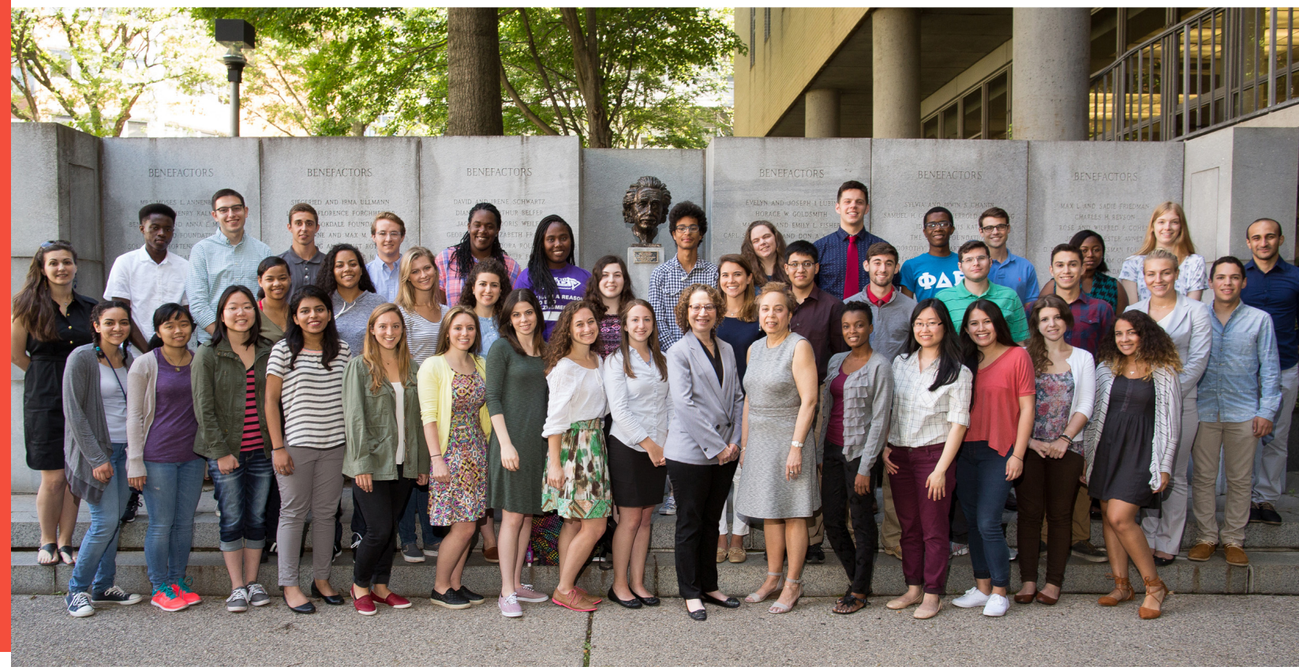

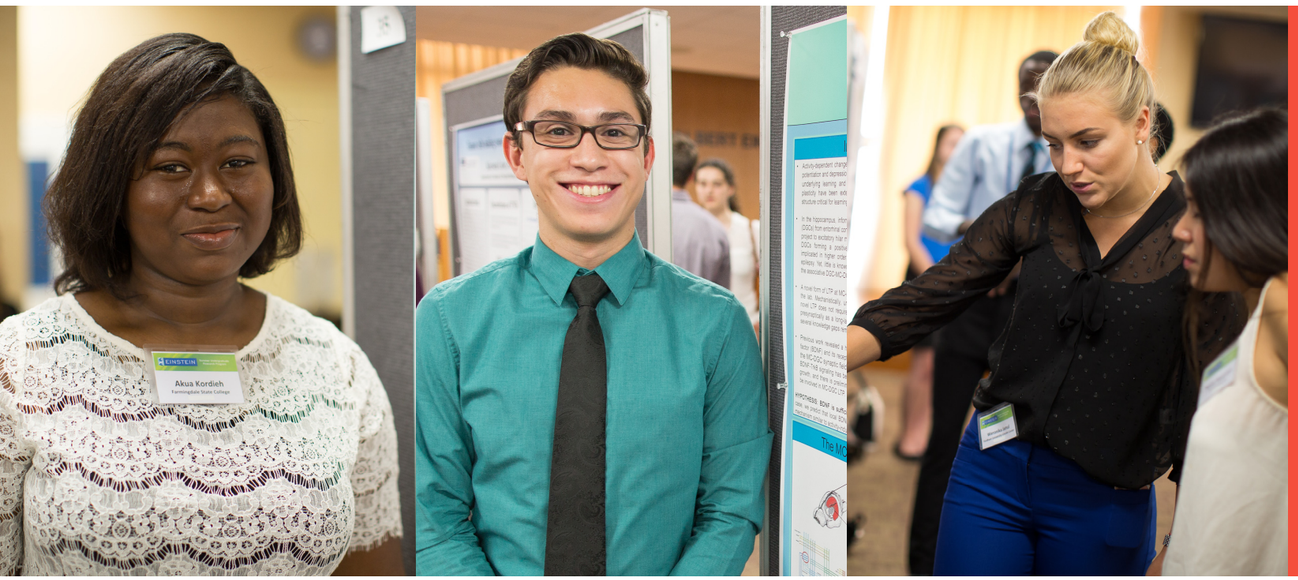

2016 ABSTRACT BOOK

Summer Undergraduate Research Program

Graduate Programs
in the
Biomedical Sciences

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Albert Einstein College of Medicine
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Graduate Division of Biomedical Sciences

SUMMER UNDERGRADUATE RESEARCH PROGRAM

2016

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

2016 Summer Undergraduate Research Program

Student Name	Undergraduate School	Mentor Name
Yewande Alabi	*University of California, Santa Cruz*	Charles Query
Gehad Ali	*Rutgers University*	Bridget Shafit-Zagardo
Ariella Applebaum	*Stern College for Women*	Joan Berman
Redwan Bhuiyan	*Wesleyan University*	Kira Gritsman
Martina Brave	*Hamilton College*	Antonio Di Cristofano
Kiera Brennan	*Rutgers University*	Julie Secombe
Daniel Carrera	*University Of Arizona*	Pablo Castillo
Kiersten Carter	*CUNY-Lehman College*	Dianne Cox
Joel Castillo	*Fordham University*	Elyse Sussman
Benjamin Cattau	*Haverford College*	Meelad Delawty
Olivia Chen	*Fordham University*	Saleem Nicola
Rachel Cheung	*Carleton College*	Wei-li Liu
Mark Choiniere	*Franklin Pierce University*	Jonathan Backer
Urieliz Cintron-Torres	*University of Puerto Rico in Ponce*	Hannes Buelow
Cassandra Dean	*Oberlin College*	Bernice Morrow
John DeBella	*Johns Hopkins University*	Florence Marlow
Rachel Gozland	*Yeshiva University*	Kostantin Dobrenis
Jordana Gross	*Stern College for Women*	Nicholas Sibinga
Anniya Gu	*Syracuse University*	David Shechter
Soulin Haque	*Fordham University*	Kimberly Reidy
Alyssa Hurley	*Western New England College*	David Spray
Adam Ibrahim	*SUNY Albany*	Michael Levi
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Akua Kordieh	*Farmingdale State College*	Nicholas Baker
Lauren Kozakiewicz	*Colgate University*	Bridget Shafit-Zagardo
Isaac Krasnopolsky	*Yeshiva University*	Vern Schramm
Eric Lee	*Lawrence University*	Thomas Ow
Stuart Levitin	*Yeshiva University*	Ertugrul Ozbudak
Katthia Loriston	*SUNY Postsdam*	Maria Abadi
Jessica Mayer	*Yeshiva University*	Myles Akabas
Eileen McAuley	*Bucknell University*	Matthew Gamble
Daniella Miller	*Yeshiva University*	Bernice Morrow
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Sara Wiener	*Stern College for Women*	Wei-li Liu

MYC Overexpression Increases Variant U2 snRNP Levels

Yewande Alabi, Brian Kosmyna, Britta Will and Charles Query
Department of Cell Biology - Albert Einstein College of Medicine, The Bronx, NY 10461
Summer Undergraduate Research Program

The spliceosome is a large and complex ribonucleic protein (RNP) that consist of 5 small nuclear RNPs (snRNPs): U1, U2, U4, U5 and U6 that base pair with and recognize splice site sequences in pre-mRNA. The spliceosome is the molecular machinery responsible for the two subsequent transesterification reactions that remove intronic regions of RNA and join the flanking exons together. Errors in splicing have been linked to multiple diseases and cancers; however not a lot is known about the mechanism by which splicing errors lead to disease.

MYC acts as a transcription factor and encodes for a protein that is vital in multiple cellular processes such as apoptosis, cell cycle regulation and cell transformation.[1] It has been shown that overexpression of MYC plays a role in the development of hematopoietic tumors, leukemias and lymphomas .[1] Recently, the Query lab has shown that many eukaryotes have U2 snRNA variants. Based on previous studies, it has been suggested that there is a correlation between MYC and U2 variants. Our project aims to investigate the role of MYC overexpression in variant U2 snRNAs and snRNP assembly.

MYC was overexpressed in U937, a cell line derived from a patient with AML, via transfection. Post transfection, MYC overexpression vectors were inhibited with JQ1. Cells were analyzed using fluorescence microscopy and cell sorting. RNA was isolated for subsequent RT-qPCR reactions to measure U2 variant levels. snRNP assembly was tested for in K562, U937 and Karpas cell lines through RNA immunoprecipitation of core splicing assembly factors like the SM core and SF3B1.

Preliminary RT-qPCR data suggest MYC overexpression may cause increased levels of U2 snRNP variants.

Our hypothesis suggests a mechanism by which MYC overexpression may lead to errors in splicing. If MYC increases variant U2 snRNP assembly, nucleotide changes in variant U2s may alter branch point and 3' splice site recognition by the spliceosome, which could potentially lead to catastrophic downstream effects. In the future we plan to test for MYC levels in various cell lines as well as variant U2 levels to find if there is a correlation. Afterwards, we plan to perform a snRNP-IP and RT-qPCR to test for increased snRNP assembly.

Acknowledgements

I would like to thank the Query Lab, especially Dr. Charles Query and Brian Kosmyna for providing a well organized and guided research experience. I would also like to thank the Will Lab, especially Dr. Britta Will for guidance in all aspects of cell culture and Mariana Ferreira for help with FACS.

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References:
[1]Koh, C. M. & Bezzi, M. *et al*. MYC regulates the core pre-mRNA splicing machinery as an essential step in lymphomagenesis. *Nature* (2015)

Characterization of Neuronal-specific AKT3 Conditional Knockout Mice

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

Multiple sclerosis (MS) is an autoimmune, demyelinating disease caused by infiltrating T cells that attack the myelin sheath and other components of the central nervous system (CNS) resulting in symptoms that range from fatigue, vision impariment, tingling, numbness, muscle weakness, paralysis, cognitive, sexual and bladder dysfunction (Compston, 2002; Thompson, 2001). MS is one of the most common causes of neurological disability in young adults affecting approximately 2.5 million people worldwide. To date, established MS therapies are solely immunomodulatory and do not aid in protection against CNS inflammatory insults and do not aid in repairing CNS damage. Our laboratory aims to identify and characterize potential neuroprotective and neuroregenerative pathways for future therapeutic interrogation.

