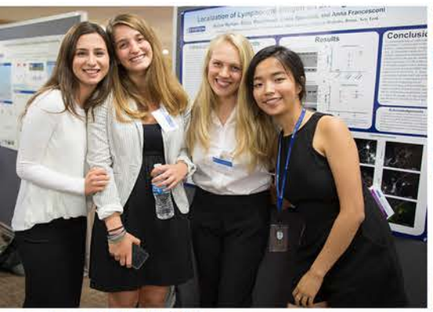

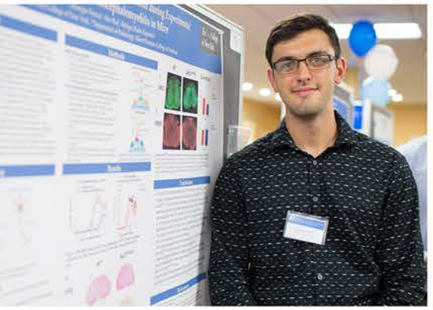

2017
ABSTRACT BOOK

Summer Undergraduate Research Program

EINSTEIN
Albert Einstein College of Medicine
OF YESHIVA UNIVERSITY

Graduate Programs in the Biomedical Sciences

Albert Einstein College of Medicine
OF YESHIVA UNIVERSITY

Graduate Division of Biomedical Sciences

SUMMER UNDERGRADUATE RESEARCH PROGRAM

2017

Victoria H. Freedman, Ph.D.
Associate Dean for Graduate Programs
Director, Summer Undergraduate Research Program

2017 Summer Undegraduate Research Students

Student Name	Undergraduate School	Mentor Name
Farida Ahmed	Fordham University	Dr. Michal Melamed
Madiha Baig	Fordham University	Dr. Kimberly Reidy
Austin Castellanos	Haverford College	Dr. Mahalia Desruisseaux
Daniel Charlton	Whitman College	Dr. Vinayaka Prasad
Dee-Anne Cush	CUNY-John Jay College	Dr. Betsy Herold
Victoria Dawkins	Xavier University of Louisiana	Dr. Titto Augustine
Cole Erickson	Lawrence University	Dr. Thomas Ow
Giuseppe Fiorica	CUNY- City College	Dr. Bridget Shafit-Zagardo
Ashleigh Francis	CUNY- Lehman College	Dr. Titto Augustine
Jeremy Garcia	CUNY-Queens College	Dr. Dianne Cox
Alice Garrastegui	Syracuse University	Dr. Louis Hodgson
Elizabeth Gaylord	University Of Pittsburgh	Dr. Jessica Mar & Dr. Johanna Daily
Zoe Goldstein	Johns Hopkins University	Dr. Libusha Kelly
Adam Haimowitz	Yeshiva University	Dr. David Shechter
Elizabeth Hammond	University of South Caroliina	Dr. Hernando Sosa
Aakash Jhaveri	Hofstra University	Dr. David Spray
Calvin Law	SUNY- Stony Brook University	Dr. Nicholas Sibinga
Clarissa Lett	SUNY- Stony Brook University	Dr. Jonathan Lai
Marjorie Liebling	Yeshiva University	Dr. Jessica Mar
Marie Mazzeo	CUNY- Hunter College	Dr. William Jacobs, Jr.
McKenze Moss	Xavier University of Louisiana	Dr. Kami Kim
Nicole Nyman	Carnegie Mellon University	Dr. Anna Francesconi
Ryan Peer	Middlebury College	Dr. J. Tiago Goncalves
Kaitlyn Petersen	Baylor University	Dr. Bridget Shafit-Zagardo
Michelle Piazza	SUNY- Binghamton University	Dr. Saleem Nicola
Jillamika Pongsachai	New York University	Dr. Teresa DiLorenzo
Yemko Pryor	Oberlin College	Dr. Julie Secombe
Dormarie Rivera	University of Puerto Rico-Ponce	Dr. Ganjam Kalpana
Fiona Roediger	Angeles	Dr. David Spray
Gavriella Shandler	Oberlin College	Dr. Elyse Sussman
Natalia Tumidajski	Mercy College	Dr. Yair Botbol
Rebecca Weitz	Yeshiva University	Dr. Michael Aschner
Emily Williams	Skidmore College	Dr. Jonathan Backer & Dr. Anne Bresnick
Siwen Xie	Fordham University	Dr. Nicholas Baker
Hannah Yoo	Vanderbilt University	Dr. Pablo Castillo
Joy Zou	Boston College	Dr. Kelvin Davies

Prevalence of Poor Health Literacy in Patients with CKD in the Bronx

Farida Ahmed[1,] Anjali Singla[2], Rushang Parikh[2], Jacqueline Kreimerman[2], Kerri L. Cavanaugh[3], Michal L. Melamed[2] ,[1]*Fordham University, Bronx, NY, USA* [2]*Albert Einstein College of Medicine/Montefiore Medical Center, Bronx, NY, USA;* [3]*Vanderbilt University, Nashville, TN, USA;*

Background and Objectives

Patients with chronic kidney disease (CKD) are required to utilize disease management skills but may be restricted by their health literacy. Limited health literacy correlates with worse kidney disease and overall poorer health outcomes. Our study aimed to identify the prevalence of low health literacy in the CKD populations.

Design, measurements, participants and setting

Our study is a prospective cohort. Enrolled were 98 patients from the Bronx, NY, living with CKD stages 3 and 4. Demographic, socio-economic, and clinical factors associated with health literacy were measured using the Rapid Estimate of Adult Literacy in Medicine (REALM) questionnaire.

Results

We found that 32% of our patients with CKD in the bronx have poor health literacy. Our results found that inadequate health literacy were more likely to be people whom have not graduated from high school. We also found that baseline eGFR was lower for patients with inadequate health literacy compared to those with adequate health literacy. We found no associations between health literacy and age, race, marital status, or household income.

Conclusion

We have concluded that limited health literacy is common in patients with CKD in the Bronx and is not associated with lower eGFR. Our patients, participants in a clinical trial, may have had higher health literacy; therefore, our 32% with low health literacy may be an underestimation.

Effect of *APOL1* Variants on Risk for Preeclampsia

Madiha Baig[1,2,3], BS, Stacy Rosenblum[1,2], MD, Rebecca Hjorten[1,2], MD, Zhongfang Du[2], MD, Kimberly J Reidy[1,2], MD

[1]Pediatrics/Nephrology, The Children's Hospital at Montefiore, [2]Albert Einstein College of Medicine and [3]Fordham University

Certain *APOL1* gene variants are associated with kidney disease in people of African descent. Any combination of two variant *APOL1* alleles (G1,G1; G1,G2; G2,G2) is termed as high risk (HR) and having only one or no alleles is termed as low risk (LR). *APOL1* is highly expressed in the placenta and transgenic mice that express the *APOL1* G2 variant in the placenta develop preeclampsia. In our study, we hypothesize that the HR *APOL1* variants in either the mother or fetus would be associated with increased risk of preeclampsia. In order to test our hypothesis, we performed genotyping using Taqman assays. In addition, we used Hematoxylin and Eosin (H&E) staining to examine the histology of HR and LR placentas derived from preeclamptic pregnancies. Genotyping results showed that fetal, but not maternal, *APOL1* HR genotype was associated with increased risk of preeclampsia, present in 1/5 of the severe preeclamptic births in the cohort. Histologic analysis of the placentas is ongoing. Based on these results, we conclude that fetal *APOL1* HR gene variants increase risk of maternal preeclampsia. Future studies would include examination of the mechanisms by which fetal *APOL1* gene variants affect risk for preeclampsia.

Role of Thromboxane A_2 Receptor (Thromboxane Prostanoid - TP) in Experimental Cerebral Malaria

Austin Castellanos, Oscar Bate Akide Ndunge, Mahalia Desruisseaux
Department of Pathology, Albert Einstein College of Medicine, Bronx, NY, USA

Cerebral malaria (CM) is one of the most severe manifestations of malaria infection, resulting in neurological complications in both adults and children. Most children recover after early anti-malarial treatment; however approximately 20% of the survivors sustain persistent neurological sequelae (memory impairment, seizures, ataxia). In CM, there is mechanical obstruction of the brain microvasculature caused by accumulation of *P. falciparum*-parasitized red blood cells (PRBCs), leukocytes, and platelets. Excessive release of immunomodulators by the immune system of the host are thought to be critical to the neurological dysfunction that occurs with CMs. A better comprehension of the pathogenetic role of platelets during CM may lead to adjunctive therapies aimed at dampening such deleterious effects. Thromboxane a2 (TxA_2) is secreted by activated platelets and plays a role in the recruitment of monocytes leading to increased cytokine and chemokine secretion. Increased secretion of TxA2 and activation of the TP receptor results in neuroinflammation.

In an effort to investigate the role of TP in the pathogenesis of CM, we are using infected wild type (WT) C57/Bl6 mice with *Plasmodium berghei*-ANKA (PbA) and compared to TPKO mice infected with PbA and to uninfected WT and TPKO mice. Mice were analyzed for changes in locomotor coordination, gene and protein expression. Rapid Murine Coma Behavior Scores (RMCBS) and Balance Beam data demonstrated impairment in locomotor coordination in WT PbA mice compared to TPKO PbA mice. qPCR results suggested the presence of neuronal injury in WT PbA compared to TPKO, with reduced mRNA expression of N-acetyl aspartate synthetase and aspartoacylase, genes involved in the synthesis of N-acetyl aspartate. No differences were observed between WT PbA and TPKO PbA mice in the temperature, weight, and RMCBS grid assays. These data suggest that TP plays a role in the neuroinflammation, neural cell injury, and subsequent neurological sequelae that occurs with CM. Deletion of the TP receptor may have neuroprotective effects during CM. Special thanks to the SURP Program at Albert Einstein and the Department of Medicine for funding this program. We also acknowledge Ms. Arianna Caradonna for assisting with the experiments.

Effects of HIV-1 Tat W11A Mutation on the Expression, Transactivation and Encapsidation of Tat in Exosomes

Daniel Charlton, Gliselle Nieves-Molina and Vinayaka R. Prasad
Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, NY

HIV-1 Tat protein is an essential transcriptional regulator of HIV-1 replication. Tat is secreted out of HIV-1 infected cells as a free protein and can be taken up by nearby uninfected by stander cells. Tat transcriptionally transactivates cellular genes, many of which are known be pro-inflammatory. Tat protein enhances the processivity of transcription through recruitment of positive transcription elongation factor P-TEFb, which allows for the efficient production of primary viral transcripts by RNA Polymerase II. Tat has also been shown to be neurovirulent, induce synaptodendritic injury, and has been detected in the brain of patients with HIV associated neurocognitive disorders. HIV-1 Clade B infected cells are known to release exosomes, which have been shown to encapsidate Tat. These exosomes subsequently mediate the delivery of inflammatory signals to other cells. Despite the presence of significant biological evidence demonstrating that Clade C is less neuropathogenic than Clade B, the question of if Clade C Tat is also encapsidated in exosomes has not yet been addressed. While Clade B is the best studied HIV-1 clade, Clade C infects the largest population. Previous site-directed mutagenesis studies identified the W11 residue in Tat as being critical to the secretion of Tat out of HIV-1 infected cells.

In this study, we examined if the W11 residue in Tat-B and Tat-C played a significant role in Tat expression, Tat transactivation, and the encapsidation of Tat in exosomes. We used site-directed mutagenesis to construct two recombinant mutants: pcDNA 3.1 Tat-B (ADA) myc W11A and pcDNA 3.1 Tat-C (BL43) myc W11A. We then expressed both wild type and mutantTat-B and Tat-C proteins in HeLa cells transfected with Tat expression plasmids. We first examined the effects of the W11A substitution on Tat expression by lysing the transfected HeLa cells and analyzing by SDS-PAGE and Western immunoblot. We then examined the effects of the W11A substitution on Tat transactivation by directly transfecting TZM-bl reporter cells, which are HeLa derived cells transfected with an HIV-LTR driven Firefly luciferase gene, with Tat expression plasmids and then utilizing a luciferase assay to measure the Tat transactivation levels. Lastly, we examined the effects of the W11A mutation on the encapsidation of Tat in exosomes by ultracentrifuging transfected HeLa cell media to purify exosomes and then analyzing by SDS-PAGE and Western immunoblot for both Tat protein and the Alix exosome marker.

