2016 West African outbreak and unpredictability of reemergence emphasizes the need for a broad spectrum therapeutic. Development of pan-Ebola therapeutics is challenging due to the antigenic diversity among the viral glycoproteins (GP) for which bispecific antibodies (bsAbs) targeting conserved epitopes could solve. Recently, antibodies targeting conserved epitopes on GP that exhibit broad neutralizing activity against multiple ebolaviruses have been isolated from survivors. Using these antibody sequences, we developed a bsAb with broad neutralizing activity. Development of bsAbs with an engineered 'open' IgG3 (IgG3C-) hinge sequence, which allows increased fab flexibility, previously showed an increased neutralizing ability. We hypothesize engineering our ebolavirus targeting bsAbs with this flexible hinge-region will confer an increased neutralizing ability. We developed a bsAb with the IgG3 hinge region and tested for neutralization activity using a recombinant vesicular stomatitis virus (rVSV) filovirus system. Our results show that the IgG3C- hinge did not affect the broad neutralizing activity of our bsAb.

The ATM Signaling Pathway Mediates Apoptosis in *sf3b1* Mutant Zebrafish

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Splicing factor 3b subunit 1(SF3B1) is a key component of the spliceosome, a large protein complex essential for regulating gene expression. Genomic mutations in this protein have been linked to various cancers and neurodegenerative diseases. Using a zebrafish *sf3b1* loss-of-function model, previous studies have demonstrated that homozygous mutants exhibit severe DNA damage and apoptosis, especially in neural tissue. DNA damage can trigger several signaling pathways via the activation of distinct kinases, such as Ataxia Telangiectasia Mutated (ATM) in response to double strand breaks, and ATM and RAD3-related (ATR) in response to single strand breaks or replication stress. The specific signaling pathway that triggers cell death in *sf3b1* mutants remains unknown. Levels of phosphorylated Checkpoint Kinase 2 (Chk2), a direct substrate of the activated ATM kinase, are elevated in *sf3b1* mutants compared to their wild type siblings, suggesting the ATM pathway is active. To determine if ATM signaling mediates the apoptotic phenotype in *sf3b1* mutants, we treated whole embryos at 6 hours post fertilization (hpf) with an ATM kinase inhibitor, KU60019, then assessed levels of apoptosis by immunostaining for the pro-apoptotic marker, active-Caspase 3 in 24 hpf embryos. Zebrafish *sf3b1* mutants treated with the ATM kinase inhibitor displayed less apoptosis than vehicle control treated mutants. This result indicates ATM acts as a key mediator of apoptosis in *sf3b1* mutants. Our results suggest that ATM inhibition could be a novel therapeutic for degenerative diseases with SF3B1 mutations.

I would like to thank Teresa V. Bowman and Sara Nik for their guidance, assistance, and vision for this project. I would also like to thank all the members of the Bowman lab for their constant support and advice. Additionally I would like to acknowledge the Summer Undergraduate Research Program at the Albert Einstein College of Medicine for providing me with this opportunity

Interaction of TDRD6 and Arginine Methylated Nucleoplasmin Tail

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Cells must manufacture proteins in order to survive. The Central Dogma of biology states that DNA must undergo transcription in order to produce RNA, which is then translated into protein. During the cleavage stage of early embryogenesis in *Xenopus*, however, the zygotic genome is transcriptionally silent, i.e. no new RNA is synthesized from the zygotic genome. Yet, the newly fertilized egg cells still undergo numerous rapid rounds of DNA replication and division in the complete absence of zygotic gene products. This period is of development is under "maternal control"—explicitly governed by maternal factors (RNA, protein, and metabolites) which were deposited into the egg during oogenesis. In other words, newly translated proteins are derived only from maternal mRNA during the cleavage stage. Maternal control of development lasts until zygotic genome activation (ZGA) which occurs after the 12th cleavage in *Xenopus*, where after development is transferred to zygotic control.

Xenopus egg cells contain maternal messenger ribonucleoproteins (mRNP) particles that function to store maternal mRNA in a translationally repressed state. After fertilization, the mRNP particles are somehow remodeled to activate translation of their stored maternal transcripts, but how this occurs remains an open question. Amongst a handful of proteins comprising maternal mRNPs is TDRD6 (Tudor domain containing 6). TDRD6 contains six extended Tudor domains (eTUD) followed by a long, intrinsically disordered C-terminal tail. The role of TDRD6 in mRNPs is not well understood. Various Tudor domain proteins have been shown to interact with binding partners containing arginine-methylated motifs that interact with the Tudor domain's "aromatic cage," exemplified by the X-ray crystal structure of TDRD1 (another TDRD protein family member) in complex with an arginine-methylated E2F transcription factor peptide.