AKT is a serine/threonine kinase of the AGC kinase family, existing in 3 isoforms: AKT1, AKT2, and AKT3 (Pearce et al, 2010). AKT1 and AKT2 are involved in normal cell growth and metabolism, respectively. AKT3 is the most highly expressed AKT isoform in the CNS where it plays a role in brain development and neuroregeneration (Easton et at, 2005; Tschopp et al, 2005), (Miao et al 2016). Previous studies in our lab showed that during myelin oligodendrocyte glycoprotein (MOG)-induced experimental autoimmune encephalomyelitis (EAE), a mouse model used to study various aspects of MS including CNS inflammation, demyelination, and axonal damage, mice lacking AKT3 ($AKT3^{-/-}$) had earlier disease onset and higher clinical scores relative to WT mice. Additionally, $AKT3^{-/-}$ mice had more demyelination and increased axonal damage relative to their WT counterparts. Furthermore, whereas in the spinal cord of naïve $AKT3^{-/-}$ and WT had equivalent levels of microtubule associated protein 2 (MAP2), a protein essential for the early development and continued maintenance of neuronal structure, $AKT3^{-/-}$ mice had decreased MAP-2 levels during EAE indicating neurodegeneration. Taken together, these data led to the hypothesis that AKT3 plays a neuroprotective role in neurons and is protective during EAE.

To address the role of AKT3 in neurons we generated conditional knockout (CKO) mice using $AKT3^{fl/fl}$ mice crossed with neuronal-specific Cre mice. Using immunohistochemistry (IHC), we observed differences in the expression of major brain proteins between $AKT3^{fl/fl}$ and $Cre3^{+}AKT3^{fl/fl}$ CKO mice. Naïve $Cre3^{+}AKT3^{fl/fl}$ CKO mice display decreased expression of MAP2 in the CA1 region of the hippocampus using two monoclonal antibodies to MAP2, one to the native structure and one to a phosphorylation-dependent epitope in the N-terminal end of MAP2 (AP-18). We also observed in the hippocampus of $Cre3^{+}AKT3^{fl/fl}$CKO mice an overall decrease in the expression of neurofilament protein as assessed by SMI31 (phosphorylated neurofilament-H), and SMI32 (nonphosphorylated neurofilament H), and myelin basic protein (MBP, SMI99) mice compared to $AKT3^{fl/fl}$ mice. Neurofilament H is a neuron-specific intermediate filament expressed in the cell body, dendrites and axons providing structural support and linking neurofilament proteins to the microtubule network. Phosphorylated neurofilament H is highly expressed in axons where it bundles neurofilaments and regulates axon diameter. MBP is an integral CNS protein involved in the myelination of axons. In the cerebellum, purkinje cells in $Cre3^{+}AKT3^{fl/fl}$ CKO mice had decreased expression of SMI31 and SMI32. IHC data also suggests decreased immunostaining of purkinje cell bodies and dendritic arborization.

Ongoing studies will confirm conditional deletion of AKT3 in neurons using flow cytometry and western blot analysis. Additionally, $AKT3^{fl/fl}$ mice will be mated with Synapsin-Cre mice to further confirm that conditional deletion in neurons results in the observed phenotype. We will also perform EAE on these mice and examine CNS inflammation, demyelination, and axonal damage.

HIV- TAT REGULATION OF HUMAN MONOCYTE PROTEINS THAT CONTRIBUTE TO HIV ASSOCIATED NEUROCOGNITIVE DISORDERS

Ariella S. Applebaum[1,2], Lillie Lopez[2], Bezawit Megra[2], Loreto Carvallo[2], and Joan W. Berman[2,3]

[1] Stern College for Women, Yeshiva University, New York, NY

[2] Department of Pathology, Albert Einstein College of Medicine, Bronx, NY

[3] Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, NY

Abstract

Despite the development of combined antiretroviral therapy (cART), HIV continues to be a major health issue, with 37 million people infected worldwide (UNAIDS: Report on the Global AIDS Epidemic 2010, Geneva, World Health Organization) and 40-70% of HIV-infected individuals developing HAND. HAND, HIV-associated neurocognitive disorders, develops even when successful cART suppresses peripheral virus. HIV enters the CNS parenchyma within 2 weeks of initial HIV exposure due to infected monocyte transmigration across the blood-brain barrier (BBB) in response to chemotactic signals. Increased chemotactic signals, such as CCL2 and CXCR12, present during HIV infection of the brain, facilitate significant transmigration of additional infeced and uninfected monocytes into the CNS. Junctional proteins on the monocytes, including ALCAM and JAM-A, undergo homo- and heterotypic interactions with proteins expressed on blood microvascular endothelial cells of the BBB to transmigrate and bring the virus into the CNS. Once inside the CNS, the monocytes differentiate into macrophages and, even in the presence of cART, release chemokines, cytokines, neurotoxic host factors, and neurotoxic viral factors, such as Tat. This results in demyelination, pruning, and neuronal injury and loss associated with HAND. HIV Tat is a small nuclear protein that activates viral transcription and is immediately produced after HIV infection, even with successful cART. In this study, we examined the role of Tat in the regulation of monocyte junctional proteins and chemokine receptors both transcriptionally and on the surface of four THP-1 cell lines. Stable THP-1 cell lines, human monocytes, were previously generated to express Tat-Flag by infection with lentivirus. We performed FACS analysis of these cells to determine surface protein. Results indicated increased chemokine receptors, CXCR7 and CCR2, and decreased junctional proteins, JAM-A, ALCAM, and PrPc. We further examined Tat-regulation of these genes through Western blot analysis for CXCR7, PrPc, ALCAM, and CCR2. We found a decrease in total cellular PrPc and ALCAM, with an increase in CCR2 and CXCR7 in response to Tat. Tat was also shown to decrease PrPc mRNA in our THP-1 cell lines through qRT-PCR. FACS, qPCR, and Westerns must be repeated to verify results, as data represents an n=1 or 2. Our data suggests that regulation of various proteins by Tat may contribute to increased transmigration across the BBB and result in HAND for the HIV-infected population.

Acknowledgements

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Einstein Summer Undergraduate Research Program

Testing for PI3K Isoform-selective Dependencies in a "Two-Hit Model" of Acute Myeloid Leukemia

Redwan Bhuiyan[1], Leanne Ostrodka[2], Michael Samuel[3], Taneisha Sinclair[3], and Kira Gritsman[3]

[1]Wesleyan University, Middletown, CT, [2]Children's Hospital at Montefiore, Bronx, NY
[3]Department of Medicine, Department of Cell Biology, Albert Einstein College of Medicine, Bronx NY

Acute myeloid leukemia (AML) has a 25% survival rate, mostly due to the toxicity of current standard treatments and to relapse after these treatments. Furthermore, chemotherapy fails to target leukemic stem cells (LSCs), which are thought to contribute to relapse. It has been hypothesized that AML follows a "two hit" model, in which at least two types of mutations, Class I and Class II, are required for full transformation. Class I mutations increase cell proliferation and survival, while Class II mutations inhibit differentiation and promote self-renewal. Mutations in NRAS and c-kit, both of which activate intracellular signaling, are examples of Class I mutation. The AML1-ETO fusion protein, which is formed as a result of the t(8;21) chromosomal translocation and affects transcriptional regulation, is an example of a Class II mutation. In patients, AML1-ETO usually coexists with a signaling mutation, such as c-kit or NRAS. The phosphoinositide-3-kinase (PI3K)/Akt signaling pathway is frequently over-activated in AML, and inhibition of this pathway affects the proliferation and survival of leukemic cells *in vitro.* Hematopoietic cells express four catalytic subunits of Class I PI3K (p110α, p110β, p110γ, and p110δ). Previous studies in our laboratory suggest that these isoforms have redundant roles in normal hematopoietic cells, but play specific roles in specific subtypes of AML. For example, Ras-mutated myeloid leukemias depend on the p110α isoform. AML1-ETO9a is a splice isoform of AML1-ETO that is expressed in AML patients, and can induce AML when introduced into mouse hematopoietic cells by retroviral transduction. Surprisingly, our laboratory recently found that mouse hematopoietic cells expressing AML1-ETO9a depend upon the p110δ isoform of PI3K. This raises the question of how effective the isoform-selective approach to inhibition of PI3K would be in the context of two different mutations coexisting in the same patient.

To determine how t(8;21) patients with a signaling mutation would respond to isoform-selective PI3K inhibitors, we examined the roles of the PI3K isoforms in two *in vitro* model systems of the "two-hit model": Kasumi-1 cells, a human AML cell line that has both t(8;21) and c-kit mutations (a Class I mutation), and mouse cells carrying both AML1-ETO and an activating mutation in NRAS. In Kasumi-1 cells we examined the effects of isoform-selective PI3K inhibitors on leukemia cell proliferation, apoptosis and differentiation. We observed that Kasumi-1 cells are sensitive to both p110δ and p110α inhibitors, which induce cellular changes consistent with myeloid differentiation. To investigate the roles of the PI3K isoforms in leukemic stem cells with both AML1-ETO9a and NRAS mutations, we transduced immature bone marrow cells isolated from lox-STOP-lox-NRAS-G12D (LSL-NRAS) knock-in mice, whose hematopoietic cells can be induced to express the G12D activating mutation, with a retrovirus expressing AML1-ETO9a. We confirmed excision of the STOP cassette in the bone marrow of LSL-NRAS mice, and then tested their *in vitro* proliferation, differentiation, and self-renewal activity in the serial replating assay, with and without isoform-selective PI3K inhibitors. We found that mouse hematopoietic cells carrying both AML1-ETO9a and NRASG12D initially formed more immature colonies, and that serial replating of these cells caused them to become sensitive to selective inhibitors of either p110δ or p110α. To determine genetically whether the oncogenic effects of AML-ETO9a and NRAS are mediated by p110α, we deleted p110α in hematopoietic cells in the LSL-NRAS genetic background, retrovirally transduced hematopoietic cells from these mice with AML-ETO9a, and performed the serial replating assay. We found that conditional deletion of p110α significantly reduced colony formation by AML1-ETO9a+NRASG12D cells, suggesting that p110α has a significant role in the proliferation of these cells. Thus, if we can continue to identify isoform-selective dependencies in these genetic contexts that mimic the genetics seen in a subset of AML patients, then it may be possible to specifically target leukemic cells in such patients using isoform-selective PI3K inhibitors, without significant toxicity to normal hematopoietic cells.

I would like to thank all the members of the Gritsman lab for lending me their support and expertise throughout the course of this program. I am especially grateful to Dr. Leanne Ostrodka, for overseeing my project and encouraging me through the project's ups and downs, to Dr. Shayda Hemmati, Dr. Imit Kaur, and Taneisha Sinclair for patiently answering my ceaseless questions, and to Dr. Gritsman, whose mentorship has inspired me to strive harder, work smarter and grow as a team member and a scientist.