Although more trials are necessary to confirm our results, we demonstrated that the W11A substitution may decrease Tat-B and Tat-C expression. We also demonstrated that the W11A substitution may decrease Tat-C transactivation and that the W11A substitution may decrease the encapsidation of Tat-B in exosomes. Our preliminary data suggests that the W11 residue may be critical for stable Tat expression and transactivation. Our results also suggest that the W11 residue in Tat-B may be required for encapsidation in exosomes, and therefore that this W11 residue may affect the ability of HIV-1 Tat to reach its target cells.

Complement Mediated Cell Death in Mice Vaccinated with □gD- a Candidate Vaccine Against the Herpes Simplex Virus

Dee-Anne Cush, John Jay College; Clare Burn, Ph.D. Candidate, Albert Einstein College of Medicine; Betsy Herold, MD, Albert Einstein College of Medicine

Herpes simplex virus infection is a major global health problem. HSV-1 and HSV-2 cause painful recurrent oral and genital lesions and establish latency in the sensory neurons of the host. HSV type 1 is more commonly associated with oral mucocutaneous lesions whereas HSV type 2 is more commonly associated with genital lesions and is linked to the HIV epidemic. Despite the large HSV infection burden, no successful subunit vaccines have been developed. The Herold and Jacobs laboratories collaboratively developed a novel live, single-cycle HSV candidate vaccine, by deleting glycoprotein D (gD) on the viral envelop, a protein necessary for viral entry into host cells. This vaccine, designated HSV-2 □gD, protects against both HSV-1 and HSV-2 infection in mice and guinea pigs. Protection is mediated by antibodies capable of inducing antibody-dependent cell-mediated cytotoxicity (ADCC) through activation of the murine Fc □RIV.

Complement-dependent cell lysis and complement-dependent neutralization are other antibody-dependent mechanisms by which the immune system might protect against infections. Complement proteins interact with the Fc component of antibodies to elicit a cascade of protein interactions that ultimately result in the formation of a membrane attack complex (MAC). In complement-dependent neutralization, complement proteins interact with neutralizing antibodies preventing viral entry into the cell. The percent cell death was measured in infected cells given mouse serum and complement in the complement-dependent cell lysis experiment, and in the complement-dependent neutralization assay, viral entry into the host cell was measured in cultures given virus, serum and complement. Results from the complement-dependent cell lysis experiment showed an increase in percent cell death upon the addition of complement and though not definitive, results from the complement-dependent neutralization assay suggest that complement might increase the neutralizing ability of the antibodies. These results suggest that the activation of complement by antibodies generated by HSV-2 □gD immune serum help mediate cell death in the complement-dependent cell lysis, and possibly enhance neutralization activity in complement-dependent neutralization.

Role of innate immune response mediators in reovirus treatment of colorectal cancer

Ashleigh Francis*, Victoria Dawkins*, Radhashree Maitra, Sanjay Goel, and Titto Augustine
* These authors contributed equally to the work

Department of Medicine, Albert Einstein College of Medicine, Bronx, NY 10461
Summer Undergraduate Research Program

Colorectal cancer (CRC) is the second leading cause of death in the US and third most common in incidence. Oncolytic reovirus, a naturally-occurring double stranded RNA (dsRNA) virus, preferentially induces apoptosis in Kras mutated CRC, which is approximately 45% of population. Viral oncolysis of mutant cells is dependent on the suppression of phosphorylation of the RNA-activated protein kinase (PKR) protein. Toll-like receptors-3 (TLR3) and -7 (TLR7), retinoic acid-inducible gene-I (RIG-I)-like receptors [RLRs; RIG-I and melanoma differentiation-associated gene 5 (MDA5)], and the multi-protein NLRP3 inflammasome complex serve as pattern-recognition receptors (PRRs) that sense RNA viruses and induce robust host innate immune response. However, no study exists exploring these PRRs in relation to reovirus administration in Kras mutant and wild type (WT) CRC cells. **Methods:** We analyzed 3 human derived CRC cell lines, namely HCT116 (Kras mutant), its isogenic derivative Hke3 (Kras WT), and LIM2405 (Kras WT). Cells were cultured and treated with reovirus at 5 MOI (multiplicity of infection) for 6 and 48 hours. Harvested cells were subject to FACS analysis for TLR3, TLR7, MDA5, RIG-I, NLRP3, and PKR and Western blotting (WB) and probed for NLRP3. **Results:** Using flow cytometry (FACS), we observed overall that the expression level of RIG-I, TLR3, TLR7 and NLRP3 was extremely low, while over 90% of cells expressed MDA5 and PKR. TLR3 expression boosted up after treatment with reovirus for 48 hrs. Reovirus intervention did not lead to a significant change in the expression pattern of any of the 6 markers studied. WB examination for NLRP3 revealed decreased protein expression upon treatment with reovirus for 6 and 48 hrs in the Kras mutant HCT116 and Kras WT Hke3 cells; however, the decrease was greater in the mutant cells. Densitometry analysis of the NLRP3 WB showed a 50 and 15% decrease at 6 hours, and 62 and 45% decrease at 48 hours, in HCT116 and Hk3, respectively. We observed an inconsistent pattern in the LIM2405 cells, with a decrease at 6 and increase at 48 hours.

Our preliminary analyses show that the constitutive expression of TLR3, TLR7, MDA5, and NLRP3 is low, in the range of 1-3%. Overall, there is no significant change by FACS in the expression of the PRRs on exposure to reovirus. However, WB show a change in NLRP3 on reovirus exposure, with an interaction of this change and Kras status, with mutant cells demonstrating a greater decrease. Further work continues with WB and RT-PCR of all the PRR proteins.

We acknowledge Dr. Goel's lab who have been accepting us with open arms. We would also like to thank Dr. Jinghang Zhang of Flow Cytometry core facility for her immense help with FACS. Lastly, we thankfully acknowledge the Diversity Summer Student Research Program for allowing us the opportunity to work in an esteemed research facility and for funding our learning experience.

Impact of miRNA-375 in Head and Neck Cancer Cells on Sensitivity to Chemotherapy

Cole Erickson, Thomas Harris PhD, Carlos Thomas, Geoffrey Childs PhD, Nicole Kawachi PhD, Jeffrey Segall PhD, Lizandra Jimenez PhD, Michael Prystowsky PhD, Thomas J. Ow M.D.

Department of Pathology, Einstein College of Medicine, Summer Undergraduate Research Program, Bronx, NY

Noncoding microRNA regulates protein abundance and can lead to inhibition of tumor suppressors and enhancement of oncogenes. Several miRNAs have been seen to be over or under expressed in many types of cancer cells. Head and neck squamous cell carcinoma is the sixth most common cancer and is responsible an estimated 11,000 people in the United States each year. Low levels of miRNA-375 in previous studies have correlated with lower survival probability, increased distant metastasis, and increased tumor recurrence. The control tumor cells used for experimentation came from the UMSCC1 cell line. UMSCC1 cells that were transfected to express high levels of miRNA-375 (10,000 fold) were used as the experimental cell line. When treated with chemotherapeutic drugs, we expected to see more cell death in the high 375 cell line. The drugs used in this study were Cisplatin (CP) and 5-Fluorouracil (5-FU). Nine concentrations of CP were tested at a range from 0.007 to 50 uM and in another experiment 9 concentrations of 5-FU were tested at a range from 0.6 to 15 uM. Five days after treatment, using a cell viability counting kit (CCK-8) and a spectrophotometer, the cell viability values were compared to control wells containing no drug. CP treated cells showed over 50% cell death starting at 5.5 uM for both the control and high 375 cell lines. 5-FU treated cells showed over 50% cell death starting at 0.9 uM in the high 375 cell line, while 50% cell death occurred between 2 and 3 uM in the control cell line. Our results suggest that miRNA-375 expression may enhance response to 5-FU. If safe ways of increasing miRNA-375 in the body are developed, this might allow for lower doses of chemotherapy to be used, or faster rates of cell death will possibly occur with the current levels of 5-FU being given.

I would like to thank Dr. Thomas Ow, Carlos Thomas, Geoffrey Childs and Thomas Harris for mentoring me for the summer research. I would also like to acknowledge the Einstein SURP program for selecting and funding students for these research opportunities.

Characterization of the Akt3^{Nmf350} Mutation during Experimental Autoimmune Encephalomyelitis in Mice

Giuseppe Fiorica, Alex Ray, Bridget Shafit-Zagardo
Department of Pathology, Albert Einstein College of Medicine Summer Undergraduate Research Program

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS). It is commonly believed to be initiated by infiltrating CD4+ T cells leading to CNS inflammation, demyelination, and axonal damage. The disease affects about two million people worldwide. Because there is no cure for MS, many therapies are aimed at reducing the frequency of relapses and slowing disease progression. One goal of our laboratory is to characterize protective signaling pathways in both the CNS and the immune system, so that novel therapies can be designed to target molecules that may foster survival of CNS cells during MS.

Akt/Protein kinase B is a serine/threonine protein kinase that controls a diverse array of cellular functions, such as growth, metabolism, proliferation, and survival. There are three Akt isoforms: Akt1, Akt2, and Akt3. Akt1 plays a critical role in cell survival, while Akt2 controls glucose metabolism. Akt3 is the major isoform expressed throughout the CNS. It plays an important role in post-natal brain development and neurodegeneration. Previous experiments from our lab showed that during myelin-oligodendrocyte glycoprotein–induced experimental autoimmune encephalomyelitis (MOG-induced EAE), an inflammatory CNS disease model sharing many pathological aspects with MS, mice deficient in Akt3 had a more severe disease course, increased CNS inflammation, more demyelination, and higher levels of axonal damage when compared to wild-type (WT) mice. These results highlighted a protective role for Akt3 during EAE.

Mice that possess a D219V missense mutation in Akt3 have been shown to possess higher levels of enzymatic activity when compared to WT mice. To better characterize this mutation, termed Nmf350, and understand its efficacy in neuroprotection, clinical scores from WT and Akt3^{Nmf350} mice were taken throughout a 15-day EAE disease course. Immunohistochemistry (IHC) was performed on spinal cord tissue from the mice to assess T cell infiltration (CD3), microglia/macrophages (Iba1), axonal damage (SMI32) and demyelination (SMI99). Akt3^{Nmf350} mice were found to have a delayed disease course and reduced clinical scores when compared to WT mice. Additionally, tissue from Akt3^{Nmf350} mice showed less demyelination, less axonal damage indicated by fewer SMI32+ axonal swellings, and possessed higher CD3^{+} cells when compared to WT mice at 10 days with clinical scores. These results suggest that the enhanced activity of the Akt3 kinase in the Akt3^{Nmf350} mice may aid in neuroprotection.

Ongoing studies will seek to identify specific T-cell populations that may be responsible for the less severe and delayed disease course seen in the Akt3^{Nmf350} mice. Western blot analysis will also be performed on these mice to identify which, if any, downstream targets of Akt3 are preferentially phosphorylated in mice possessing the Nmf350 mutation and what roles they may play in protecting against EAE.