During the cleavage stage, histones must be released and deposited onto the rapidly replicating DNA to establish embryonic chromatin. Nucleoplasmin (Npm2) is the predominant storage chaperone for histones H2A and H2B in *Xenopus laevis* eggs. It has a pentameric form and is composed of a core domain and an intrinsically disordered C-terminal tail. Npm2 contains three acidic patches that are post-translationally modified, including phosphorylation and glutamylation, to promote histone binding. Over 90% of Npm2 is arginine methylated near the end of its C-terminal tail, yet a function for this methyl mark has not yet been described.

Comparing the sequences of the arginine-methylated motifs of both Npm2 and E2F revealed that Npm2 shares 100% identity with the binding motif of E2F in the TDRD1 structure. Furthermore, TDRD6 presence is highest in the oocyte and egg during early embryogenesis, but significantly decreases after ZGA. Therefore, we hypothesize that after release of its histone cargo, the Npm2 Rme C-terminal tail can interact with maternal mRNPs by binding one or more of the TDRD6 Tudor domains to de-repress the translational block of maternal mRNPs during early embryogenesis. In this capacity, Npm2 would function as a developmental timer: release of histones from Npm2 marks the completion of S-phase and Npm2 binding TDRD6 signals protein expression and cell cleavage and progression to the next cell cycle. Our approach to test this hypothesis was to purify each individual TDRD6 Tudor domain and perform protein pulldown assays with the C-terminal 19 residues of the Npm2 tail with and without arginine methylation.

Results:

All 6 eTUDs (extended Tudor domains) expressed by E. coli bacteria were insoluble under non-denaturing conditions and could not be extracted by Nickel purification and stepwise dialysis. However, complete denaturation of all proteins using Urea and injection of the protein into NDSB folding buffer, followed by slow dialysis, produced a significant increase of soluble protein for eTUDs 2, 5, and 6, as shown by size exclusion chromatography. The proteins of the remaining domains proved too insoluble to be purified, thus far. A binding assay of Npm2-C19 with and without Arginine methylation pulled down eTUDs 2 and 5, while 6 was inconclusive. However, upon further examination by Western blot using a Strep II tag primary antibody (present on all 3 eTUDs tested) showed that Npm2-C19 with Arginine methylation pulled down eTUDs 2, 5, and 6, while Npm2-C19 without Arginine methylation did not pull down any eTUDs. While further experiments must be done, a connection is now established between the binding of the eTUDs and the methylated Arginine residue of the Npm2 tail.

This study was supported by the Albert Einstein College of Medicine Summer Undergraduate Research Program. Thank you to the entire Shechter Lab for the resources, mentorship, and assistance this summer, and specifically Benjamin Lorton for his constant support and guidance throughout this project.

Next Generation Luciferase Reporter Assays for Mtb Drug Susceptibilities

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Tuberculosis is an infectious disease that affects a quarter of the global population and is caused by the infectious agent, *Mycobacterium tuberculosis.* Cases are most prevalent in underdeveloped communities where the most effective diagnostics and treatments may not be available. Prior research has shown that bacterial lysogens containing hybrid bacteria-bacteriophage genomes can be efficiently mutated through specialized transduction. We seek to use this technique to create a cheap and efficient diagnostic test capable of detecting drug susceptibility in which *M. tuberculosis* cells are made to express a fluorescent reporter, from cosmids packaged in lysogen factories. To that end, we set out to demonstrate that stable lysogens could be generated in the closely related species, *Mycobacterium smegmatis*, in order to develop an *in-vivo* cosmid packaging system, similar to prior work with *Escherichia coli.* Through specialized transduction, we set out to transduce mutations into mc^26 lysogens of the L5, D29, and Starstuff phages. Based on previous results with L5 Δ*attL* lysogen mutants in which phage excision was stunted, we plan to delete the lysin/holin gene system in L5, D29, and Starstuff lysogens, delete the repressor gene in L5 and Starstuff lysogens, and delete the attL site in D29 and Starstuff lysogens. Compared to wild type lysogens, these mutants should be less prone to spontaneous lysis while producing a high titer of phages containing luciferase cosmids. These cosmids could then be transduced into *M. tuberculosis* to produce a detectable glow even at low cell concentrations.