The Role of SGK1 and -3 in PI3K-driven Thyroid Cancer

Martina Brave[1,2,3], Antonio Di Cristofano[2], Arturo Orlacchio[2]
[1]Hamilton College, 198 College Hill Rd., Clinton, New York
[2]Department of Developmental Molecular Biology, Albert Einstein College of Medicine, Bronx, New York
[3]Summer Undergraduate Research Program

The thyroid gland is one of the main endocrine glands of the human body. In 2016, the American Cancer Association expects 62,450 new cases of thyroid cancer with approximately 1,980 deaths. Anaplastic thyroid cancer (ATC) is the rarest form representing 2-5% of thyroid cancer, but is the deadliest with most patients dying within 6 months of diagnosis. It arises from the follicular cells of the thyroid gland, but is composed of undifferentiated cells.

By characterizing the pathway of cell proliferation in thyroid cancer, we can further understand the biochemical components of this form of cancer as well as design potential drugs that could help patients suffering from ATC and other forms of thyroid cancer. One major signaling cascade in thyroid cancer is the PI3K signaling pathway. The pathway relies on PDK1 simultaneously activating AKT as well as the AGC kinase family. Activation of the AGC kinases depends on the PIF-pocket domain of PDK1. If this domain is mutated, that portion of the pathway is silenced, and cell proliferation is decreased. However, mutation of the PIF-pocket domain does not affect AKT activation.

SGK1 and -3 are members of the AGC kinases family. The aim of our study was to determine the role of SGKs in thyroid cancer using both chemical and genetic inhibition in mouse and human derived cell lines. The shRNA-mediated SGK inhibition was measured through western blot to determine protein expression and cell counts to create a growth curve. The effects of pharmacological inhibition of SGK were measured through cell counts.

We found that when using chemical inhibitors that target SGKs we see a clear decrease in cell proliferation. However, when using shRNA-mediated SGK inhibition it is almost impossible to keep the SGK silenced. The cells were able to rescue SGK expression and continue growing at almost the same rate as the wild type (WT). Moreover, we also found that concomitant chemical inhibition of the AKT and SGK portions of the pathway synergistically cooperate in impairing cell proliferation in human thyroid cancer cells.

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Characterizing *kdm5* Mutations in *Drosophila*

Kiera Brennan, Coralie Drelon, Julie Secombe
Department of Genetics, Albert Einstein College of Medicine, Bronx, NY

Careful regulation of gene expression is necessary for proper cellular function and differentiation, whereas dysregulation can lead to disease. Crucial to this process are transcriptional regulators- proteins that affect the expression of specific genes in a context-dependent manner. The packaging of DNA into nucleosomes (bundles of DNA wrapped around positively charged histone proteins), allows for additional regulation of gene expression. Modifying histone proteins can affect how tightly DNA is wound or it can result in changes that are recognized by proteins that can impact transcription. One such family of histone modifiers is the KDM5 family of proteins, best known for their histone demethylase activities removing methyl groups from the lysine 4 on histone 3. Mammalian cells encode four KDM5 paralogs (KDM5A, KDM5B, KDM5C and KDM5D) whereas *Drosophila* has a single KDM5 ortholog, bypassing issues related to functional redundancy among the four mammalian paralogs. Clinical importance has been attributed to KDM5 proteins in humans, as loss of function mutations have been implicated in Intellectual Disabilities (ID) and their overexpression linked to cancers including lung, breast, and prostate cancers.

In the interest of creating fly strains with mutations in KDM5 that are comparable to human ID alleles, missense mutations were generated. The presence and location of these mutations was confirmed through Sanger sequencing. In order to generate a null *kdm5* allele for use in future experiments, deletions were generated through imprecise excision of a *P* element located upstream of the *kdm5* genes. Polymerase chain reaction (PCR) and quantitative PCR (qPCR) were performed in order to investigate whether true *kdm5* null alleles had been created. All of the ID mutants were confirmed to carry the intended missense mutations, except for one due to poor sequencing resolution and a likely null *kdm5* allele was identified, evidenced by molecular mapping and undetectable levels of *kdm5* transcript.

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A Role of BDNF in Long-Term Potentiation at the Mossy Cell to Dentate Granule Cell Synapse

Daniel Carrera, Yuki Hashimotodani, Andrés E. Chávez, Kyle R. Jensen, Pablo E. Castillo
Dominick P. Purpura Department of Neuroscience Albert Einstein College of Medicine, Bronx, NY; Summer Undergraduate Research Program

Activity-dependent changes in synaptic strength, in the form of long-term potentiation and depression (i.e. LTP and LTD) are the cellular mechanisms underlying learning and memory formation. These forms of synaptic plasticity have been extensively studied in the hippocampus, a brain structure critical for learning and memory. Information enters the hippocampus via entorhinal cortex inputs that synapse onto dentate granule cells (DGCs) in the dentate gyrus (DG). DGCs in turn, project to excitatory hilar mossy cells (MCs), which then project back onto DGCs forming a positive feedback loop within the DG. Although this associative circuit has been implicated in higher order pattern separation and its dysregulation linked to epilepsy, little is known about synaptic plasticity of this circuit. A novel form of LTP at MC-DGC synapses has been recently discovered in the lab. Mechanistically, induction of this novel LTP does not require NMDA receptor-activation, but is PKA-dependent, and is expressed presynaptically as a long-lasting increase in glutamate release. However, several knowledge gaps remains about the rules that governs this LTP.

Previous work revealed a high concentration of brain-derived neurotropic factor (BDNF) and its receptor, TrkB, in the inner molecular layer (IML), the MC-DGC synaptic field in the DG. In addition, preliminary results suggest that BDNF-TrkB signaling could be involved in MC-DGC LTP. Here, we test the hypothesis that BDNF is sufficient to induce MC-DGC LTP. To this end, we performed whole cell recordings from DGCs and stimulated the MC inputs of the IML. We locally puffed BDNF (8 nM) onto the MC-DGC synapse for 5 minutes at 0.5 Hz while monitoring excitatory postsynaptic currents. To control for the effect of puffing, we puffed artificial cerebral spinal fluid (ACSF) in a blind manner in separate cells. Our results indicate a significant increase in the amplitude of EPSCs after BDNF application compared to our control. Although, these results are preliminary and more experiments must be performed, our data show that BDNF in the absence of repetitive presynaptic activity is sufficient to induce LTP.

We propose that synaptic stimulation releases BDNF, which is necessary and sufficient to induce MC-DGC LTP. We predict that BDNF-LTP should be blocked by interfering with TrkB and PKA signaling, and that BDNF-LTP and MC-DGC LTP should occlude each other. Finally, future experiments should determine whether BDNF is released from the presynaptic (MCs) or postsynaptic (DGCs) neurons and the precise location of TrkB receptors at this synapse.

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Tumor cells making connections through Tunneling Nanotubes

Kiersten Carter[1], Samer Hanna[2], Dianne Cox[2]

1 Summer Undergraduate Research Program

2 Department of Anatomy & Structural Biology, Albert Einstein College of Medicine, Bronx, NY

Cell-to-cell communication is essential for biological functions. Macrophages have been observed to interact with tumor cell through Tunneling Nanotubes (TNTs). These are membranous structures that pass materials between plasma membranes, such as signals, nutrients, mitochondrial DNA, etc. In breast tumors, TNTs are few in numbers when alone; however, when tumor cells and macrophages are co-cultured there is an increase in TNT formation. Specifically, when tumor cells are cultured in macrophage conditioned media (CM), TNTs are observed to form in greater numbers. We tested the CM to see which factors are involved in the increase of TNTs. After confirming tumor cell TNTs increased in CM, we conducted experiments isolating exosomes and EGF (Epidermal Growth Factor), which are found in CM. Exosomes are RNA and protein cargo containing vesicles that are secreted by all cell types and are also considered another form of intercellular communication.1 Our results show that there was a significant effect of TNT formation with EGF and blocking EGF in the CM reduced TNT formation. On the other hand, isolated exosomes did not have a significant effect on TNT formation. Meanwhile, in order to explore the mechanism of tumor cell TNT formation we also conducted an experiment to measure tumor cell expression of M-sec (M cell sec protein), also known as TNFAIP2 (tumor necrosis factor, alpha-induced protein2), which is known to be an important factor in TNT formation in macrophages. Preliminary data shows a decrease in TNT formation when M-sec is depleted when treated with. This suggests that M-sec levels are decreased within the MTLn3 cells. With further investigation, we will use western blot analysis to measure M-sec levels and repeat to confirm data.

Which Perception is it Anyways?: Investigating Stream Segregation using the Auditory Steady-State Response

Joel Castillo, Juin-Wan Zhou, Renée Symonds, Elyse Sussman
Department of Neuroscience, DSSROP
Albert Einstein College of Medicine

Auditory scene analysis is the ability to perceptually group distinct sound sources into manageable units. This occurs on a daily basis, as when one listens to a conversation in a noisy environment. Thus, auditory scene analysis is crucial for interpreting sound in everyday life. Previous studies have established that the perception of two sound sources depends on the frequency separation between tones. However, these studies have focused on discrete, alternating, non-overlapping tones. Our current study aimed to determine whether the same relationship held true when complex, overlapping amplitude-modulated (AM) tones were used. These tones have typically been used to elicit an auditory steady-state response (ASSR), a brain response to sounds that follows the high repetition rates of the tones presented. If two tones are presented at different presentation rates, the ASSR could be used to determine the neurophysiological representation (one or two sources) of these sounds. To validate the use of AM tones, we conducted a behavioral study to investigate participants' perception of these tones at different frequency separations. We hypothesized that the perception would shift from one to two sound sources as the frequency difference increased. Frequency separation effects held for overlapping AM tones; participants were more likely to perceive two sound sources the greater the difference in frequency. The validation of complex AM tones provides a basis for ongoing neurophysiological studies investigating the ASSR as an index for stream segregation.

Generation of expression constructs to dissect DNA binding functions of CXXC domain containing proteins Idax & Rinf in ESCs

Benjamin Cattau, Run Lei PHD, Katherine Howgego MD & Meelad Dawlaty PHD

Department of Genetics and The Institute for Stem Cell and Regenerative Medicine Research, Albert Einstein College of Medicine, Bronx NY

Idax and Rinf are CXXC DNA-binding-domain-containing proteins that regulate the Wnt pathway as well the Tet family of DNA modifying enzymes. Loss of these proteins in ESCs affects their proliferation and differentiation. However, it is not known whether the DNA binding or the Wnt signaling functions of Idax and Rinf regulate these properties of ESCs. To dissect this, we have generated tagged Idax and Rinf CXXC mutant transgenes and have sub-cloned them into mammalian expression vectors. These constructs will be tested for rescuing the phenotypes of Idax and Rinf deficient ESCs in order to define the requirements of DNA binding functions of Idax and Rinf in ESCs.