Role of innate immune response mediators in reovirus treatment of colorectal cancer

Ashleigh Francis[*], Victoria Dawkins[*], Radhashree Maitra, Sanjay Goel, and Titto Augustine
[*] These authors contributed equally to the work

Department of Medicine, Albert Einstein College of Medicine, Bronx, NY 10461
Summer Undergraduate Research Program

Colorectal cancer (CRC) is the second leading cause of death in the US and third most common in incidence. Oncolytic reovirus, a naturally-occurring double stranded RNA (dsRNA) virus, preferentially induces apoptosis in Kras mutated CRC, which is approximately 45% of population. Viral oncolysis of mutant cells is dependent on the suppression of phosphorylation of the RNA-activated protein kinase (PKR) protein. Toll-like receptors-3 (TLR3) and -7 (TLR7), retinoic acid-inducible gene-I (RIG-I)-like receptors [RLRs; RIG-I and melanoma differentiation-associated gene 5 (MDA5)], and the multi-protein NLRP3 inflammasome complex serve as pattern-recognition receptors (PRRs) that sense RNA viruses and induce robust host innate immune response. However, no study exists exploring these PRRs in relation to reovirus administration in Kras mutant and wild type (WT) CRC cells. **Methods:** We analyzed 3 human derived CRC cell lines, namely HCT116 (Kras mutant), its isogenic derivative Hke3 (Kras WT), and LIM2405 (Kras WT). Cells were cultured and treated with reovirus at 5 MOI (multiplicity of infection) for 6 and 48 hours. Harvested cells were subject to FACS analysis for TLR3, TLR7, MDA5, RIG-I, NLRP3, and PKR and Western blotting (WB) and probed for NLRP3. **Results:** Using flow cytometry (FACS), we observed overall that the expression level of RIG-I, TLR3, TLR7 and NLRP3 was extremely low, while over 90% of cells expressed MDA5 and PKR. TLR3 expression boosted up after treatment with reovirus for 48 hrs. Reovirus intervention did not lead to a significant change in the expression pattern of any of the 6 markers studied. WB examination for NLRP3 revealed decreased protein expression upon treatment with reovirus for 6 and 48 hrs in the Kras mutant HCT116 and Kras WT Hke3 cells; however, the decrease was greater in the mutant cells. Densitometry analysis of the NLRP3 WB showed a 50 and 15% decrease at 6 hours, and 62 and 45% decrease at 48 hours, in HCT116 and Hk3, respectively. We observed an inconsistent pattern in the LIM2405 cells, with a decrease at 6 and increase at 48 hours.

Our preliminary analyses show that the constitutive expression of TLR3, TLR7, MDA5, and NLRP3 is low, in the range of 1-3%. Overall, there is no significant change by FACS in the expression of the PRRs on exposure to reovirus. However, WB show a change in NLRP3 on reovirus exposure, with an interaction of this change and Kras status, with mutant cells demonstrating a greater decrease. Further work continues with WB and RT-PCR of all the PRR proteins.

We acknowledge Dr. Goel's lab who have been accepting us with open arms. We would also like to thank Dr. Jinghang Zhang of Flow Cytometry core facility for her immense help with FACS. Lastly, we thankfully acknowledge the Diversity Summer Student Research Program for allowing us the opportunity to work in an esteemed research facility and for funding our learning experience.

Tumor cell elongation and NF-κB activation via Tunneling Nanotubes

Garcia, J., Martinez, S., Hanna, S.[1], and Cox, D.[1,2]
[1]Department of Anatomy and Structural Biology, [2] Gruss-Lipper Biophotonics Center
Albert Einstein College of Medicine of Yeshiva University, Bronx, New York, USA

The interaction between tumor cells and macrophages has been known to be important in promoting tumor invasion and metastasis. One of the many cell-cell interactions between macrophages and tumor cells occurs through direct cell contact by way of thin, membranous, actin-based structures known as tunneling nanotubes (TNTs). TNTs act as bridges connecting two or more cells that can transfer cytosolic material such as proteins, ions, vesicles, viruses, and even organelles like mitochondria can be directed from one cell to another through TNTs. In this study we look at the role of heterotypic TNTs, those connecting macrophages and tumor cells, in metastasis and tumor cell invasion. Tumor cell elongation is a characteristic of an invasive phenotype acquired by cells in metastatic tumors. Therefore, using an *in vitro* 2D assay, we measured elongation in two tumor cell types: rat adenocarcinoma cells (MTLn3s) and human breast cancer cells (MDA-MB-231s), in co-culture with murine macrophages (RAW/LR5). Our results show that that tumor cells connected to macrophages through TNTs in the control co-culture showed a more elongated phenotype as compared to a co-culture with TNT deficient (sh-Msec) macrophages. Interestingly more nanotubes form between MDA-MB-231 tumor cells in the presence of macrophages. In order to investigate a possible mechanism in tumor cell elongation we looked at a role for NFkB activation downstream of EGF-EGFR. A preliminary experiment using a NFκB inhibitor (DMF) suggests that may be required for tumor cell elongation. We used MTLn3s expressing a GFP-Rel-A reporter of NFκB to monitor its translocation into the nucleus upon activation. Our data suggests that in the presence of macrophages there is an increase NFκB activation in tumor cells. Overall, our experiments demonstrate TNTs occur between macrophages and tumor cells and play a role in tumor cells acquiring a more invasive phenotype possibly through EGF activation of NFkB.

A new FRET biosensor for TC-10 reveals localized activity dynamics at invadopodia of breast cancer cells

Alice M. Garrastegui Rivera[1, 2], Sara K. Donnelly[3, 4], and Louis Hodgson[3, 4]

[1]Summer Undergraduate Research Program, Albert Einstein College of Medicine, Bronx, NY
[2]Department of Biology, Syracuse University, Syracuse, NY
[3]Department of Anatomy and Structural Biology, Albert Einstein College of Medicine, Bronx, NY
[4]Gruss-Lipper Biophotonics Center; Albert Einstein College of Medicine, Bronx, NY

Breast cancer metastasis stems from invasive cancer cells migrating and disseminating from the primary breast tumor to other parts of the body. To migrate, cancerous cells form specialized actin-rich extracellular matrix degrading protrusions known as invadopodia. Invadopodia are able to degrade the extracellular matrix by expressing membrane surface-bound proteases at the site of degradation. The proteases are targeted and delivered to invadopodia in exocytic pathways through intracellular vesicular trafficking. However, the molecular mechanisms that regulate the intracellular vesicular trafficking at invadopodia and how it may affect invasion, dissemination and metastasis are not well understood.

To better understand this disease process, we examined the role of TC-10 GTPase in breast tumor cells as it could be a key signal regulator of vesicular trafficking in tumor cells. TC-10 is a member of the Rho family p21 small GTPases that are critical for regulating cell motility and migration. The Rho family of small GTPases, including TC-10, serve as molecular switches which cycle between an activated (GTP-bound) state or inactivated (GDP-bound) state. TC-10 has been well-studied within the context of diabetes as a regulator of glucose receptor translocation to the plasma membrane in response to insulin. It is also known that GTP hydrolysis by TC-10 causes the fusion of exocytic vesicles to the plasma membrane. Nonetheless, TC-10 remains understudied in relation to other diseases, such as cancer. From preliminary studies, TC-10 appears to be involved in the vesicular trafficking, potentially important in invadopodia function. We hypothesized that TC-10 would be in its active GTP-bound state at invadopodia sites to allow the surface presentation of proteases that degrade the extracellular matrix via the fusion of the exocyst complex.

In order to research the mechanisms of invadopodia function by spatiotemporal coordination of TC-10 activities in breast cancer cells, we developed a new generation of mCerulean-mVenus FRET biosensor for TC-10. Our novel TC-10 biosensor allowed the visualization of TC-10 activity dynamics in live cell imaging. Interestingly, our TC-10 biosensor revealed highly dynamic patterns of activity at and surrounding invadopodia. This is the first observation of spatiotemporal dynamics of TC-10 activity in breast adenocarcinoma to date. Our observations are in agreement with previous studies indicating that the exocyst complex docks at the sides of invadopodia to effect surface presentation of proteases.

Developing a *P. falciparum* host-parasite interactome using statistical methods on gene expression data

Elizabeth Gaylord[1,3], Dr. Johanna Daily[2], Dr. Jessica Mar[1]
[1]Systems and Computational Biology, Albert Einstein College of Medicine, Bronx NY
[2]Department of Microbiology & Immunology, Albert Einstein College of Medicine, Bronx NY
[3]Summer Undergraduate Research Program, Albert Einstein College of Medicine, Bronx NY

Malaria, which totals more than 200 million cases per year, is caused by Plasmodium parasites. Approximately ninety percent of cases are in Africa, where the most common malaria-causing parasite is *Plasmodium falciparum*.[1] This investigation uses a set of transcriptomic data collected from 35 patients infected with *P. falciparum.* Previous studies analyzing this data have clustered the host and *P. falciparum* transcriptomes individually. The human data was clustered with respect to patient clinical information, while the *P. falciparum* expression data was clustered based on the physiological profiles of the parasite.[2,3] This project combines both datasets to study gene expression at an interactome level.

The chip-seq datasets were processed together using GenePattern software.[4] Both datasets were normalized using quantile normalization and background corrected. Thresholds were applied to filter genes that did not reach a minimum expression value and genes that showed little variation across the entire dataset.

To identify patterns within the host-parasite transcriptome, several unsupervised clustering methods were applied to the combined data. Agglomerative hierarchical clustering with complete and average linkage was used to cluster the data by gene. In addition, the gap statistic was used to predict an ideal number of clusters, which were then created by k-means clustering. Both clustering methods proved inconclusive in determining the number of clusters of genes in the dataset. For this reason, the R package clValid was used to score the different clustering methods.[5] Hierarchical clustering, k-means clustering, and partitioning around medoids (PAM), with up to sixteen clusters, was performed on a subset of the 300 most differentially expressed genes from the human and *P. falciparum* datasets. Non-negative matrix factorization was also performed using GenePattern.[4]

To find host-parasite genes with similar expression patterns across the patient set, Spearman's correlations were calculated for all human-malaria gene pairs. A subset of pairs with strong positive or negative correlations was removed and the resulting genes underwent pathway enrichment analysis to investigate the biological processes showing correlations between the parasite and host.

The goal of this project is to perform an interactome-level analysis to better understand host-parasite in vivo gene expression patterns. Preliminary results suggest that the human-parasite dataset has two or three gene clusters. Within these clusters are several modules of genes with strong positive or negative correlations. These correlations may demonstrate human or *P. falciparum* responses to infection, and could aid in the discovery of novel gene functions and biological pathways. Through these analyses, we may also be able to identify the different ways the host-parasite subgroups are regulated that lead to their distinct responses, including distinct subgroups of host-parasite combinations that could be targeted through more personalized treatments that capitalize on their transcriptomic differences.

[1] "Malaria." World Health Organization, World Health Organization, Apr. 2017.
[2] Feintuch, Catherine Manix, et al. "Activated neutrophils are associated with pediatric cerebral malaria vasculopathy in Malawian children." MBio 7.1 (2016): e01300-15.
[3] Milner Jr, Danny A., et al. "Transcriptional profiling of *Plasmodium falciparum* parasites from patients with severe malaria identifies distinct low vs. high parasitemic clusters." PloS one 7.7 (2012): e40739.
[4] Reich, Michael, et al. "GenePattern 2.0." Nature genetics 38.5 (2006): 500-501.
[5] Brock, Guy, et al. "clValid, an R package for cluster validation." Journal of Statistical Software (Brock et al., March 2008) (2011).