Characterization of Conditional Knockout of AKT3 in CD4+ T cells during Experimental Autoimmune Encephalomyelitis

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Multiple sclerosis (MS) is an autoimmune-mediated disease of the central nervous system (CNS) of unknown etiology believed to be a result of infiltrating CD4+ T cells and monocytes. MS effects approximately 2.4 million people worldwide and is characterized by inflammation and demyelination of the brain and spinal cord, resulting in motor and cognitive deficits. Currently there is no cure for MS. Most therapies are immunomodulatory and target symptoms of inflammation. In order to develop better therapies, it is important to understand the cellular pathways that are neuroprotective and aid in the survival, repair, and repopulation of damaged CNS cells.

Protein kinase B (PKB), also known as AKT, is a serine/ threonine kinase that is part of the AGC kinase family. AKT family members are involved with a variety of cellular functions such as growth, proliferation, and survival. Among the three main isoforms AKT1, 2, and 3, AKT 3 is the major isoform present in the brain. Though AKT3 signaling has not been widely investigated, earlier studies have shown that MS patients have altered AKT3 expression. Previous experiments from our laboratory found that during myelin-oligodendrocyte glycoprotein-induced experimental autoimmune encephalomyelitis (MOG-induced EAE), an inflammatory model used to study MS, $AKT3^{-/-}$ mice have a more severe disease course, increased axonal damage, increased CNS inflammation with reduced T cell suppression, fewer T_{regs} cells, and more demyelination in spinal cord compared to wild-type (WT) mice. Conversely, we found that mice with enhanced AKT3 kinase activity (NMF350 mice), had a delayed influx of inflammatory cells into the CNS, decreased CNS inflammation, and less axonal damage compared to WT controls. The results from both these studies suggest that AKT3 signaling has a protective effect during EAE.

In order to better characterize the role of AKT3 signaling in T cells and to possibly identify specific T cell types responsible for the delay in disease seen in NMF350 mice, mice with conditional deletion of AKT3 in CD4+ T cells ($CD4Cre^{+}Akt3^{fl/fl}$ conditional knockout mice; CD4-CKO mice) were studied and compared to $CD4Cre^{-}Akt3^{fl/fl}$ mice. We hypothesized that without AKT3 expression, CD4-CKO mice would have a more severe disease course and show differences in levels of pro and anti-inflammatory cytokine expression at both the mRNA and protein level when compared to $CD4\text{-}Cre^{-}$ mice. Unlike our hypothesis, our results showed that CD4-CKO and $CD4\text{-}Cre^{-}$ mice did not differ in disease course or disease onset. We did observe a significant increase in IFN-γ mRNA and protein levels in the corpus callosum of the CD4-CKO mice, but not in the spinal cord, relative to $CD4Cre^{-}$ mice. Future studies will continue to investigate the role of AKT3 in MOG-induced EAE in the corpus callosum of these mice.

This project was funded by the NIH and NMSS and was done at Einstein Montefiore Campus as part of the Summer Undergraduate Research Program (SURP). Thank you to Jennifer Guarino, Dr. Juwen DuBois, Dr. Bridget Shafit-Zagardo, and to SURP for their help on this project.

Identifying genes that mediate persistence to Isoniazid in vivo: Engineering a shuttle phasmid for transposon mutagenesis of *Mycobacterium*

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Tuberculosis (TB) is one of the deadliest diseases worldwide and although it was first identified as an infectious disease in the 1880's, there is still much to be uncovered about its causative agent, *Mycobacterium tuberculosis* (*Mtb*). Treatment of TB is grueling on patients and involves chemotherapy for anywhere from six months up to two years with a cocktail of drugs for varying amounts of time. As with most drug treated microorganisms, *Mtb* is growing highly resistant to treatment but what is also astonishing is the presence of persistent bacilli which survive treatment with frontline drugs such as Isoniazid (INH). One of the major problems with studying mycobacteria is that they are highly resistant to genetic manipulation and will not readily be transformed as other bacteria are. Therefore, the development of the shuttle phasmid system which makes use of bacteriophage transduction has been immensely helpful. A shuttle phasmid vector is a chimera that is replicated as a plasmid in *E.coli* and as a phage in mycobacteria and were the first recombinant DNA vectors to be engineered for mycobacteria. The plasmid contains a mariner transposon sequence so that upon infection of the phasmid into mycobacteria, the transposon will randomly insert itself into the cell's genome and disrupt the function of genes. Our goal is to engineer a new phasmid and use this system to mutagenize various *Mtb* to screen for genes that mediate persistence. Transposon mutagenesis is a powerful tool for analyzing gene function and can help us identify novel persistence genes as better drug targets or those that can be targeted synergistically with the current drug regimen.

I would like to thank my mentor Jacqueline Copeland for guiding me through this project, Dr. William R. Jacobs Jr. for the opportunity to work in his lab as well as the other members of the Jacobs lab. Additionally, thank you to the Howard Hughes Medical Institute for funding this research.