The Effects of Optogenetic Stimulation of Dopamine Neurons on Omission-Induced Extinction Behavior

Olivia Chen and Andrew Kirvin-Quamme, Cindy M. Reyes, Saleem M. Nicola, Ph.D.
Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY, Summer Undergraduate Research Program

Cues associated with external rewards, like drugs of abuse, are known to cause a release of dopamine (DA) in the Nucleus Accumbens (NAc). This release of DA is due to dopaminergic afferents from the Ventral Tegmental Area (VTA). Our lab has shown that many NAc neurons are excited by cues and that these excitations precede the onset of cued approaches toward predicted rewards. Further, these excitations predict the probability of future behavioral responses to the cue, and use of DA receptor antagonists has shown that these cue-evoked excitations are DA-dependent and necessary for behavioral responses to reward-predictive cues. Previous studies have suggested that DA is sufficient to prevent extinction behavior; we aim to further study this by using in vivo stimulation and recording in the VTA and NAc, respectively. We hypothesized that when extinction of cued-approach behavior occurs in response to repeated omission of a reward, DA-dependent cue-evoked firing also decreases and that this decrease can be prevented by an increase in DA release.

To test this we performed intracranial virus injection surgery on Long-Evans rats expressing Cre-recombinase in dopaminergic (tyrosine hydroxylase-positive) neurons (*TH::Cre* rats). An adeno-associated virus containing Cre-inducible channelrhodopsin-2 (ChR2) was injected into the VTA. Expression of ChR2 allows for externally controlled excitation of DA neurons in the target area via photostimulation with blue light. After surgery, we trained the animals on a conditioned stimulus (CS) task where an auditory cue (CS+) predicted the availability of reward if the animal entered the reward receptacle within 5 seconds of cue onset. We then fitted the animals with fiber optic implants in the VTA and microelectrode arrays in the NAc, allowing simultaneous optogenetic stimulation and recording. Next, animals underwent one session of a reward omission paradigm where neurons were recorded but no photostimulation was issued. The session included a 30-minute baseline of reward administration in response to the CS+, followed by 60 minutes of reward omission. The next day, animals participated in a similar session where they received photostimulation in place of the omitted sucrose reward.

By recording at the time of optical stimulation we will be able to determine if reduction of cue-evoked excitations is prevented by DA release as hypothesized. We will then be able to suggest a mechanism by which cue-evoked excitations in the NAc are modulated during extinction behavior.

A Tale of Two Tumor Suppressors: A Direct Interaction between PBAF and p53

Rachel Cheung, Charles Kenworthy, Robert A. Coleman, Wei-Li Liu
Department of Anatomy & Structural Biology, Albert Einstein College of Medicine, Bronx, NY

PBAF, or polybromo/BRG1-associated factor, is a member of the SWI/SNF family of ATP-dependent chromatin remodeling complexes with multiple other functions, including centromere cohesion, transcriptional repression, and DNA repair. Recent whole-exome surveys have shown that deleterious mutations in SWI/SNF components are found in high frequencies in single cancer types, including ovarian clear cell carcinoma and renal cell carcinoma. *TP53*, which encodes for the most frequently mutated protein in human cancer (p53), had an estimated average mutation frequency of 26% across multiple human cancers. In the same study, SWI/SNF's average mutation frequency was estimated at 19%, suggesting these chromatin remodelers play a role in tumor suppression.

Beyond mutational frequencies, PBAF has been tied to tumor suppression through two of its unique subunits, BRD7 and BAF180. BRD7 has been shown to co-immunoprecipitate with the tumor suppressor and transcription factor p53, and both BRD7 and BAF180 have been implicated in p53 target gene regulation. However, the direct relationship between p53 and PBAF remains unclear. Understanding the interaction between these two tumor suppressors could elucidate cancer development mechanisms.

In order to better understand PBAF's role in tumor suppression, we investigated its relationship with p53. We hypothesized that a direct protein-protein interaction between p53 and PBAF existed. We used U2OS cells virally overexpressing HaloTag and FLAG-Tag conjugated to BAF180 in order to simulate overexpression of PBAF, as well as U2OS cells virally overexpressing just the HaloTag and FLAG-Tag as negative control. We upregulated p53 in these cells by treating them with Nutlin-3a, a cis-imidazoline analog that blocks a mechanism of p53 inhibition. We then conducted a co-immunoprecipitation against the FLAG-tag by using these cells to isolate BAF180, and performed Western blot analyses to see if the isolated component of PBAF interacted with p53.

One of our BAF180WT co-immunoprecipitation samples produced a p53 band, indicative of an interaction between PBAF and p53. Different immunoprecipitation conditions failed to produce this band, suggesting the interaction between PBAF-p53 is highly dynamic. Future studies could investigate how cancerous mutations of BAF180 affect PBAF's interaction with p53. In addition, exploration of the localization of PBAF on p53 target genes could inform how PBAF modulates other tumor suppressive functions.

In Vitro Binding of p85α to Rab5 Isoforms

Mark Choiniere[1]; Samantha Heitz[2]; Anne Bresnick[3]; Jonathan Backer[2, 3]

Franklin Pierce University[1]; Departments of Molecular Pharmacology[2] and Biochemistry[3], Albert Einstein College of Medicine, Bronx, NY; Summer Undergraduate Research Program (SURP)

Phosphoinositide 3-kinases (PI3K), play a role in a variety of cellular functions such as cell growth, survival, and motility. p85α is the regulatory subunit of the Class 1A PI3-Kinases, and forms a dimer with p110β catalytic subunits. Rab5 is a small monomeric GTPase that helps mediate early endocytosis in the cell as well as vesicular fusion. In a variety of cancers, such as breast cancer and prostate cancers, the PI3K signaling pathway becomes deregulated. Both the p110β catalytic subunit and p85α regulatory subunit have been suggested to bind to Rab5 in co-immunoprecipitation experiments. We set out to test this using highly purified p85α, to determine whether or not the interactions are direct. In order to test these interactions, we immobilized recombinant GST-Rab5 on glutathione beads, and loaded them with GDP or the non-hydrolyzable GTP analog GTPγS. We then incubated them with either p85α and p85α/p110β expressed in HEK293 cells, or purified recombinant p85α and p85α/p110β. In both assays, we observed GTP-dependent binding to p85α/p110β but not to p85. In additional experiments, we are testing whether the Rab5 binding domain from Rabaptin-5 could serve as a more robust positive control for GTP-dependent binding. We conclude that p85α does not bind directly to activated Rab5.

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A novel addition to a cell adhesion complex that regulates PVD dendrite development

Urieliz M. Cintron-Torres[1], Maisha Rahman[2], Hannes E. Bülow[2, 3]
Department of Biology, University of Puerto Rico in Ponce, Ponce, PR[1]
Summer Undergraduate Research Program (SURP), Albert Einstein College of Medicine[1]
Department of Neuroscience[2] and Department of Genetics[3], Albert Einstein College of Medicine, Bronx, NY

The presence of well-developed dendrites in the nervous system plays an important role in intracellular communication and responses to stimuli to control functions of the body. Importantly, nervous system defects as a result of defective dendrites may lead to neuropsychiatric diseases such as autism and schizophrenia. Caenorhabditis elegans is an excellent model to study dendrite development due to its simple nervous system, transparent body, and because all of its genome is already known. PVD are a pair of somatosensory neurons in the body of C. elegans that are responsible for response to external stimuli, such as harsh touch and cold temperatures. PVD dendritic branches have a menorah-like structure, which distinguishes them from other neurons in C. elegans. Previous studies have shown that this structure is patterned by the conserved SAX-7/L1CAM and MNR-1/Menorin cell adhesion complex and DMA-1, a leucine rich transmembrane receptor. We have now found that LECT-2, which is a diffusible protein secreted from the muscle of the worm, serves an important role in patterning the highly organized structure of PVD dendrites together with the SAX-7–MNR-1–DMA-1 complex. LECT-2 was discovered twenty years ago, but its developmental role is not yet characterized. To elucidate which other genes could contribute to PVD arborization, a screen for genes that modify a hypomorphic lect-2 allele has been performed and several new mutants have been identified. I will conduct a series of complementation tests to determine which of these mutants affect new genes involved in PVD dendrite development. Our finding is that the enhancer 6C2 and mnr-1 failed to complement each other meaning that this mutant could potentially be a new allele of mnr-1 that interacts with lect-2. More complementation tests will be performed with other enhancers. The mnr-1 allele and all the genes that fail to complement, will be sequenced for identification.

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Investigating the roles of T-box genes in inner ear development

Cassandra Dean, Stephania Macchiarulo, Bernice Morrow

Department of Genetics, Albert Einstein College of Medicine

Inner ear development is still not yet fully understood despite hearing loss being the most common sensory defect in humans, affecting 1 in 1,000 individuals by early childhood. Many of the genes that drive inner ear development in particular are still unknown, while other genes such as *Tbx1* (*T-box protein 1*) have been thoroughly investigated. T-box genes are a family of transcription factors which play an important role during development and have been linked to several human disorders including 22q11.2 deletion syndrome (22q11DS), which occurs in 1/4000 live births. *Tbx1* has also been shown to play a significant role in repressing neurogenesis in the cochleovestibular ganglion (CVG) as have other genes such as *Notch1*. It is not known, however, what genes or pathways may act downstream of *Tbx1* to regulate neurogenesis. Thus, we decided to take a candidate gene approach by examining the relationship between *Tbx1* and Notch pathway genes, particularly *Jag1* (*Jagged1*). Secondly, since no research has previously investigated the roles of other T-box genes in inner ear development, *Tbx2* and *Tbx3*, we decided to inactivate them individually and together to ascertain whether they have necessary and/or redundant functions in inner ear development, as well as whether they might genetically interact. To address these aims, I performed phenotypic analyses in these mutants. Further, I designed and tested RNA probes to various inner ear genes used to determine their pattern of expression in these inner ear during development. These probes will eventually be used to determine what cell types are affected in our mutants of interest. Overall, we aim to use mutations in T-box genes to reveal their roles in inner ear development and therefore better understand the human disorders the gene family is linked to. *NeuroD* expression in the CVG expands in *Tbx1;Jag1* conditional double mutants at E10.5, indicating a synergistic enhancement of the *Tbx1* null phenotype. *NeuroD, Lmo4, Notch1*, and *Sox2* RNA probes all show expressionin the inner ear, as well as other structures, that closely resemble previously published expression data. Inner ear development is more greatly disturbed by mutation of *Tbx2* than *Tbx3*. *Tbx2* appears to be partially compensating for *Tbx3* mutation causing a phenotype close to wild-type. *Tbx2*and *Tbx3* may be genetically interacting.