Balsalazide Metabolism by the Gut Microbiome

Zoe Goldstein[1], Reese Hitchings[2], Libusha Kelly[3]
[1]Department of Systems and Computational Biology, [2]Albert Einstein College of Medicine, Bronx, NY
[3]Summer Undergraduate Research Program

Balsalazide (BSZ) is an anti-inflammatory prodrug most commonly used to treat inflammatory bowel disease (IBD) and ulcerative colitis. The structure of BSZ includes an azoreductase bond between the 5-aminosalicylic acid and the active drug. BSZ relies on colonic bacteria to split the azo bond and liberate the active drug into the gut. Differences in the efficiency of BSZ in individual patients may be driven by enzymatic differences in the gut microbiomes of patients and thus we measured BSZ decomposition by the gut microbiome**. We hypothesized that over a 24-hour period, BSZ will decompose in the gut microbiome at a linear rate, corresponding to the rate at which the azo bonds are broken by the metabolism in the gut microbiome.** To test this hypothesis, we used high-performance liquid-chromatography (HPLC) and mass-spectrometry (MS) to detect the concentrations of BSZ over time. One fecal sample was used to create fecal homogenates; one homogenate was injected with five different concentrations of BSZ. Samples were incubated at 37° Celsius for 24 hours, and the amount of drug found in a sample is determined based on the drug's molecular weight of 437.31 g/mol. If this molecular weight is detected in the sample, the HPLC will show a peak in its generated graph**. Our findings suggest that as the concentration of drug in the fecal homogenates increased, there is a linear correlation between the concentration of drug and the amount of drug found in the fecal sample.** The area under the curve resulting from the HPLC peaks corresponds to the percentage of drug concentration in the given sample. We observed that the metabolic enzymes slow down as the BSZ product accumulates in the gut, which explains the non-linear trend in decomposition observed in the fecal samples. Future research will analyze BSZ concentration turnover in larger sample sizes, and apply that data of the gut microbiome to the eventual individualization of BSZ treatments. **Studying the metabolic enzymes of the gut microbiome is the first step towards personalized therapies for people treated with BSZ.**

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

A first step in cracking the histone code: Examining the crosstalk between lysine acetylation and arginine methylation using a novel and easy one-step enzymatic assay

Adam Haimowitz, David Shechter
Department of Biochemistry, Albert Einstein College of Medicine, Bronx, NY,
Summer Undergraduate Research Program

A class of proteins known as histones regulates expression of DNA. DNA will wrap itself around histone proteins (H2A, H2B, H3, and H4) forming a nucleosome. Histones are subject to several posttranslational modifications (PTMs): methylation, acetylation, and phosphorylation. The combination of PTMs labeled histones forms the histone code and is responsible for the regulation of gene expression. Deregulation of PTMs is responsible for numerous diseases and the cellular mechanism leading to this is poorly understood. Histone methylation is often found on lysine and arginine residues. Methyl writers for arginine are known as protein arginine methyltrasnferases (PRMT). Each PRMT reaction uses *S*-adenosyl-L-methionine as the methyl donor, which gets converted to S-adenosyl-L-homocysteine (SAH). In this study we probed the cross-talk between PTMs, more specifically the impact of lysine acetylation onto methylation rates at neighboring arginine residues. Focusing on histone H_4, we hypothesized lysine K5 acetylation modulates PRMT1 and PRMT5 activities. A novel assay was used to precisely detect methylation of arginine R3. Our preliminary data provide a strong foundation to unravel the histone code, which will have profound impact on our ability to treat diseases resulting from deregulation of PTMs.

MCAK in Bacteria Cells to Determine Microtubule Interaction and Depolymerization Activity

Elizabeth Hammond, Ana Asenjo and Hernando Sosa

Summer Undergraduate Research Program
Albert Einstein College of Medicine
Department of Physiology and Biophysics
Bronx, NY

Kinesins are a superfamily of motor proteins that use ATP hydrolysis to generate force and movement along microtubules or depolymerize microtubules in eukaryotic cells. A subfamily of kinesin proteins, called kinesin-13, work as microtubule depolymerases. MCAK, a kinesin-13 found in humans, is localized in the spindle poles and kinetochores where it helps to separate sister chromatids during mitosis. Kinesin-13 proteins loosely bind to microtubules until they reach the end and begin to depolymerize, however, the exact mechanism with which this occurs is unknown. Recent studies in the Sosa lab on Klp10A, a kinesin-13 found in drosophila, have defined the structure of key intermediates in this protein's depolymerizing mechanism. This information is not available for human kenisin-13s.

In this work, a shorter construct of MCAK was studied to determine if MCAK and Klp10A act with similar mechanisms. When examining the structures of the two proteins it can be seen that they are very similar structures despite having several residue differences. There is an 84% homology and 64% identity in the motor domain. The functionality of MCAK was studied in order to determine if those differing residues resulted in structural and functional differences. The MCAK construct was expressed and purified, samples were examined using electron microscopy and were seen to be interacting with both free tubulin and microtubules forming the intermediates of successful depolymerization and a similar structure. A microtubule depolymerization assay is being performed to compare their activities. We plan to pursue cryo-electron microscopy to obtain higher resolution structures of hsMCAK-tubulin complexes.

The authors thank the entire Sosa lab, Sharp lab and Analytical Imaging Facility as well as Albert Einstein College of Medicine Summer Undergraduate Research Program for funding.

Astrocyte connexin43 controls permeability of the blood brain barrier by stabilizing endothelial cell tight junctions

Aakash V. Jhaveri[1,2],Sandra Veronica Lopez-Quintero[1], Eliana Scemes[1] and David C. Spray[1]
[1]Albert Einstein College of Medicine, Bronx, NY, [2]Hofstra University, Hempstead, NY

Previous studies indicate that both astrocytes and endothelial cells (ECs) play an integral role in maintaining the tightness of the blood brain barrier (BBB). However, the underlying mechanisms by which astrocytes regulate the tightness of the endothelial cell layer are still largely unknown. That gap juncitons between astrocytes could influence endothelial tight junctions was suggested by increased vascular permeability in mice in which both astrocyte gap junction proteins (Cx43 and Cx30) were deleted (Ezan et al., 2012). We now report studies in which we have used an in vitro cell culture system to quantify permeability of astrocyte-endothelial cocultures on permeable membrane supports. To test the role of astrocyte Cx43 in establishing tight BBB, we compared brain microvascular endothelial cells (ECs) cocultured with immortalized wildtype or Cx43 deficient astrocytes. Our data demonstrate that EC-WT astrocyte cocultures form barriers that are less permeable than wither EC cells alone or coculture of EC with a neuroblastoma cell line (Neuro2A) and display a tighter barrier to permeation of the fluorescent dye 10kDa Lucifer Yellow dextran than did EC-Cx43 null cocultures. Moreover, in response to focal sonication (insonation), EC tight junctions were disrupted, and EC-Cx43 null astrocye cocultures displayed transient permeability enhancement not seen in the EC-WT astrocyte coutlures... Together, these data provide evidence that Cx43 helps maintain BBB integrity by stabilizing EC tight junctions.

Support: R01 NS092466 and SURP

AIF1 Promotes NFκB Pathway in Bone Marrow Derived Macrophages and RAW 264.7 Cells in the Context of Atherosclerosis

Calvin Law[1,2], Lander Egaña-Gorroño[1], Prameladevi Chinnasamy[1], Nicholas E.S. Sibinga[1].
[1]Wilf Family Cardiovascular Research Institute, Department of Medicine (Cardiology), and Department of Developmental and Molecular Biology, Albert Einstein College of Medicine, Bronx, NY. [2]Albert Einstein College of Medicine Summer Undergraduate Research Program (SURP).

Introduction: Atherosclerosis is characterized by the formation of focal plaques in the arterial wall and the subsequent chronic inflammatory processes by which the plaques grow in size and complexity and progressively occlude the arterial lumen. Plaque formation is a complex process involving multiple cell types such as macrophages (MΦs), which promote lesion formation by taking up oxidized- LDL (oxLDL), becoming foam cells, and secreting pro-inflammatory cytokines which drive atherogenesis. Nuclear Factor-κB (NFκB)-mediated signal transduction in MΦ has been established at different stages of atherosclerosis. In preliminary studies we found that Allograft Inflammatory Factor-1 (AIF1), a 17kDa protein positively associated with inflammatory processes and various forms of vascular disease, supports MΦ migration, phagocytosis, survival and pro-inflammatory cytokine secretion. Moreover, we found that mice lacking AIF1 had increased necrotic core formation and less NFkB activation in atherosclerotic lesions *in vivo*.
Hypothesis: AIF1 affects discrete components of the NFkB signaling pathway activity, with consequences for overall pathway activity and MΦ functions relevant to atherosclerosis.
Methods: Bone marrow derived MΦs (BMMΦs) were isolated and immortalized from wild type (wt) and *Aif1*$^{-/-}$ mice and stimulated with 50 μg/μl of oxLDL. Mouse MΦ cell line RAW 264.7 cells were transfected using the TransIT-X2 lipid-base delivery system with either *Aif1* siRNA or p3xFLAG-CMV14-*Aif1* expression vector for knock down and overexpression studies, respectively. Protein lysates were immunoblotted to assess the levels of AIF1 and total and phosphorylated (active) forms of NFκB-pathway proteins.
Results: In AIF1-deficient BMMΦs stimulated with oxLDL, we found no differences in the levels of total NFκB-pathway kinases, but interestingly, phospho-p65 NFκB levels were significantly reduced and phospho-IκBα levels increased compared to wt cells ($P<0.05$). Knock down and overexpression studies were performed using RAW 264.7 cells. *Aif1* knock down using siRNA significantly increased phospho-IκBα levels compared to scrambled control ($P<0.05$). On the other hand, AIF1 overexpression significantly increased phospho-p65 NFκB levels compared to untransfected control ($P<0.05$).
Conclusions: These data indicate that both in BMMΦs and RAW 264.7 cells, AIF1 limits phospho-IκBα inhibitory complex levels and subsequently promotes phospho-p65 NFκB levels *in vitro*. Because the NFkB pathway has been closely linked to both cytokine expression and cell survival signaling, these results point to a critical role for AIF1 in pro-inflammatory MΦ functions. Future studies will be conducted with the aims of identifying the precise steps of the pathway controlled by AIF1 and the mechanisms by which AIF1 exerts control over NFκB signaling.

Acknowledgements
I am thankful to the members of the Sibinga Laboratory for their guidance as well as to the Albert Einstein College of Medicine Summer Undergraduate Research Program (SURP). This project was supported by NIH - 1R01HL128066.

Engineering Bispecific Antibodies for Chikungunya Virus

Clarissa Lett, Jose Quiroz, Margaret Kielian, Courtney A. Cohen, John M. Dye, Jonathan R. Lai
Department of Biochemistry, Albert Einstein College of Medicine
Summer Undergraduate Research Program
Bronx, NY

Chikungunya fever outbreaks are currently on the rise and there are no approved treatments or vaccines. In recent years, antibodies that neutralize Chikungunya virus have been identified and mapped onto the viral glycoproteins. To improve the neutralizing capabilities of these antibodies we engineered three bispecific antibodies CHK-152x166, CHK-152x265, and CHK-m242x265 comprised of antibodies that target different segments of the glycoprotein or unique domains on the same segment, CHK-152, CHK-166, CHK-m242, and CHK-265. Following this strategy we hope to enhance neutralizing and binding activity while decreasing potential competition among individual monospecific antibodies in a cocktail. The bispecific antibodies were tested against their parent antibodies for both binding and neutralization using Bio-layer Interferometries (BLIs) against the immature glycoprotein (p62-E1), Vesicular Stomatitis Virus displaying the CHIKV glycoprotein with a GFP reporter (VSV-CHIKV), Plaque Reduction Neutralization Tests (PRNTs) using the CHIKV 181/25 vaccine strain, and authentic live virus microneutralization assays under Biosafety Level 3 conditions by our collaborators at the United States Army Medical Institute of Infectious Diseases (USAMRIID). The results of these tests showed that the bispecific antibodies were able to bind and neutralize better than their parent antibodies, validating their potential as immunotherapeutics against CHIKV. Overall CHK-m242x265 was the most effective neutralizer and binder in these preliminary studies. In future studies these antibodies will be further tested *in vitro* against the authentic virus and in mice to determine *in vivo* efficacy at USAMRIID.

Acknowledgements

C.L. was supported by the Einstein Summer Undergraduate Research Program; J.Q. was supported by the Einstein Medical Scientist Train Program (T32-GM007288-44); J.R.L. acknowledges funding from the National Institute of Health (R01-AI125462-01A1).

Understanding Bayesian Networks for Single Cell Transcriptomic Data

Marjorie C. Liebling[1], Shuonan Chen[2], Samuel E. Zimmerman[2], Daniel Piqué[2], and Jessica C. Mar[2,3]

[1]Stern College for Women, Yeshiva University, New York, NY, [2]Department of Systems and Computational Biology, [3]Department of Epidemiology and Population Health, Albert Einstein College of Medicine, New York, NY

Traditionally, biologists have studied the genomics of bulk cell population in which gene expression values are collected for various genes for a number of individuals. While this method is often very informative of patterns of genetic behavior across various populations, it has the potential to fail to recognize any heterogeneity that may or may not exist within a given population of cells. To understand the biological ramifications of many diseases, it is crucial to study the genetics of such abnormalities with single cells as much of the heterogeneity lies from one cell to another. By studying single cell data in addition to bulk cell population data, we may gain insight into the heterogeneity in which the transcriptome is controlled, not just from one individual to another, but from a single cell to another single cell in the same population.

Single cell data, however, can be difficult to interpret at the transcriptome level because of the newness of the field and the slow emergence of bioinformatic tools, which is especially the case for genetic networks. The goal of this study is to produce an interactive interface to describe the behavior of single cell genetic relationships through stage specific networks. Through this network visualization method, the user can interpret how the genes in the dataset work together across various stages.

This particular dataset originates from mesoderm cells which have blood forming potential. Mesoderm cells form in the early stages of embryonic development, known as gastrulation and have the capacity to evolve into endothelial, smooth muscle, or blood cells. The presence of blood is an important marker for embryogenesis, as it is necessary to support the developing organs. Gene expression values from mice mesoderm cells were obtained between the E7.0 and E8.5 stages .

After discretization of the data, the R package, bnlearn, which uses a sampling method called bootstrapping to learn the network from the data. Several different networks are produced to model the behavior of the dataset by randomly choosing a root gene. Each individual network is scored according to its likelihood of its truly representing the data. These steps are repeated hundreds of times until a sufficient tally of highest scoring networks is obtained. All edges that ranked higher than the threshold were chosen as the edges to be used in the final network.

Contingency tables representing the probability of a gene being off or on given the status of its parents were obtained using Bayes Theorem which states, $P(B|A) = \frac{P(B \cap A)}{P(A)}$ The tables were obtained by computing the frequency of the child being OFF or ON given different combinations of parent states in each of the stages and in all of the stages combined.

To determine the strength of two connected genes, a contingency table for each edge was produced representing the probability of the child being OFF or ON given the status of a single parent gene. The interaction was inhibitory and activation-based using an odds ratio statistic which was used to rank the edges by variability across stages. Edge heterogeneity was plotted in a histogram to reveal the percent

edges in just one, two, three, four, five, or all of the stages of cell development. Overall, activating edges remain activating through five of the stages, whereas inhibiting edges remain inhibiting for only two of the stages. Thus, it becomes clear that there is more heterogeneity across the stages among the inhibiting edges.

In conclusion, the data was put through many statistical tests to reveal significant edges of the network and the relationship that exists between the parent and child. The goal of our project was to create a real-time visualization of gene-gene interaction across various stages of the cell development using single cell data which allowed us to gain insight into the workings of cells from a single population. The majority of significant edges had an activation relationship and maintained this relationship throughout five stages. Few significant edges were inhibiting. Those that were, however, generally maintained this relationship throughout only two stages of cell development. We deduce that while inhibiting edges are highly heterogeneous across the stages, activating edges are not and maintain this relationship. Future directions of this project include generalizing the application so any scientists researching Bayesian networks may upload and interactively visualize the distinguishing characteristics of his network.

Combating tuberculosis: Targeting the arginine biosynthesis pathway as a potential drug target and human vaccine candidate

Marie T. Mazzeo[1], Sangeeta Tiwari[2] and William R. Jacobs, Jr[2,3]

[1] Summer Undergraduate Research Program, Albert Einstein College of Medicine, Bronx, NY, USA, [2] Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, NY, USA, [3] Howard Hughes Medical Institute, Bronx, NY, USA.

Tuberculosis (Tb), caused by *Mycobacterium tuberculosis*, remains one of the top ten causes of mortality in the world and is the leading cause of death due to an infectious disease. The devastation of this pandemic necessitates improved methods of prevention and treatment. Furthermore, effective treatment for Tb must sterilize emergent drug-resistant "persister" *Mtb* subpopulations. Previous data has demonstrated that arginine starvation sterilizes *Mtb in vitro and in vivo*, suggesting that it is a valuable drug target. However, drug screening against this target in a Biosafety Level 3 facility is cumbersome and *Mtb* grows slowly. Therefore, we decided to use *Mycobacterium smegmatis* as a model organism because it is fast growing and shares a large degree of genetic homology with *Mtb*. We constructed allelic exchange substrates (AES) and used specialized transduction to establish the mutant strain $mc^2$1756 with a gene deletion in MSMEG_3772, the homolog of Rv1656 (*argF*), which encodes an ornithine transcarbamylase in the arginine biosynthesis pathway. Observing the desired phenotype of bactericidal or sterilizing effects upon deletion of *argF*, or other genes in the *de novo* arginine biosynthesis pathway, demonstrates the potential of these strains as a drug-screening model.

Furthermore, arginine auxotrophs have the potential for improved vaccine development. The *Mycobacterium bovis* strain bacilli Calmette-Guerin (BCG), has been used as a vaccine for nearly a century; however, it provides limited protection and can disseminate in immunocompromised individuals. We anticipate that the disruption of arginine biosynthesis will result in further attenuation, increased safety, and will have the potential for vaccine development. We have isolated and amplified AES to make gene deletions in various steps of the arginine biosynthesis pathway, Rv1654, Rv1652- Rv1656 and Rv1658-Rv1659, for future deletions in BCG. Further deletion mutants in the arginine biosynthesis pathway of *Mycobacterium tuberculosis* will also provide a promising direction for future research.

I would like to thank my mentor, Dr. Sangeeta Tiwari, for all of her guidance on this project, and Dr. William R. Jacobs Jr. for providing me the opportunity to work in his lab. Additionally, I would like to thank the other members of the Jacobs Laboratory, and the Phage Finders program, for their participation and support, and HHMI for funding this project.

Evaluation of Cell-Free DNA as a Correlate of Malaria Severity

McKenze Moss[1,2], Anne Kessler[1], Li-Ming Ting PhD[1], Kami Kim MD[1]
[1]Albert Einstein College of Medicine, [2]Xavier University of Louisiana, Departments of Medicine, Pathology and Microbiology & Immunology

Malaria is a life-threatening disease caused by the parasite *Plasmodium falciparum* and is transmitted to humans through the bite of infected female mosquitoes (WHO, 2017). Sub-Saharan African countries account for majority of the reported cases of Malaria and is responsible for the most malaria-related deaths globally. Symptoms of mild malaria consist of fever, headaches and other flu-like symptoms. Cerebral malaria is characterized by factors such as high parasite burden, seizures, and coma (Idro MD, 2005). Children, between the ages of 6 months and 12 years, in Blantyre, Malawi with uncomplicated malaria (UM) and cerebral malaria (CM)were recruited. Children with CM were given an ophthalmology exam to test for retinopathy. In this study, we attempted to develop an assay that would quantitate the total cell-free DNA(cfDNA) from the patients' blood plasma using two different methods. Total WBC count and parasite cfDNA correlate with malaria severity. We hypothesize that plasma cfDNA (from WBC +/- parasites) may also correlate with severe malaria.

The cell-free DNA analyses were performed from frozen samples stored at -80˚C. The total cell-free DNA was quantitated without any prior purification, using the Qubit dsDNA High Sensitivity Assay Kit and the Qubit 2.0 Fluorometer following the manufacturer's protocol. Plasma samples collected from the Blantyre Malaria Project were diluted 20-fold and analyzed in duplicates. A standard curve was developed using the qPCR and purified DNA from human foreskin fibroblast (HFF) with amplification of the LINE gene. The HFF DNA was diluted 10-fold and assayed by the SYBR direct green assay. The amplification mixture contained 10μL of SYBR green, 1μL of forward human LINE primer,5'-TCACTCAAAGCCGCTCAACTAC-3', 1μL of reverse human LINE primer, 5'-TCTGCCTTCATTTCGTTATGTACC-3', and 6 μL of water.

The Qubit assay measures DNA with sensitivity down to 10 picograms of DNA. Due to the high sensitivity, the Qubit fluorometer was the best assay for measuring cfDNA. Overall malaria patient samples displayed elevated cfDNA levels compared to the healthy donor samples. Patients diagnosed with cerebral malaria displayed even higher cell-free DNA levels compared to patients having uncomplicated malaria. Cerebral malaria patients with malaria retinopathy also showed higher cfDNA levels compared to those who did not have retinopathy. The rodent models were injected with *Plasmodium berghei* and were monitored over a 7-day period. Blood samples were collected on Day 3, 5, and 7. Cell-free DNA from the blood plasma were measured using the Qubit assay. The infected mice displayed higher levels of cell-free DNA compared to the uninfected mice.

Future work includes establishing a qPCR method to measure human specific cell-free DNA, using parasite specific cfDNA to calculate human specific cell-free dna present in total cfDNA, and further investigating cerebral malaria in rodent model to determine if there is an increase in cell-free DNA levels.

Localization of Lymphocyte antigen 6H in Fragile X Syndrome

Nicole Nyman, Stacy Roudabush, Luisa Speranza, and Anna Francesconi
Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine, Bronx, New York
Summer Undergraduate Research Program

Fragile X Syndrome (FXS) is one of the most common forms of inherited intellectual disability and about 30% of those with the disorder are also diagnosed with autism, indicating that these conditions potentially share a common pathophysiology. FXS is due to transcriptional silencing of the FMR1 gene which is responsible for encoding the Fragile X Mental Retardation Protein (FMRP), an RNA-binding protein that regulates mRNA translation, trafficking, and stability by inhibiting the translation of associated transcripts. Many of the mRNAs regulated by FMRP encode proteins that are involved in lipid synthesis, metabolism, and transport. Thus, abnormal expression of FMRP might lead to the dysregulation of lipid homeostasis, specifically dysregulation of membrane rafts. Membrane rafts are specialized cell compartments that orchestrate and compartmentalize dynamic cellular processes involving signaling effectors, ion channels, and neurotransmitter receptors like nicotinic acetylcholine receptors (nAChRs).

Past research has shown that there is dysregulation of membrane rafts in the brains of adult *Fmr1* knockout mice. Particularly, there is reduced presence of Lymphocyte antigen 6H (Ly6h) in membrane rafts of *Fmr1* KO mice, in absence of changes in its total expression. Ly6h is involved in immune regulation and is encoded by a family of genes that is also linked to other autism spectrum disorders. Moreover, Ly6h was shown to act as nAChRs auxiliary protein to regulate their trafficking and channel properties. Ly6h is abundantly expressed in the frontal cortex, hippocampus, and midbrain, but its specific localization in neurons remains unclear. Together, these finding raise the question of where Ly6h is localized in neurons given its impaired recruitment to membrane rafts in *Fmr1* KO mice and whether abnormal Ly6h expression in rafts may affect trafficking and/or function of nAChRs.