Understanding the interactions of Rbpms2 and Buc in germ cell formation

John M. DeBella, Odelya H. Kaufman, Florence L. Marlow
Department of Developmental & Molecular Biology
Albert Einstein College of Medicine Summer Undergraduate Research Program

Germ cells form either by inductive cues or by inheriting a substance called the germ plasm, made up of the determinants required for germ cell development. In zebrafish, germ cells form via inheritance of germ plasm, which is first localized in stage I of oogenesis. It consists of cytoplasmic RNAs and proteins, which are required for establishing the germ line. The Balbiani body (Bb) is the structure in which the germ plasm localizes in primary oocytes, and it is responsible for delivering patterning RNAs to the vegetal pole of the embryo. Previous studies have demonstrated that *bucky ball* (*buc*) is necessary for Bb formation in oocytes. In embryos, Buc is present in and localizes to the cleavage furrows of early WT embryos, and overexpression of *buc* via injection of exogenous RNA leads to a higher number of germ cells than is typically seen. The functionality of these new germ cells has not been determined, but they have been observed to share several characteristics with WT germ cells, specifically with respect to gene expression and migration. Because Buc protein interacts with RNA binding proteins (RNAbp), including Rbpms2, a potential pathway in the formation of additional germ cells may involve recruitment of germ cell components via RNAbps. In order to understand the role of Rbpms2 in germ cell formation, techniques such as microinjection of RNAs encoding tagged proteins and visualization of germ cells through *in situ* hybridization are utilized. Through these and other approaches, we will gain insight into potential role that Rbpms2 may play in modulating Buc activity during germ cell formation.

Identifying Novel Mechanistic Targets for Therapy of Niemann-Pick Type C Disease

Rachel Gozland, Ben Harpe, Andrew Smith, Kevin Fisher, Ben Papapietro and Kostantin Dobrenis
Dominick P. Purpura Dept. of Neuroscience, Albert Einstein College of Medicine, Bronx NY
Summer Undergraduate Research Program

Niemann-Pick type C (NPC) disease is an autosomal recessive lysosomal storage disorder arising from mutations in either the *NPC1* or *NPC2* gene. The disease is characterized by the lysosomal storage of cholesterol and accumulation of other lipids within cells, which results in neurodegeneration and ultimately early death.

There is no known cure for NPC disease, but some treatments, such as Cyclodextrin (CD) and Miglustat (Mig), have been found to alleviate several symptoms and extend lifespan. However, the mechanisms of these drugs, and exactly how they act on NPC disease, are not well understood. Comprehension of these pathways of action is crucial, not only because these drugs are seeing clinical trials but also in order to improve upon these therapies and potentially identify alternative superior small molecule therapies.

One focus is to discover pathways and their constituents affected by CD, Mig, or their combined use (Combo), which has recently been tested, and to use this information to learn more about their roles in NPC disease pathogenesis and therapy. Based on our lab's previous genome wide microarray analysis of neocortex-derived material from treated and untreated mice, we conducted immunofluorescence assays to evaluate the expression of three proteins with altered gene expression not previously implicated in NPC disease: Plexin B3 and TTYH2 in untreated WT and NPC disease 8-week old mouse cerebrum; and Sez6l2 in mouse cerebellum. We employed confocal microscopy to evaluate protein localization and expression. More cells were found positive for Plexin B3 in the mutant and these corresponded to a subset of largely non-neuronal cells, resembling oligodendrocytes or microglia, in both grey and white matter where some positive staining also appeared to encircle axons. TTYH2 also more abundantly labeled the mutant tissue than the WT, which largely, but not exclusively, co-labeled with anti-NeuN positively identified neurons. Anti-Sez6l2 displayed especially strong labeling of Purkinje neurons of cerebellar cortex in WT and remaining Purkinje cells in disease mice. Purkinje cells are prominently progressively lost in this disease. More detailed analyses, including quantitative image analyses, are ongoing. Gene expression microarray studies on laser microdissected Purkinje cells are also in progress.

To gain further insights, we applied Ingenuity Pathway Analysis (IPA) on the collected microarray data of neocortical tissue. We conducted multiple comparison analyses of predicted upstream regulator effects between NPC untreated and NPC treated with CD, Mig, or Combo. Of 63 pathways linked to upstream regulators predicted significantly altered in the disease, we identified 35 predicted to return to WT status by CD, and only 25 predicted to return to WT activity levels by Combo. In contrast, Mig treatment induced no significant changes to the disease in even the original dataset. In addition, we identified 3 upstream regulators hypothesized to be affected by CD that were not originally altered in the disease. We found one other such "side effect" involved in the Combo therapy. These findings seem to imply that CD may by far be the most effective treatment of the three, and that the addition of Mig might only hinder the effectiveness of CD. Future investigation of specific upstream regulators identified may clarify if and how they are in fact affected by NPC and its treatments.

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Effect of β-catenin Inhibitors on Growth of Vascular Smooth Muscle Cells and Endothelial Cells and its Applications

Jordana N. Gross, Dario F. Riascos-Bernal, Nicholas E.S Sibinga.

Departments of Medicine/Cardiology and Developmental and Molecular Biology, Wilf Family Cardiovascular Research Institute, Albert Einstein College of Medicine, Bronx, NY. Summer Undergraduate Research Program.

Vascular remodeling occurs in response to injury and disease, as seen in atherosclerosis and restenosis. Vascular injury damages endothelial cells (ECs), triggering inflammation and thrombosis, and releasing factors that activate smooth muscle cells (SMCs). SMCs in turn migrate, proliferate, and remodel the extracellular matrix, aiming at healing the vessel wall. Recovery of the endothelium is also critical for healing. Excessive proliferation of SMCs, however, or delayed recovery of ECs, promotes formation of a neointima that blocks the arterial lumen, limiting blood flow and causing tissue ischemia. The mechanisms controlling SMC and EC activities after vascular injury are not fully elucidated. Genetic studies suggest that β-catenin is required in ECs only in the central nervous system circulation, while in SMCs, it is essential for artery formation and neointima formation after vascular injury. Based on these observations, *we hypothesize that pharmacologic inhibition of β-catenin inhibits growth of SMCs while not affecting EC growth*. **Methods:** We evaluated growth of mouse aortic SMCs, human coronary artery SMCs, and mouse cardiac ECs in culture using AlamarBlue after exposure to increasing concentrations of β-catenin inhibitors: PKF118-310, ICG001, XAV939, and Carnosic Acid. We also evaluated the effect of these inhibitors on β-catenin activity in SMCs using a TOPflash reporter system. **Results:** We found that PKF118-310, ICG001, and XAV939, but not Carnosic Acid, inhibit mouse and human SMC growth in a dose-response manner. PKF118-310 and ICG001 also inhibited mouse EC growth in a dose-response manner, but with decreased potency; while XAV939 did not affect EC growth. We also found that PKF118-310, ICG001, and XAV939, but not Carnosic acid, inhibit β-catenin/TCF activity in SMCs. **Discussion:** These studies suggest that blocking either β-catenin/TCF interaction, CBP/β-catenin C-terminal interaction, or promoting the activity of the destruction complex are effective strategies to inhibit growth of SMCs, with a less pronounced effect on ECs; however, the ECs used were not primary cells and this might explain their decreased sensitivity. We are currently testing primary human coronary artery ECs. **Conclusion:** Pharmacological inhibition of β-catenin inhibits growth of vascular SMCs in culture, providing a rationale to study these inhibitors in models of vascular injury as they hold promise as novel therapies for cardiovascular disease.

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The second acidic patch of histone chaperone Nucleoplasmin binds the maternal linker histone H1M in *Xenopus* eggs

Anniya Gu[1,2,3], Benjamin Lorton[1], Christopher Warren[1] and David Shechter[1]

[1]Department of Biochemistry, Albert Einstein College of Medicine, Bronx, NY.
[2]Syracuse University, Syracuse, NY.
[3]Albert Einstein College of Medicine Summer Undergraduate Research Program

In eukaryotic cells, DNA is wrapped around core histones H2A, H2B, H3, and H4 forming repeating units called nucleosomes. The linker histone, H1, sits between nucleosomes on segments of linker DNA. Because histones are highly charged they tend to form aggregates, which can be damaging to the cell. Therefore, specific proteins called histone chaperones bind and neutralize free histones. Histone chaperones are implicated in a variety of processes involving histone transfer such as storage and deposition. We are specifically interested in the role of histone chaperones in storing and releasing maternal linker histone, as it is essential for the establishment of an embryonic epigenetic state. In the period of development immediately after fertilization, there is an absence of transcription in the dividing zygote. All cell cleavages are instead regulated by a storage of maternal proteins and mRNA molecules. *Xenopus laevis* makes an especially good model system with which to study maternal influence on development because zygotic gene expression does not begin until the 4000-cell stage. Histone chaperones, such as Nucleoplasmin (Npm), have been implicated in the storage of histones in oocytes but little is known about histone chaperones for the linker histones.

Although Npm is primarily a chaperone for H2A/H2B, previous data in our lab indicates that Npm may also interact with H1M. In order to investigate the hypothesis that Npm is involved in chaperoning H1M, we conducted immunoprecipitation assays to determine if H1M was co-precipitated with Npm.

We observed H1M present in the Npm IP, consistent with the hypothesis that Npm interacts with linker histone. Furthermore, we conducted pull-down assays with different truncations of Npm to determine which domain of the protein interacts with H1M. Previous research in our lab indicates that Npm, made up of a core domain, acidic patches (A1, A2, and A3) and a disordered tail domain, binds core histones H2A/H2B at acidic patch, A2, and preliminary results suggest that H1M binds Npm in the same acidic A2 patch as the core histones. Competition assays between linker histone and the core histones indicate that H2A/H2B has a higher affinity for Npm than H1M.

APOL1 High Risk Alleles of Mother and Baby with Preeclamptic Birth Complications

Soulin Haque[1,2*], Bianca L. Ruiz, BS[1*], Rebecca Hjorten, MD[3], Stacy Rosenblum, MD[4], Joseph Myrie, BS[1], Masako Suzuki, PhD[1], Sandra Reznik, MD, PhD[1], Cheryl Winkler PhD[5], Jeffrey Kopp, MD[5], Frederick Kaskel, MD[1] and Kimberly J. Reidy, MD[1,3]

[1]Albert Einstein Medical College, Bronx, NY, United States
[2]Department of Biology, Fordham University, Bronx, NY United States
[3]Pediatric Nephrology, Children's Hospital at Montefiore, Montefiore Medical Center, Bronx, NY, United States
[4]Neonatalogy, Children's Hospital at Montefiore, Montefiore Medical Center, Bronx, NY United States
[5]National Institute of Health, Bethesda, MD, United States

Preeclampsia, characterized by high blood pressure, is a condition that complicates many pregnancies. There is an increased risk for African Americans, who are three times more likely to die from preeclampsia than Caucasians. African Americans also have an increased risk of developing end stage renal disease (ESRD). Variations in the APOL1 gene, termed G1 and G2 (HR) alleles, were found to be significant risk factors for ESRD. Pre-eclampsia results in part from microangiopathy in the glomerulus of the kidney, and previous studies have shown that 50% of the risk of the preeclampsia is due to genetics. We hypothesized APOL1 high risk variants would be associated with increased risk of preeclampsia. Mother and baby samples of placenta and umbilical cord, respectively, with preeclamptic birth complications were used. DNA was extracted and genotyped for APOL1 gene from the samples. Preliminary results indicate: Of the 19 mothers genotyped, 2 had high-risk alleles (11%) a proportion not dissimilar to general population. However, of the 24 babies genotyped, 7 carried the high risk alleles (29%), which is significantly different from general population (p-value 0.01, chi-square). When comparing birth outcomes, births with babies with APOL1 high-risk status have a lower gestational age, 32 versus 37 weeks (p-value 0.02, Mann–Whitney U test). While limited by small sample size in this preliminary study, our results suggest that high-risk APOL1 status may be present at a higher proportion in babies born from births complicated by preeclampsia and that they are more likely to have poorer perinatal outcomes.