To begin investigating these questions, I used immunolabeling and epifluorescence microscopy to characterize Ly6h localization in cortical and hippocampal neurons where both Ly6h and FMRP are highly abundant. To test whether impaired recruitment of Ly6h to membrane rafts might be due to compromised trafficking to the plasma membrane, I used live labeling with Ly6h-specific antibodies or biotin to examine surface expression of Ly6h in neurons and brain slices in which FMRP expression was acutely suppressed by RNAi or absent (*Fmr1* KO mice). Preliminary results from these experiments indicate that Ly6h surface expression is not compromised in FMRP deficient cells suggesting that its stability/clustering within membrane rafts might instead be affected. Future studies will aim to confirm these preliminary results and to explore the impact of Ly6h on trafficking and function of nAChRs in *Fmr1* KO neurons.

The Role of Microglia on the Dendritic Development of Adult-Born Neurons in the Dentate Gyrus

Ryan Peer, Gjystina Lumaj, M. Agustina Frechou, Kelsey McDermott, J. Tiago Gonçalves
Department of Neuroscience and Stem Cell Institute, Albert Einstein College of Medicine, Bronx, NY; Summer Undergraduate Research Program

Microglia are the primary immune cells of the central nervous system, and they play an active role in the development and homeostasis of the CNS. However, their function extends beyond their role in orchestrating the brain's inflammatory response. Microglia have been found to monitor synaptic function and are involved in the phagocytic elimination of synapses and the remodeling of neuronal circuits. This is especially important in the context of adult hippocampal neurogenesis. Impaired integration into neural circuits and altered neuronal morphology can be linked with developmental disorders such as schizophrenia and autism. Therefore, it is essential to understand the mechanisms that control proper neuron development. Previously our lab has shown that adult-born neurons in the dentate gyrus (DG) of the hippocampus undergo a period of dendritic overgrowth and pruning, and that this process is regulated in a homeostatic fashion. Because microglia are involved in the pruning of synapses, we question how microglia might affect the dendritic development of these adult-born neurons in the DG.

In order to image newborn dentate gyrus cells (DGCs), we injected the mouse with a GFP-expressing retrovirus (RV-GFP) in the DG subgranular zone. The RV-GFP is based on the Muloney murine leukemia virus and selectively labels newborn DGCs because it only integrates into dividing cells at the time of injection. In order to selectively ablate microglia during the period of dendritic development of these labeled adult-born DGCs, we utilized a CX3CR1-Cre/ERT2 x CSF1R inducible conditional knock-out mouse, which excises floxed CSF1R alleles in cells that express CX3CR1 when induced with tamoxifen. The mouse underwent tamoxifen treatment for 30 days, after which the mouse was perfused, and the brain sliced and prepped for imaging. We imaged 400 μm slices of the perfused brain using 2-photon microscopy on the Bergamo Thorlabs microscope. The image sequences were then imported into FIJI Image J and the dendrites were reconstructed utilizing the Simple Neurite Tracer feature. Dendrite reconstructions were then analyzed for total dendrite length, total number of endings, and dendrite branch order which was compared to previously acquired data of adult-born DGC dendrite reconstructions of wild type mice.

Our findings showed that both the average total length of the dendrites and the average total number of endings were significantly less in the mouse with ablated microglia than that of wild type mice. Similarly, the dendritic order was significantly altered in microglia ablated mice compared to that of the control. These results suggest that microglia are important for facilitating proper dendritic development of adult-born DGCs and that ablation of microglia results in dendritic defects. However, these findings do not elucidate whether the presence of functioning microglia directly impacts dendritic pruning specifically. The lab's previous work shows that the peak time period for dendritic pruning in adult-born DGCs takes place between 14-21 days post infection. Therefore, by ablating microglia specifically during this period after the neurons' birth, we can better understand microglia's role in this process. Further investigation of microglia's role in dendritic development in adult-born DGCs will give valuable insight into the process of hippocampal neurogenesis and the connection between the brain and the immune system.

Gene Expression Profile of $Axl^{-/-}$, $Gas6^{-/-}$, and DKO Mice During Cuprizone Treatment and Recovery

Kaitlyn Petersen[1], Juwen DuBois[2], Bridget Shafit-Zagardo[2]
[1]Department of Neuroscience, Baylor University, Waco, TX,
[2]Department of Pathology, Albert Einstein College of Medicine, Bronx, NY
Summer Undergraduate Research Program

Multiple sclerosis (MS) is an immune-mediated disease of the CNS characterized by demyelination, inflammation, and lesions in the brain and spinal cord. The receptor tyrosine kinases (RTKs) Tyro3, Axl, and MerTK (TAMs) and their respective ligands, Gas6 and Protein S1 (ProS1) are important for the innate immune response and homeostasis. While only Gas6 directly activates Axl, ProS1 binding to Tyro3/MerTK can indirectly activate Axl through receptor heterodimerization. Several studies in mouse models of MS have shown that Gas6/Axl signaling is neuroprotective and is important for myelination. Therefore, to assess the contribution of this signaling pathway to the development of MS, $Gas6^{-/-}Axl^{-/-}$ (DKO) mice were generated. Using the cuprizone (CPZ) model of demyelination/remyelination we found that DKO mice undergo more severe demyelination, have increased axonal damage, and have increased motor deficits compared to WT mice on CPZ. Proinflammatory cytokines TNF-α, IFN-γ, and IL-6 were elevated in both the DKO and WT corpus callosum at 4-, 5- and 6-weeks CPZ treatment and persisted after 1-week recovery. Tyro3 and MerTK mRNA expression in DKO mice remained lower than WT mice on CPZ and during recovery. However, ProS1 expression was significantly elevated in DKO compared to WT mice on CPZ, and returned to baseline after 1 week of recovery.

In this project we aimed to determine the expression of the TAMs, Gas6, and Pros1 in the single knockout mice after 3 weeks of recovery from CPZ. Additionally, we wanted to determine whether TNFR-1α and -1β played a role in regulating the expression of the pro-inflammatory cytokines in DKO mice during CPZ treatment and recovery. We hypothesized that single knockout mice would be more similar to WT mice in terms of TAM expression. We also predicted that TNFR-1α expression in DKO mice would be significantly elevated at the same time points as TNF-α, IFN-γ, and IL-6 while TNFR-1β, which functions to regulate apoptosis, would remain unchanged.

We extracted RNA from the corpus callosum of WT, $Axl^{-/-}$, $Gas6^{-/-}$, and DKO mice at 0-, 4-, 5-, and 6-weeks of CPZ treatment as well as at 1- and 3-weeks of recovery. qRT-PCR was used to determine TNFR-1α, TNFR-1β, Tyro3, Axl, Mer, Gas6, and ProS1 expression. TNFR-1α was found to be significantly upregulated at 4-, 5-, and 6-weeks CPZ in WT, at 4- and 5-weeks CPZ in DKO, and at 5- and 6-weeks CPZ in $Axl^{-/-}$ mice compared to naïve. Additionally, TNFR-1α expression in $Axl^{-/-}$ mice was significantly higher than WT and DKO at 6-weeks CPZ treatment. These results were expected as the proinflammatory cytokines were also upregulated at these time points. TNFR-1β expression in $Axl^{-/-}$, $Gas6^{-/-}$, and DKO mice remained similar to WT levels throughout treatment and recovery. No differences in Tyro3, MerTK, Gas6, or ProS1 were observed in $Axl^{-/-}$ mice at 3 weeks of recovery compared to their naïve counterparts. Similarly, no differences in Tyro3, MerTK, Axl, or ProS1 were observed in $Gas6^{-/-}$ mice at the same time points. These data suggest that the pathology in the $Axl^{-/-}$ and DKO mice are consistent with prolonged inflammation via TNFR-1α signaling.

Effects of a dopamine uptake inhibitor on D1 or D2 antagonist treated rats in a cued sucrose-reward task

Michelle Piazza, Marcin Kazmierczak, Saleem M. Nicola, Ph.D.
Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY, Summer Undergraduate Research Program

Dopamine is a key neurotransmitter implicated in drug addiction, primarily through its roles in motivation and reinforcement. These processes can be modeled through the use of a cue-based reward task in which rats learn to associate a discriminative auditory stimulus (DS) with the prediction of a sucrose reward but a neutral stimulus (NS) with the absence of reward. As such, the DS reinforces response behavior and increases motivation for future response while the NS does not, causing rats to respond to only the DS.

Using this model, it has been established that systemically blocking D1 receptors with SCH23390 leads to a gradual decline in response to the DS. In this experiment, rats were injected with SCH, placed back in the home cage for 1 hour and then placed in the operant chamber for 2 hours of testing. However, a follow up study found that when rats spent this 1-hour wait period in the testing chamber to eliminate pre-session arousal, response started and remained low without the gradual decline. Furthermore, when rats were re-excited 1 hour into testing by the experimenter touching the rat for 5-10 seconds, there was a spike in DS response. Taken together, these results suggest that arousal through physical touch is capable of overpowering the response suppression caused by SCH. However, treatment with the D2 antagonist haloperidol caused consistent response suppression from the beginning of the session that was unresponsive to arousal.

Based on the aforementioned results, it is hypothesized that the ability of arousal to increase cue responding in D1 antagonist-treated animals is due to an arousal-induced increase in dopamine that activations D2 receptors. In contrast, in the presence of a D2 antagonist, arousal cannot increase responding because dopamine cannot activate D2 receptors. To better characterize this dichotomy, the present study sought to reproduce the suppression of cued response behavior using either the D1 antagonist SCH23390 or the D2 antagonist haloperidol. We then used the dopamine uptake inhibitor GBR12909 to pharmacologically model the effects of physical arousal seen in prior studies. We predicted that SCH23390 would produce a gradual decline in responding whereas haloperidol would cause cue response behavior to start and remain low. Furthermore, we predicted that co- administration of GBR12909 with each antagonist would increase responsiveness only in D1 antagonist-treated animals.

As expected, the results of this study showed a gradual decline in responding in SCH23390 treated animals, which was reversed by GBR12909, and a consistent suppression in haloperidol-treated animals. However, counter to our hypothesis, we found that GBR12909 also increased responsiveness in animals that received haloperidol. After comparing the data from each testing day, we hypothesize that this may be due to lasting effects of repeated psychostimulant treatment with GBR. Furthermore, there may be more neuromodulators involved in arousal than just dopamine. Reproducing this study using a between rather than within-subjects design would be an appropriate follow up to clarify this unexpected effect.

This study is supported by funding from the Albert Einstein Summer Undergraduate Research Program and the National Institute of Drug Abuse.

Development of Human Proinsulin-Linked Antibodies for Targeting Proinsulin to Dendritic Cells via Human DEC-205 Receptors

Jillamika Pongsachai[1,2], Jennifer Schloss[1], Nitin Amdare[1], Riyasat Ali[1],& Teresa P. DiLorenzo[1]
[1]Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, NY, 10461
[2]New York University, New York, NY 10003

Type 1 diabetes mellitus (T1D), previously known as juvenile diabetes, is a chronic autoimmune disease usually diagnosed in children and young adults. T1D is characterized by increased blood glucose levels (hyperglycemia), which are due to the insulin deficiency that occurs as a consequence of losing the pancreatic islet beta cells. T1D has become a major problem because the triggering event of this disease is unclear and its incidence is increasing worldwide. A doubling of new patients less than five years of age and a 70% increase in the number of patients 5-15 years old are expected between 2005 and 2020 in high-incidence countries, such as the United Kingdom and North America. An immunotherapeutic strategy for the prevention and reversal of T1D is necessary. We believe that an effective strategy would be one that eliminates pathogenic T cells specific for beta cell antigens and promotes the growth of regulatory T cells capable of controlling the level of pathogenic T cells. A study reported that delivery of a peptide to the receptors (DEC-205^{+}) on dendritic cells (DCs) could prevent experimental autoimmune encephalomyelitis in mice and reverse early symptoms of the disease. DCs are known to have multiple tolerance mechanisms, which include T cell deletion, T cell unresponsiveness, and the induction of Foxp3^{+} regulatory T cells from Foxp3^{-} cells. Delivering a beta cell antigen to DCs can potentially lead to a way to prevent or reverse T1D. The purpose of this research project is to develop the anti-human DEC-205 (anti-hDEC-205) antibody (3G9) linked to human proinsulin (hIns) for targeting hIns to DCs, as insulin is one of the main antigens targeted by T cells in T1D. To produce the 3G9/hIns antibody, we first amplified hIns from pPCR-script, its original vector, and ran the PCR product on an agarose gel. To facilitate proper proinsulin folding, the primer used for amplification included a flexible sequence to link proinsulin and the heavy chain of 3G9. Proper folding is important for the expression of the antibody to be produced in 293T cells. We performed gel extraction and digested the hIns insert with XhoI and NotI restriction enzymes. Then, we purified the insert and ligated it to a plasmid encoding 3G9. We transformed the ligation mixture into NEB 10-beta *E. coli* cells and purified DNA from multiple antibiotic-resistant colonies. Sequencing results showed that a plasmid encoding 3G9 linked to hIns was engineered successfully. The presence of the hIns insert was verified using double digestion and gel electrophoresis. The 3G9/hIns antibody was successfully produced after transfection into 293T cells, with a resulting concentration of 0.156 mg/ml. In future studies, the 3G9/hIns antibody will be used to induce insulin presentation by dendritic cells via human DEC-205 receptors. The effect of insulin-presenting DCs on T cells and its ability to prevent or reverse T1D in non-obese diabetic (NOD) mice transgenic for human DEC-205 will be examined.