Pericytes: An integral piece of the neurovascular connectome

Alyssa C. Hurley[1, 2, 3], Randy F. Stout, Jr.[1], David C. Spray[1]
[1]Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY
[2]Summer Undergraduate Research Program, Albert Einstein College of Medicine, Bronx, NY
[3]Department of Neuroscience, Western New England University, Springfield, MA

The blood-brain barrier serves as a protective layer for the brain by preventing the entry of toxic molecules that may be present in the bloodstream. This barrier is formed by a network of cells known collectively as the neurovascular unit (NVU), which is composed of several different cell types including astrocytes, pericytes, endothelial cells, smooth muscle cells, neurons, and macrophages. Many of these cell types communicate with other cells of the same or different types through gap junctions. These channels directly connect adjacent cells and allow passage of molecules smaller than 1kDa from the cytoplasm of one cell to the other. In the brain, the transfer of ions facilitates synchronization of neuronal activity, and exchange of calcium and second messenger signaling molecules provides long range signaling in non-neuronal cells. It is thought that calcium signaling may be central to pericyte control of vascular tone, but few studies have tested if signals can be transmitted between pericytes and other cells that make up the NVU. We hypothesized that functional gap junction channels formed of connexin43 (Cx43) connect pericytes, endothelial cells, and astrocytes *in vitro.* We tested for the presence of functional gap junction connections between pericytes and other cells using multiple approaches including scrape-loading dye transfer assay and electrophysiology, and tested for the presence of Cx43 gap junctions using immunostaining. We found that pericytes, astrocytes and endothelial cells are robustly coupled *in vitro,* but pericytes were most strongly coupled. When pericytes and other cells were cocultured, the pericytes increased dye spread, suggesting that *in vivo* they may provide lower resistance pathways/conduits through their high level of Cx43 gap junction coupling. Future tests will be aimed at examining the strength of coupling between pericytes and endothelial cells in culture and testing if pericytes are coupled to other cells within the NVU *in vivo*. These studies indicate that there is a large difference in the gap junction coupling strength between different cell types of the NVU and raise the possibility that gap junctions forming the neurovascular connectome play an important role in pericyte regulation of blood flow in the brain.

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The Use of Cepheid Xpert Carba-R Screening Kit to Directly Screen Blood Culture Samples for The Presence Carbapenemase Genes.

Adam Ibrahim[1], Philip Gialanella[2], Wendy Szymczak[1], Michael H. Levi[1]

Department of Pathology, Division of Clinical Microbiology, Montefiore Medical Center and Albert Einstein College of Medicine, Bronx, NY.
Summer Undergraduate Research Program.

The prevalence of carbapenem-resistant *Enterobacteriacae* (CRE) in health-care settings has become a major public health concern. Rapid detection of bacteria with carbapenemase genes in patients either colonized or infected with these bacteria would be of great clinical value. The Cepheid Carb-R Assay has been FDA-approved for detection of carbapenemases from cultures and in rectal swabs. In this study we wanted to determine if the Carb-R assay could be used to detect CRE from blood cultures containing Gram negative rods. Using known bacterial isolates with two of the CRE enzymes, KPC and VIM, we determined the sensitivity of the Xpert Carba-R by testing the level of detection for each of these enzymes (LOD). Additionally, mock blood cultures were prepared using Bactex FX system and aerobic cultures containing human blood and each of these organisms. Finally, a series of real time blood cultures were screened with Carb-R and compared to the standard susceptibility results. The LOD of KPC gene in pure culture sample was ~$3.04*10^3$ CFU/mL and in blood culture samples, it was ~$5.1—5.4*10^2$ CFU/mL. The LOD for the VIM gene in blood culture sample was ~9.0 and 8.9 $*10^4$ CFU/mL. All 10 patients` positive blood cultures screened using the Cepheid Xpert Carba-R Assay were negative for all five carbapenemase gene. Nevertheless, the assay was not inhibited by the presence of human blood. Additionally, Carb-R results from patient cultures matched the results of standard phenotypic susceptibility testing of carbapenem antibiotics in the BD-Phoenix. These results indicate that Carb-R works with human blood cultures and after more extensive testing may be a useful and rapid adjunct to standard susceptibility testing.

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The Effects of Pathogen-derived Antigen on Memory CD8$^+$ T-cell Clustering and Pathogen Control *in vivo*

Weronika Janul, Marie Boutet and Gregoire Lauvau
Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, NY
Summer Undergraduate Research Program

CD8$^+$ T lymphocytes belong to blood leukocytes. They can undergo rapid proliferation upon pathogen recognition, kill tumors and virus-infected cells and ultimately become long-lived memory cells. Memory CD8$^+$ T cells are considered unique and essential for long-term immunity due to their ability to produce cytokines and chemokines. Cytokines signal in cells involved in host defense, and chemokines attract additional cells to sites of infection. In this study, we focused on the mobilization of memory CD8$^+$ T cells in a model of mice immunized and challenged with the intracellular bacterium *Listeria monocytogenes (Lm)*. CD8$^+$ T cells possess T cell receptors (TCRs) that can recognize specific peptide antigens bound to a MHC class I molecule, leading to the initiation of an immune response against this antigen. Here we have used T cell antigens expressed by *Lm*, namely the chicken ovalbumin model antigen (Ova) and the herpes simplex virus 2 (HSV-2) gB antigen. Activation of naive, antigen-inexperienced CD8$^+$ T-cells, a process also known as "priming", occurs as soon as a stimulatory antigen is displayed on the class I MHC molecule. Expression of the adhesion Molecule 1 (ICAM-1) during T cell activation facilitates the interaction between TCR and MHC, which in turn results in clustering of activated CD8$^+$ T cells. In the case of memory CD8$^+$ T cells, the mechanisms of their activation do not follow all the same cues. Memory cells can rapidly sense inflammatory cytokines and chemokines, with no need to recognize pathogen-derived antigens. Yet, protection of immunized host by memory CD8$^+$ T cells still requires pathogen-derived antigen recognition. During this process, memory CD8$^+$ T cells rapidly form clusters and prevent pathogen spreading inside infected tissues. We asked whether the presence of pathogen-derived antigen is required for the clustering of the memory cells in the spleen. We hypothesized that the presence of *Lm* antigens would cause the clustering of *Lm*-specific memory CD8$^+$ T cells. Mice received naïve T cells that recognize Ova (OT-I) or gB (gBT-I) and one day later were primary immunized with *Lm* expressing Ova and gB T cell antigens. After one month, these mice were further challenged or not (control), with *Lm* expressing OVA. We predicted that gB memory cells would not cluster like the OT-I cells. To test this hypothesis we performed FACs analysis in order to check T cell activation and immunofluorescent staining of spleen tissue sections to visualize the T cells using Confocal microscopy. FACS analysis revealed that there were far more OT-1 cells present in both unchallenged and challenged mice compared to gBT-I cells. Immunohistochemistry analysis revealed that OT-1 memory cells were visible within the follicle in the unchallenged condition, and gBT-1 memory T-cells were visible on the exterior of the follicle. Interestingly, in the challenged condition, both OT-1 and gBT-1 cells were visible on the exterior of the cell exhibiting the same behavior, despite having different TCRs. This experiment did not provide sufficient evidence, compared to previous work on memory T cell clustering, to answer the question posed. Further experiments are necessary in order to determine whether or not the presence of pathogen-derived antigen is required for the clustering of the memory cells in the spleen. Conclusively, the many fine details of immune response, such as cell clustering, are what configure the immune system. By understanding these mechanisms of protective immune response, we will gain better understanding of vaccines and T cell therapies.

Association between serum anion gap and history of kidney stones in a young adult population: results from NHANES 2007-2012

Naama Kipperman[1,2,3], Matthew K. Abramowitz , MD, MS[2], Michal L. Melamed, MD, MHS[2]

1. Bucknell University, 710 Moore Ave., Lewisburg, PA

2.. Department of Epidemiology & Population Health, Albert Einstein College of Medicine, Bronx, NY, USA

3. Summer Undergraduate Research Program, Albert Einstein College of Medicine, Bronx, NY, USA

Metabolic acidosis, or lower than normal blood pH, is a common acid/balance imbalance resulted from progressive impairment of kidney function. One widely implemented clinical tool for detecting such acidosis is the serum anion gap (AG), which is calculated using electrolyte measurements from laboratory data. Above-normal levels of AG have been associated with disordered acid/balance metabolism, and more specifically, early stages of chronic kidney disease.

Studies over the past decade have documented a marked increase in kidney stone prevalence across the general population. Obesity, diabetes, hypertension and metabolic syndrome are considered risk factors for stone formation, which, in turn, increases risk of developing CKD and other renal disorders. Insulin resistance, a measure of disordered metabolic function, is a known correlate of kidney stone formation. High levels of AG have been associated with insulin resistance, with a more pronounced association in obese populations. We suspect that a higher AG, which is known to characterize both disordered metabolism and impaired renal function, might be present in individuals who report a history of one or more kidney stones, given that stones are a risk factor for the aforementioned disorders.

In this study, we explored a potential association between three measures of serum anion gap with kidney stone history in a young adult population of ages 20-50 years (n=6592), using statistical analyses of the nationally representative NHANES cross-sectional data from 2007 to 2012. Additionally, we evaluated this association in a subgroup of obese individuals (n=2421). The anion gap was calculated in traditional manner, after adjustment for serum albumin, and after adjustment for serum albumin and other electrolytes.Kidney stone analysis was based on self-reported history of kidney stones from NHANES participants (“Have you ever had a kidney stone?”). Our statistical analyses did not show a significant difference in any of the three measures of AG between stone-formers and non-formers. Additionally, despite documented associations between AG, obesity, and insulin resistance, the serum anion gap did not significantly differ between obese-stone formers and obese non-formers. Further analyses will exclude individuals who report taking thiazide diuretics, as well adjust for dietary variables known to affect acid load.

Mutations in Ribosomal Protein genes in *Drosophila melanogaster*

Akua Kordieh, Nicholas Baker (PhD)

Department of Genetics, Albert Einstein College of Medicine

Mutations in most Ribosomal proteins (Minute mutations) dominantly slow growth rate in the fruit fly, Drosophila melanogaster. Specifically, flies that are heterozygous for RpS3, are observed to have shorter and relatively thinner bristles and take longer to develop. Mutation in the XRP1 gene is able to reverse delayed growth. However, it was unknown whether this shortened growth time is complemented by an increase in cellular growth activity so that adult flies maintain their size. Otherwise, adults of RpS3Plac92/XRP1M273 should be smaller. Thus, the aim of this experiment was to identify the extent to which XRP1 mutations restore growth or the extent to which they reduce size of adult flies.

Genotypes investigated in this experiment and their controls were created by crossing different strains. These are RpS3Plac92/+, RpS3Plac92/XRP1M273, XRP1M273/+, frt82/+ and XRP1M273 RpS3Plac92/DfEDxxx. The flies were made to grow on yeast-glucose food medium to ensure adequate nutrition and their thoraces and wings were prepared for microscopy. The length of the four scutellar bristles on the thoraces and the area of the wings were measured because these correlate with body size.

In the adult males, it was observed that the mean wing area of RpS3Plac92/XRP1M273 is significantly smaller than the mean wing area of RpS3Plac92/+ flies. However, in the adult females, there is no significant difference. Further investigation on the sizes of phenotypes expressed by the two genotypes is being done by measuring and comparing the scutellar bristles on the thoraces.

I would like to thank DRSSOP for the Research Opportunity and Dr Baker for the project and the privilege of working in his laboratory. I would also like to thank Gerard Rimesso for his immense assistance, Jorge Blanco and Marianthi Kiparaki.

Characterization of Pro-inflammatory Cytokine Expression in $Gas6^{-/-}Axl^{-/-}$ Double Knockout (DKO) Mice Following Exposure to Cuprizone

Lauren Kozakiewicz[1], Juwen DuBois[2], Alex Ray[2], Bridget Shafit-Zagardo[2]

[1]Colgate University, 13 Oak Drive, Hamilton, NY 13346. [2]Department of Pathology, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461

Multiple sclerosis (MS) is a neurodegenerative disease characterized by inflammation, demyelination, and axonal damage. Protection of the axons and cells in the central nervous system (CNS) through reduction of the inflammatory response is the target of current clinical therapies. Our studies show one signaling pathway that regulates the innate immune response is activated through Axl, a member of the TAM family of receptor tyrosine kinases, and its ligand, growth arrest-specific protein 6 (Gas6). The TAM family, Tyro3, Axl, and Mertk, are found on the surface of several cells of the CNS including microglia, astrocytes, and neurons, and are activated by their respective ligands, Gas6 and ProteinS1α (ProS). Both Gas6 and ProS signal through the TAM family, however, Gas6 is the sole ligand for Axl. Gas6 is a vitamin K-dependent growth factor expressed in the CNS and regulates cell survival, homeostasis, mitogenesis and the innate immune response. Studies have shown that the Gas6/Axl signaling pathway is necessary for maintaining axonal integrity, aiding in remyelination, and regulating the release of pro-inflammatory cytokines. Since ProS1 can activate Tyro3 and Mertk, and activate Axl by heterodimerization, we generated $Gas6^{-/-}Axl^{-/-}$ double knockout (DKO) mice to further understand the role of this pathway in axonal maintenance, remyelination and inflammation. Using the cuprizone model of demyelination/remyelination, oligodendrocytes in the corpus callosum die after 4-6 weeks of ingestion of 0.2% (w/w) cuprizone in powdered chow and demyelination and glial inflammation are observed. While the number of microglia were equivalent at these time points, we hypothesized that there would be a resulting increase in pro-inflammatory cytokine expression in the corpus callosum of mice lacking Gas6/Axl signaling. Using qrt-PCR, pro-inflammatory cytokiness TNFα, IL-1β, IFNγ, IL-23, and inhibitory cytokine, TGFβ, were examined in the corpus callosum of DKO and wild type (WT) mice after 4, 5, and 6 weeks of cuprizone treatment and compared to naive controls. Relative to WT mice exposed to the same conditions, DKO mice had increased IFNγ mRNA at 5- and 6-weeks, and decreased IL-23 mRNA at 6-weeks cuprizone treatment. This data supports a role for Gas6/Axl in dampening inflammation which may delay remyelination following 6-weeks cuprizone withdrawal and recovery. In fact, the lab has determined that relative to WT mice the corpus callosum of DKO mice have diminished remyelination and increased axonal damage following 6-weeks cuprizone treatment and 3-weeks recovery. Because TAM signaling can be activated through ProS1, we examined whether ProS1 expression was elevated in the corpus callosum of DKO mice, in order to compensate for the loss of Gas6. ProS1 mRNA was increased at 4- and 6-weeks cuprizone. These data suggests a role for Gas6/Axl signaling during demyelination/remyelination and the inability of ProS1 to compensate for the loss of Gas6. Whether TAM receptors can alter the immune response towards an anti-inflammatory phenotype is being extensively examined, and warrants further studies for potential therapeutic interventions to aid in remyelination.

Inhibition of Ebola RNA Polymerase with Transition State Analogues

Isaac M. Krasnopolsky, Ross S. Firestone, Scott A. Cameron, and Vern L. Schramm

Department of Biochemistry, Albert Einstein College of Medicine Summer Undergraduate Research Program, 1300 Morris Park Avenue, Bronx, NY 10461

Immucillin-A (ImmA) is a 9-deazapurine nucleoside hydrolase analogue that has been shown to inhibit filovirus growth *in vivo*. This novel drug is currently in phase 1 clinical trials and has potential to open new therapeutic pathways for Ebola and other filoviruses. It is hypothesized that this inhibitory effect is due to ImmA being triphosphorylated at its 5´position within the host cell. The nucleoside triphopshate analogue is then believed to be erroneously incorporated into the growing viral RNA genome, ultimately resulting in RNA polymerase chain termination. To test this hypothesis, an enzymatic synthetic scheme to produce Immucillin-A-5´-triphosphate has been developed. Adenosine kinases from *Homo sapiens* and *Anopheles gambiae* were expressed and purified to catalyze the first 5´-phosphorylation step. The second two reactions were catalyzed by the commercially available adenylate kinase (myokinase) and pyruvate kinase, respectively. Early results show promising data indicating the potential for high-yield synthesis of Immucillin-A-5´-triphosphate. Hepatitis C virus RNA Polymerase (HCV-RNAP) is being expressed to further test the mechanism of RNA polymerase inhibition. Viral RNA sequencing following an ImmA inhibition assay will allow us to confirm or restructure the currently proposed mechanism of action.

Improving Cisplatin in HNSCC by Targeting Inhibitors of Apoptosis with Navitoclax

Eric Lee, Carlos Thomas, Michael B. Prystowsky, MD, PhD, Thomas J Ow, MD, MS
Department of Pathology, Albert Einstein College of Medicine, Bronx, NY 10461,
Summer Undergraduate Research Program

Apoptosis is a cellular pathway that results in programmed cell death. The pathway can be initiated via both intrinsic or extrinsic signals. Extrinsic signals include apoptosis ligands, the lack of growth factors, or separation from the extracellular matrix. The intrinsic pathway is often initiated by DNA damage, which activates molecules that commence the apoptosis signaling network. A key step in the process is a shift in the balance between pro-apoptotic molecules (e.g. BAX and BAK), which form pores in the mitochondrial membrane when activated, and pro-survival/anti-apoptotic molecules, such as Bcl-2 and Bcl-XL. As the balance tips towards apoptosis activation, pores in the mitochondrial membrane release oxygen free-radicals and cytochrome c, which trigger the caspase signaling cascade, causing indiscriminate protein degradation and subsequent cell death.

Cisplatin is an anti-cancer drug that crosslinks DNA during replication, causing irreparable DNA damage and activating the intrinsic apoptosis pathway. Since cancer cells, including Head and Neck Squamous Cancer Cells (HNSCC), generally proliferate at a faster rate than normal tissues, cisplatin can be an effective choice for therapy. However, when cisplatin is used as a single agent in treatment, resistance to the drug has been a reoccurring challenge. An effective solution may be to use a novel drug combination treatment to potentiate cisplatin's apoptotic effects. Navitoclax is an experimental cancer treatment drug that inhibits anti-apoptotic proteins Bcl-2 and Bcl-XL and subsequently inducing apoptotic signaling. We hypothesize that when navitoclax is used in addition to cisplatin the result should be an increase in the efficacy of cisplatin.

To test our hypothesis, we examined the response to navitoclax and cisplatin in seven HNSCC cell lines. MTT assays were used to compare the survival of cancer cells 48-hours after drug incubation to determine the half maximal inhibitory concentration (IC50). We studied a range of cisplatin doses in combination with 2 and 4 uM of navitoclax, and compared results to that of single agent and untreated controls.

Baseline cisplatin IC50s were established for each cell line and ranged from 2.7 to 11.83 uM. When exposed to 4 uM of navitoclax during combination treatment, all cell lines exhibited a decreased cisplatin IC50. The new IC50s ranged from 2.15 to 9.92 uM, and the difference from the baseline IC50s averaged 1.25 uM. A similar decrease was observed when 2 uM of navitoclax was used.

Combination treatment with navitoclax resulted in increased cisplatin efficacy, as predicted by our hypothesis, and the decrease in cisplatin IC50's suggested an additive interaction. Further research is needed to confirm these results by repeating the MTT assay with a finer range of navitoclax concentrations and to evaluate possible synergistic interaction between the two drugs.

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Gene-Environment Interaction Controls the Penetrance of Vertebral Defects in Zebrafish

Stuart Levitin, Sevdenur Keskin, Nicolas Selemon, Ertugrul Ozbudak

Department of Genetics, Albert Einstein College of Medicine. Bronx, New York. Summer Undergraduate Research Program.

Segmentation of the vertebral column during embryonic development (somitogenesis) is controlled by a segmentation clock in the presomitic mesoderm (PSM). In zebrafish, a negative feedback loop creates oscillating levels of core genes *her1* and *her7*, causing rhythmic segmentation of the PSM. Several mutations affecting the signaling pathways involved in somitogenesis can lead to vertebral defects such as congenital scoliosis. *Her7*$^{-/-}$ (Hu2526), one such mutation in zebrafish, leads to vertebral defects that are not completely penetrant. In this study, we sought to demonstrate that environmental factors including temperature, hypoxia, and hyperglycemia increase noise in somitogenesis signaling pathways and influence the penetrance and severity of *her7*$^{-/-}$ in homozygous zebrafish. After individually controlling environmental temperature, activity of the hypoxia-inducible factor-1 (HIF-1) and glucose levels during somitogenesis, we used whole-mount *in situ* hybridization with anti-digoxigenin labeled xirp probes to evaluate the embryonic expression of somite defects. In untreated mutants, we found that left and right somites developed malformations independently of each other (with a correlation coefficient of 0.17), suggesting stochastic gene expression in somitogenesis. We found that decreased temperatures increase expressivity of the *her7*$^{-/-}$ mutation, resulting in a greater number of malformed somites and higher incidence of malformation in early somites. Initial results indicate that hypoxia and hyperglycemia also result in a greater number of deformed somites. These results suggest that gene-environment interactions may increase noise contributing to the malformation of somite boundaries, leading the development of more severe forms of congenital scoliosis. Further research must be done to investigate the effects of other environmental factors on the severity of congenital scoliosis and to identify other mutations involved in somitogenesis pathways similarly affected by gene-environment interactions.