Drosophila kdm5^{140} Mutant and Ecdysone Hormone Pathway

Yemko Pryor, Coralie Drelon PhD, and Julie Secombe PhD
Genetics Department, Albert Einstein College of Medicine, Bronx, NY

The KDM5 family of histone demethylases is important in cancer research as well as research on intellectual disability. These proteins are histone modifiers that remove methyl groups from lysine 4 on histone 3 (H3K4) at the transcriptional start site of actively transcribed genes. In addition to its demethylase activity, KDM5 proteins have other domains that influence transcription. One of these is a PHD motif that binds to H3K4me3 and functions independently of the JmjC domain-encoded demethylase activity. The PHD domains of two mammalian KDM5 proteins, KDM5A and KDM5B, affect the activity and transcription of the estrogen and androgen nuclear receptors respectively, which has been linked to breast and prostate cancer progression. To examine the link between KDM5 and nuclear hormone function, we are using the model organism *Drosophila*, which encodes a single, essential *kdm5* gene. Ecdysone is the steroid hormone in insects that directs postembryonic development. The level of ecdysone spikes between different stages of the *Drosophila* life cycle and triggers the transcription of many primary and secondary response genes the regulate development through these stages. Ecdysone binds to the EcR/USP nuclear receptor complex in order to initiate transcription of its target genes. Ecdysone also promotes lipid metabolism during different stages of development, providing the energy for growth and metamorphosis. The *Drosophila kdm5* null mutant, *kdm5*^{140}, generated within our lab is developmentally delayed and appears to have more lipid accumulation than the wild type. For these reasons we decided to test whether *kdm5*^{140} mutants show defects in the in the ecdysone pathway in order to gain a better understanding of the interaction between KDM5 and nuclear hormones. Results from the Nile Red staining on 3rd instar larval fat bodies demonstrates elevated levels of lipid accumulation in *kdm5* mutants compared to the wild type. Quantitative real-time PCR data collected show a statistically significant decrease in two major ecdysone primary response genes *Br-C* and *E75*. These results suggest a possible connection between KDM5 and the ecdysone pathway. Additional data show that EcR expression is similar in UAS-*kdm5*^{RNAI} cells and wild type cells, suggesting that KDM5 may not affect of EcR itself, but may affect a different portion of the ecdysone pathway.

Determination of isoform specific functions of INI1/SMARCB1 variants: Implications for their role in cancer

Dormarie E. Rivera, Estah Bock, Ganjam V. Kalpana
Department of Genetics, Albert Einstein College of Medicine, Bronx, NY
Summer Undergraduates Research Program (SURP)

Integrase interactor 1 (INI1), also known as hSNF5 and SMARCB1, is a nuclear protein and a component of the SWI/SNF complex involved in chromatin remodeling. INI1 is a tumor suppressor mutated in rhabdoid tumors, a pediatric malignancy of the kidneys, brain, abdomen, liver, and other regions, with poor prognosis. The majority of INI1 mutations found in rhabdoid tumors are deletions or nonsense mutations. However, missense mutations such as, the P48S (Substitution of aa P to S at position 48), are also found in these cancers. Two major isoforms of INI1 exists that differ from each other by 9 amino acid (GKKTKPNTK) lysine-rich stretch, formed due to alternative splicing. Unpublished research in our lab, which examines the INI1 P48S mutant knock-out in mice, found an increase in the spliced isoform b (INI1b) expression in mouse tumors. Additionally, these tumors had an increased cytoplasmic localization of INI1. Since INI1 is a nuclear protein, it is intriguing to see this increased cytoplasmic localization selectively in tumors. At this point it is unclear if this increased cytoplasmic localization is due to isoform-specific function of INI1 or if it is the effect of P48S mutation. To understand the altered localization of INI1, we transfected MON cells, a rhabdoid-derived cell line lacking INI1, with different isoforms of INI1 with and without P48S mutation and carried out immunofluorescence analysis. We found that while the INI1a isoform is mostly nuclear, the INI1b isoform was also found in the cytoplasm. Interestingly, when P48S mutation was present, both the isoforms exhibited localization similar to INI1b isoform, showing increased cytoplasmic localization compared to the INI1a wild type. This data indicates that the P48S mutation disrupts INI1 nuclear localization, which may affect its tumor suppressor function.

Analysis of Nav1.8 positive sensory nerve fiber innervation in intact load bearing bones

Fiona Roediger[1,2,4,5], Zeynep Seref-Ferlengez[1,4], Gary J. Schwartz[2,3], David C. Spray[2,3], Mia M. Thi[1,2,4]

Departments of [1]Orthopedic Surgery, [2]Neuroscience, [3]Medicine, [4]Laboratories of Musculoskeletal Orthopedic Research at Einstein-Montefiore (MORE), Albert Einstein College of Medicine and Montefiore Medical Center, Bronx, NY; [5]UCLA Department of Integrative Biology and Physiology

Bone, including periosteum, endosteum, and marrow, is richly innervated by sensory and autonomic fibers, indicating the existence of a sophisticated neuronal regulatory mechanism. Nerve fibers enter the mineralized bone through the microvasculature and have been shown to establish direct connections with individual osteoblasts and osteoclasts, which provide the basis for a direct neural regulation of bone metabolism. However, the importance of this innervation to the skeletal physiology is not well understood. Specifically, the role of sensory nerve fibers in the regulation of bone mass and metabolism has yet to be thoroughly explored. Impairment of sensory or sympathetic nerves have been shown to affect bone integrity. These findings support the importance of neuronal regulation for skeletal homeostasis. There is also compelling evidence for neuronal regulation of load-induced bone formation and existence of neural mechanisms that facilitate functional skeletal adaptation by enabling cross-talk between loaded and unloaded bones. Thus the need for methods that provide 3D information to study bone is key to better understanding these mechanisms. The aim of the present study was to perform a comprehensive analysis of the sensory innervation in intact load bearing bones and place these fibers in a three dimensional reconstruction of the bone. This was done using Nav1.8-Cre-tdTomato reporter mice, where expression of tdTomato is driven by the promoter region of Nav1.8, a tetrodotoxin-resistant sodium channel present in large subsets of peripheral sensory neurons including both spinal and vagal afferents; a novel bone clearing technique; and confocal microscopy with voxel image analysis. Our results confirmed that this technique works. We saw that sensory nerve fibers richly innervate mineralized bone tissue and are located in close proximity with osteocyte lacunae. In the future, image resolution could be increased by using higher power objectives and two-photon microscopy.

Processing of Unattended Sounds During a Selective Attention Task

Gavriella Shandler, Renée Symonds, Elyse Sussman
Albert Einstein College of Medicine, Bronx, NY

Auditory scene analysis is the process by which we organize complex sound environments into individual meaningful elements. Our lab has shown that once the sound is organized we have the ability to attend to a single group, or stream, of these sounds. Some studies have shown that when we selectively attend to a single stream, we process less of the streams we are not attending to. However, these studies used only one feature (e.g., frequency) to segregate streams, which may not match a natural listening environment well enough to show the true depth of processing of unattended sounds. The current study uses stimuli that have multiple features to more closely mirror a complex auditory scene in order to investigate the processing of unattended sounds. Participants in the current study selectively attended to one stream of sounds and responded to deviants in that stream, while deviants occurred in the unattended streams. In one condition, these deviants always preceded the target deviants, thus acting as cues. We predict that the unattended sounds in a selective attention task are processed sufficiently to elicit a brain response and impact behavioral performance. We found that the unattended deviants elicit mismatch negativities, showing that they were processed. The deviant cues shortened response time, suggesting that the unattended cue worked effectively as an implicit warning that the target would occur. It is notable that the order of conditions mattered. For participants who listened to the uncued condition first, there was no change when cues were added. However, for participants who listened to the cued condition first, switching to the uncued condition increased response time. This suggests that the strategy that subjects adopted in response to their learning of the implicit cues was resistant to change.

Regulation of Macroautophagy in CD4 T Cells

Natalia Tumidajski and Yair Botbol, PhD
Department of Pathology, Albert Einstein College of Medicine, Bronx, NY
Diversity Student Summer Undergraduate Research Program

Macroautophagy is a cellular process of degradation of proteins and organelles through the lysosome. In CD4 T cell, autophagy is induced following engagement of T cell receptor. Whereas the molecular mechanisms underlying autophagy regulation have been extensively studied in nutrient starvation model, its regulation in CD4 T cells remains unknown. We sought to determine the role of known regulators of autophagy during activation-induced autophagy in T cells. For that purpose, we focus on analyzing ULK complex and its activity and also the role of AMPK on autophagy in T cell. We have first reproduced the activation-induced autophagy phenotype and shown that ATG13 expression was significantly increased while ULK expression was not modulated. However, the effects of inhibition of either ULK or AMPK on T cell autophagy were not totally conclusive as we have observed variations among our experiments. Nevertheless, our preliminary data suggest that ULK complex might be a positive regulators and initiators of autophagy in CD4 Th1 cells following TCR engagement.

I would like to thank Diversity Student Summer Research Opportunity Program (DSSROP) at Albert Einstein College of Medicine for funding this research project and the opportunity to have tremendous research experience. Especially I would like to thank Ms. Nilda Soto for her time and effort to make this opportunity possible. I would like to express my deepest gratitude and special thanks to my mentors, Dr. Macian and Dr. Yair Botbol for guidance, dedication, and great inspiration. Their enthusiasm and effort, wealth of knowledge, generosity with resources is unparalleled, and it will make a lasting impact on my life. Also, I would like to thank members of Dr. Macian lab including Samuel Dowling and Cara Reynolds for their kindness, and support.

skn-1, src-1, src-2 knockout in *C. elegans* alter susceptibility to MeHg toxicity

Rebecca Weitz[1,2], Mahfuzur R. Miah[3], Megan Culbreth[2], Michael Aschner[2,3]
[1]Yeshiva University Stern College for Women, [2]Department of Molecular Pharmacology, Albert Einstein College of Medicine, [3]Department of Neuroscience, Albert Einstein College of Medicine

Methylmercury (MeHg) is a highly toxic organometal found in the environment. Humans are exposed to MeHg through consumption of contaminated fish and plants, as well as through occupational exposure and anthropogenic mercury emissions. It has been previously shown that MeHg causes an oxidative stress response in our cells. The transcription factor, nuclear factor erythroid 2-related factor 2 (Nrf2), acts to combat this oxidative stress by moving into the nucleus and promoting cytoprotective gene expression. When Nrf2 levels are high, Fyn and other Src family kinases move into the nucleus and phosphorylate Nrf2. This results in Nrf2 export out of the nucleus and a subsequent down regulation of the oxidative stress response. The animal model *C. elegans* has homologs for these genes and has many conserved molecular pathways shared across species. In this study, we examined strains that had mutations for *skn-1* (Nrf2 homolog), *src-1* (Src homolog), and *src-2* (Fyn homolog). We hypothesized that without *src-1* or *src-2* present, there will be persistent *skn-1* activity, leading to greater protection against MeHg toxicity**.** To establish relevant dose exposure for our strains, we performed a lethality assay. We exposed L1 stage worms to doses of MeHg ranging from 0 - 100 μM and treated for one hour. After treatment, 20-30 worms were plated in triplicate and the percent of worms alive 48 hours later was recorded. After establishing LD_{50} values for each strain (wildtype: 46.61μM, *skn-1* KO: 39μM, *src-2* KO: 45.23μM, *src-1* KO: 35.68μM), we chose 20μM as the sublethal dosage for subsequent experiments. We performed qPCR assays to examine gene expression levels comparing MeHg treated and untreated worms. This assay was used to investigate how src family kinases differ between strains and how the cytoprotective genes differ due to treatment and genetic background. We were specifically interested in *gst-4, sod-1, src-1,* and *src*-2 (*gst-4* and *sod-1* are cytoprotective *skn-1* target genes, while *src-1* and *src-2* are src family kinases). Our results from the qPCR thus far have been inconclusive. Perhaps the dose level is too low and/or the time point is too far from the time of treatment. Additional replicates are needed for this assay. Our results from the lethality assay were consistent with previous research showcasing that knockout of *skn-1* led to increased susceptibility against MeHg toxicity when compared to wildtype. Contrary to our hypothesis, knockout of *src-2* was comparable to wildtype and knockout of *src-1* led to increased susceptibility to MeHg. It is conceivable that knockout of *src-1* or *src-2* led to compensatory upregulation of *src-2* or *src-1*, respectively. Because of the dramatic leftward shift of the *src-1* knockout and the comparability of the lethality curves of the *src-2* knockout to the wildtype, we speculate that *src-2*, the homolog to Fyn, is the stronger *src* family inhibitor of *skn-1*. Further replication of the qPCR, as well as other additional assays, are required to justify our speculation.

Acknowledgments: This research is supported by NIH ES R0110563; Caenorhabditis Genetic Center (CGC, MN, USA) and the Albert Einstein College of Medicine Student Undergraduate Research Program. Special thank you to the entire Aschner lab for their continuous guidance and support.

Regulation of Myosin-IIA Heavy Chain Phosphorylation by S100A4

Emily Williams, Jonathan Backer, and Anne Bresnick

Non-muscle myosin-IIA (MIIA) is a major regulator of contractility in motile cells. The MIIA monomer consists of two regulatory light chains, two essential light chains, and two heavy chains. The formation of MIIA filaments is regulated by phosphorylation of both the regulatory light chain, as well as the heavy chain. While several kinases can phosphorylate the MIIA heavy chain, protein kinase C (PKC) phosphorylates the heavy chain on a serine near the C-terminal end of the coiled coil (Ser1916). It has been shown that PKC-mediated S1916 phosphorylation mediates the disassembly of MIIA filaments. MIIA filament dissasembly is also stimulated by calcium-dependent binding of S100A4. S100A4 binds to a region of the MIIA heavy chain that encompasses the PKC phosphorylation site. We know phosphorylation of MIIA by PKC does not inhibit S100A4 binding, because the side chain of S1916 points away from the S100A4/MIIA binding interface. However, it is not clear how S100A4 binding affects PKC phosphorylation of MIIA. Using the Raw 264.7 macrophage cell line, we activated PKC by treating the cells with PMA and ionomycin, and then blotted whole cell lysates and MIIA immunoprecipitates with an antibody that recognizes PKC substrates. We also blotted MIIA immunoprecipitates for S100A4, to determine whether S100A4 binding was regulated under these conditions. Our results suggest that acute activation of PKC increases phosphorylation of MIIA in macrophages. Future experiments will determine whether the loss of S100A4 in Raw 264.7 CRISPR knockout cells affects PKC-mediated phosphorylation of MIIA.

Development and Symmetry of Mutants with Cell Competition Eliminated

Siwen Xie, Zhejun Ji, Nicholas E. Baker
Department of Genetics, Albert Einstein College of Medicine, Bronx, NY
SURP/DSSROP

Ribosomes have known for translation. Ribosomal proteins are not only a structural component of ribosome but also involve in growth regulation and cell competition. $Rp^{-/+}$ cells are known as *Minute* cells in *Drosophila,* and are out-competed from the mosaic tissues by wildtype cells located in proximity and actively undergo apoptosis. However, a point mutation in RpS12 protects *Minute* cells from cell competition in Minute RpS12 double-mutant cells. In addition, RpS12 regulates *Xrp1*, a putative transcription factor that is responsible for 212 genes whose expression is altered in $Rp^{-/+}$ cells. While $Rp^{-/+}$ flies have delayed growth, the *Xrp1*, $Rp^{-/+}$ double mutants suppress this delay. Our goal for this summer was to study the phenotypes of $Rp^{-/+}$, *Xrp1* double mutants to obtain some clues about potential functions that *Xrp1* has for cell growth and competition. To investigate the contribution of growth rate to the developmental delay, the wing size of *Minutes* and $Rp^{-/+}$, *Xrp1* double mutants, $RpS12^{G97D/-}$ mutants and wildtype flies were measured, and asymmetry was assessed by calculating percent difference between the two wings of each fly. The results suggest that growth rate may not be the only factor controlling developmental rate. Contrary to the hypothesis of de la Cova et al. (2004), *RpS12* mutant flies lacking cell competition did not show increased asymmetry, suggesting that cell competition does little to promote symmetry in normal development. $RpS12^{G97D/-}$ Drosophila had significantly smaller wings than control flies. Future experiments are necessitated for the yet unknown pathway of $RpS12^{G97D/-}$ behind this phenotypic difference. Finally, thanks to Fordham CSTEP for funding, SURP/DSSROP programs, Baker's Lab funded by NIH for scientific guidance.

Cannabinoid-mediated long-term depression of excitatory transmission at Schaffer Collateral-CA1 synapse

Hannah Yoo[1,2], Hannah Monday[1], Pablo E. Castillo[1]
[1]Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461, [2]Summer Undergraduate Research Program, Albert Einstein College of Medicine, Bronx, NY 10461

Synaptic plasticity is a biological mechanism in which a synaptic connection is strengthened or weakened by activity. Long-term potentiation (LTP) and long-term depression (LTD) are two forms of synaptic plasticity that play pivotal roles in learning and memory. Long-term plasticity can be characterized by changes in either postsynaptic or presynaptic components. Postsynaptic changes entail modifications in the number or function of postsynaptic receptors, whereas presynaptic changes involve increases or decreases in the amount of neurotransmitter release. It is well established that endocannabinoids (eCB) mediate presynaptic LTD (eCB-LTD) by activating the $G_{i/o}$ protein-coupled receptor cannabinoid receptor type 1 (CB1). Upon activity, eCBs are released from the postsynaptic neuron and target presynaptic CB1 receptors to reduce neurotransmitter release in a long-lasting manner. This form of plasticity is widely expressed in the brain and could play an important role in memory formation, drug addiction, depression and normal brain development. Moreover, eCB signaling could be a potential target for treatment of epilepsy, anxiety and other neurodegenerative disorders including Alzheimer's and Hungtington's.

Previous studies have demonstrated that eCB-LTD can occur at inhibitory synapses in the hippocampus. This LTD requires local presynaptic protein synthesis and can be mimicked by exogenous activation of CB1 receptors. Whether a similar form of plasticity also occur at hippocampal excitatory synapses is less clear. To address this issue, we examined excitatory inputs in the CA1 area which are known to play critical roles in memory formation. CA1 pyramidal cells receive excitatory inputs from CA3 region via Schaffer collateral path (Sch-CA1) and from entorhinal cortex via perforant path (PP-CA1). Sch-CA1, but not PP-CA1, synapses express presynaptic CB1 receptors. For this study, we specifically studied Sch-CA1 pathway. Using extracellular field recording potentials in acute slices of the rat hippocampus, we tested the effect of transient CB1 receptor activation on Sch-CA1 and PP-CA1 synaptic transmission. We found that 25-min bath application of the CB1 agonist WIN (5 μM) induced a form of chemical LTD at Sch-CA1. WIN-LTD was associated with an increase in paired-pulse ratio, suggesting a presynaptic mechanism of expression. Unexpectedly, WIN-LTD was not blocked by the protein synthesis inhibitor cycyloheximide (80 μM). Our findings suggest that eCB signaling can mediate mechanistically diverse forms of plasticity at CB1-expressing synapses in the CNS. We are currently examining the molecular mechanism underlying LTD at Schaffer collateral-CA1 synapses and studying other excitatory inputs such as the PP-CA1 synapse to understand the synapse-specificity of cannabinoid mediated LTD.

Supported by NIH grants R01-MH081935 and R01-DA17392

Identification of Direct Binding Between BK Potassium Channels and ADPGK and its Potential Role in Glycolysis

Joy Zou, Yi Wang, and Kelvin P. Davies
Department of Urology and Department of Physiology and Biophysics, Albert Einstein College of Medicine, Bronx, New York – Summer Undergraduate Research Program

BK potassium channels (encoded by the *slo* gene) are activated (opened) by changes in membrane electrical potential and/or by increases in concentration of intracellular calcium ($[Ca^{2+}]$). They regulate several important physiologic functions, such as smooth muscle tone and neuronal excitability. The importance of the BK channel in normal bladder function has been shown unequivocally through the use of $slo^{-/-}$ knock-out mice which have enhanced myogenic and nerve-mediated detrusor contractility and increased voiding frequency. These observations have led to many groups investigating the potential of BK channels as targets for treating bladder pathophysiology, such as overactive bladder. Recent studies by our group and others has suggested that BK plays a role in regulating metabolism and intercellular signaling, which may be more relevant to its function in the non-contractile tissues where it is expressed, such as the bladder urothelium. Although several BK interacting proteins have been identified, the goal of the present studies was to utilize the yeast two-hybrid screen to specifically identify BK protein-protein interactions that might be related to its involvement in metabolism

The yeast two-hybrid screen suggested a novel interaction between BK and ADP-dependent glucokinase (ADPGK), an enzyme involved in glycolysis. In order to confirm the interaction between the BK channel and ADPGK we co-transfected Hek293 with plasmids expressing *hslo-cmyc* (human slo gene tagged with cmyc) and *adpgk-gfp* (*adpgk* tagged with *gfp*). We also co-transfected Hek293 cells with plasmids expressing *hslo-cmyc* and *gfp* to detect any unspecific binding. Protein was isolated from the cell-lines after approximately 12 hours and used for co-immunoprecipitation (coIP) with the anti-cMYC antibody. An SDS-PAGE gel was run containing the protein lysates, cMYC-immunoprecipitated proteins, supernatant and a molecular weight marker, followed by semi-dry transfer of protein to a PDVF membrane. Western blotting was performed using anti-GFP as the primary antibody to detect ADPGK-GFP and GFP and then stripped and probed a second time using anti-cMYC as the primary antibody to detect hslo-cMYC. Visualization of immunoprecipitated proteins was also accomplished using silver staining.

Western blot CoIP analysis confirmed an association between the BK channel and ADPGK, with proteins of the correct molecular weight also observed from the silver stain. This work represents the first report directly associating the BK channel with an enzyme involved in glycolysis and supports our recent studies using metabolomics that inhibiting the BK channel activity can impact metabolism.

Special acknowledgement to:
Summer Undergraduate Research Program
Lilly Innovation Fellowship Award
National Institute of Diabetes and Digestive and Kidney Diseases; Diacomp Grant