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Placenta Accreta in an Urban Setting

Katthia B. Loriston, Rita Astur, PA, Crissanna Tang, PA, and Maria Abadi, MD.
Department of Pathology, Jacobi Medical Center, Bronx, NY 10461
Albert Einstein College of Medicine Summer Undergraduate Research Program

Introduction: Placenta accreta is an abnormal implantation of placental tissue directly onto or into the smooth muscle of the uterus. Placenta accreta may present as painless vaginal bleeding; however, excessive hemorrhage may lead to hypovolemic shock, which can result in the death of the mother and the baby. Frequent risks factors associated with an abnormal placental implantation include: previous deliveries by cesarean section, placenta previa, advanced maternal age (> 35 years of age) and prior uterine surgery. Women with placenta accreta undergo emergent hysterectomy. In the United States, the incidence and prevalence of placenta accreta is rising, mostly due to previous caesarean sections.

This study aimed to identify and describe common risk factors for placenta accreta in an urban setting between 2011 and 2016.

Materials and Methods: A retrospective study of medical records at Jacobi Medical Center and North Central Bronx Hospital between 2011-2016, was conducted to identify cases of pregnant women with abnormal placental implantation. Data include gestational age, prior cesarean sections, placenta previa, ultrasound findings and symptoms. The data were recorded in an Excel spreadsheet database. In addition, correlation between previous cesarean delivery and presence of placenta previa was explored.

Results: There were 18 cases of abnormal placental implantation between 2011 to 2016. The risks factors for this population were: 9 (50%) women were older than 35 years of age, 4 (22%) had placenta previa, 3 (17%) had positive ultrasound findings. The most common symptoms were bleeding (11%), pain (11%). infection (6%), and elevated blood pressure (6%),

Conclusion: Placenta accreta is a growing threat to maternal health, mostly due to an increase in cesarean deliveries. This study suggests that it is important to identify potential modifiable risk factors that may aid in the prevention of placenta accreta.

The Effects of Antimalarial *Plasmodium falciparum* Equilibrative Nucleoside Transporter 1 (PfENT1) Inhibitors on Human Purine Transporters

J. Yael Mayer[1], Noa Applebaum[2], Yvett Sosa[3], Myles Akabas[3,4]

1 Stern College for Women, 2 Ma'ayanot Yeshiva High School for Girls, 3 Department of Physiology, 4 Departments of Neuroscience and Medicine, Albert Einstein College of Medicine

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

Malaria is a serious public health threat, with roughly 200 million infections and 500,000 deaths reported annually. The majority of fatal cases occur in regions of sub-Saharan Africa, and are largely caused by *Plasmodium falciparum*. Effective antimalarial drugs, such as chloroquine and artemisinin, have been developed. However, the emergence of drug resistant parasites has reduced the efficacy of chloroquine and threatens the efficacy of artemisinin. Thus, it is necessary to develop new antimalarial drugs.

Malaria parasites are purine auxotrophs; they rely on purine acquisition from the host. The primary equilibrative nucleoside transporter, PfENT1, is utilized in all erythrocytic stages. Previous research showed that *pfent1* knockout parasites are not viable when grown in media containing physiological purine concentrations (<10 μM). This implicates PfENT1 as the primary purine transporter and suggests that it may be an excellent drug target.

One hundred seventy-one PfENT1 inhibitors were identified using a yeast-based high throughput screen; nine of the most efficacious were chosen for further characterization. We performed parasite cytotoxicity assays to determine the efficacy of these compounds. The concentration dependence of parasite killing was assessed after 48 hours of growth by measuring DNA content, using a SYBR Green assay.

We determined the specificity of the nine compounds for PfENT1 relative to the human red blood cell (RBC) purine transporters, human equilibrative nucleoside transporter 1 (hENT1) and human facilitative nucleoside transporter 1 (hFNT1). Adenosine is transported by hENT1 but not by hFNT1 and conversely, hypoxanthine is transported by hFNT1 but not hENT1. We assayed the efficacy of the nine PfENT1 inhibitors against these transporters by the concentration dependence of inhibition of either [^{3}H]adenosine or [^{3}H]hypoxanthine uptake into uninfected red blood cells. The compounds collectively displayed lower IC50's against the erythrocyte purine transporters than in parasite growth inhibition. The findings indicate a higher affinity and thus specificity for PfENT1 over hENT1 and hFNT1.

Future research will seek to improve the potency of these compounds for PfENT1. In previous experiments, PfENT1 inhibitors killed the *pfent1* knockout parasites, albeit with lower affinity than against wild-type parasites. This suggests that the compounds hit a secondary target in addition to PfENT1. The dual targets would significantly decrease the likelihood of parasites developing resistance against the drugs. As the compound potency is improved through medicinal chemistry, we will attempt to sustain efficacy against both targets.

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Understanding how cancer cells dysregulate macroH2A1 alternative splicing: a role for RNA Polymerase II elongation rate

Eileen V. McAuley, Alyssa D. Casill, Matthew J. Gamble

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

MacroH2A1 is a histone variant that is implicated in many cellular processes, including gene expression and tumor suppression. MacroH2A1, encoded by the H2AFY gene, has two splice variants: macroH2A1.1 and macroH2A1.2. The macrodomain of macroH2A1.1, and not macroH2A1.2, is able to recruit proteins modified with PAR chains, a post-translation modification catalyzed by enzymes called PARPs. Through this interaction macroH2A1.1 regulates gene expression important for tumor suppression. Many cancers bypass macroH2A1.1-mediated tumor suppression by altering macroH2A1 expression and splicing; uterine, lung, colon, rectal, breast, bladder, and prostate cancer all have significant reductions in macroH2A1.1 splicing and increases in the overall expression of macroH2A1. This inverse relationship suggests that transcriptional regulation additionally regulates the alternative splicing decision of macroH2A1.

While the exact mechanism for the dysregulation of macroH2A1 splicing in cancer cells is still unknown, using both genetic and pharmacologic approaches, we have shown that changes in the elongation rate of RNA Polymerase II (Pol II) regulate the alternative splicing of macroH2A1. Slower rates of transcript elongation favor the splicing of macroH2A1.1 while faster elongation rates promote the splicing of macroH2A1.2. We therefore hypothesize that cancer cells suppress the splicing of macroH2A1.1 in part by increasing the rate of Pol II elongation on the H2AFY gene.

To test this hypothesis, we measured the elongation rate of Pol II on the H2AFY gene in the A549 and MG63 cancer cell lines and the IMR90 normal cell line. These cell lines were chosen because their levels of macroH2A1.1 are drastically different. RT-qPCR analysis shows that while IMR90 and MG63 have high levels of macroH2A1.1, A549 and HeLa have low levels of this splice form. In order to measure the gene-specific elongation rate on H2AFY we treated the cells with high concentrations of DRB, a drug that prevents Pol II from entering productive elongation and causes Pol II to accumulate at the 5' end of the gene. Upon wash-out of DRB, Pol II can synchronously begin transcription. We collected cells at time points following DRB wash-out and isolated nascent RNA. We used RT-qPCR and primers specific to the H2AFY gene in order to follow the progress of nascent RNA production by the pioneer polymerase. We found that cancer cell line A549 has faster Pol II elongation rates in the H2AFY gene body than IMR90. This supports our hypothesis that cancer cells use an increased rate of Pol II elongation in order to suppress the expression of the splice variant macroH2A1.1. However, contrary to our hypothesis, MG63 cells, an atypical cancer cell line which has higher levels of macroH2A1.1 compared to macroH2A1.2, have the fastest rate of elongation across all the cell lines. We postulate that other factors are affecting these higher levels of macroH2A1.1 in MG63. Future experiments across additional cancer and normal cell lines will enable us to determine how prevalent regulation of Pol II elongation rate is as a mechanism to regulate macroH2A1 splicing.

Examining Pharyngeal Apparatus Development in *Tbx1-Cre/+;Foxi3*$^{f/f}$ Mouse Embryos to Explain Thymus and Parathyroid Defects

Daniella Miller, Erica Hasten, Bernice Morrow
Department of Genetics, Albert Einstein College of Medicine, Bronx, NY
Summer Undergraduate Research Program

TBX1 is a T-box transcription factor gene and its haploinsufficiency causes 22q11.2 deletion syndrome (also called DiGeorge and velo-cardio-facial syndrome). Global inactivation of *Tbx1* in mouse embryos causes perinatal lethality and embryos exhibit cardiovascular, craniofacial, thymus and parathyroid defects. These defects can be explained by abnormalities in the pharyngeal apparatus (PA) that occur early in development within Tbx1 null embryos since PA formation and segmentation is crucial for proper embryonic development. *Foxi3* is a gene downregulated in expression in *Tbx1* null mutants and is involved in PA segmentation. Foxi3 encodes a Fox-family transcription factor and Foxi3 null embryos also have PA segmentation defects. To determine if there is a genetic interaction between these two genes, we generated *Tbx1 +/-;Foxi3 +/-* embryos that have parathyroid and thymus defects at E15.5. We also generated conditional *Foxi3* null mutants using the Tbx1-Cre/+ allele and found even more severe thymus and parathyroid defects. Since the parathyroid and thymus are derived from the third pharyngeal arch, we wanted to determine if these mutants have pharyngeal apparatus defects early on in development. We performed whole mount in situ hybridization (WMISH) with RNA probes for PA markers *Dlx2* and *Fgf8* to determine if there were PA segmentation defects and results indicated that both genes are downregulated in both mutants in comparison to their controls. This finding can help in understanding the molecular mechanisms of 22q11.2 deletion syndrome and lead to further research for more targeted therapeutics.

Directed evolution of PD-1 using phage display to engineer a high affinity variant to prevent T-cell inhibition during cancer

Edward Polanco[Ψ], Julia Frei[δ], and Jonathan Lai[δ]
[δ]Albert Einstein College of Medicine Department of Biochemistry,
[Ψ]UCLA Department of Physics and Astronomy

Immunotherapies which target the cytotoxic T-cell co-stimulatory pathways have recently emerged as promising cancer treatments. In particular, blockade of the CTLA-4 and PD-1 T-cell co-inhibitory pathways through use of monoclonal antibodies has shown great promise for tumor burden reduction in clinical trials. Furthermore, these therapies are highly targeted, and thus avoid side effects of chemotherapies that occur due to off target effects. Cytotoxic T-cells, which infiltrate the tumor microenvironment, are often deactivated by the interaction of their co-receptor, PD-1 (programmed death receptor 1), with its ligands PD-L1/-L2, which cancer cells overexpress to avoid cell death. By targeting the PD-1 pathway, cytotoxic T-cell activation is enhanced allowing for the effective killing of cancerous cells. Using PD-1 as a template, we created a phage display library in which all ligand-contacting residues were allowed to vary among all twenty amino acids to select for variants of PD-1 with increased affinity for both PD-L1 and PD-L2 compared to wildtype PD-1. Clones demonstrating increased binding relative to negative control were selected for further characterization by phage ELISA. When used as a therapeutic, these high affinity PD-1 variants will disrupt the interaction between PD-1 of the T-cell and the ligands on the cancer cell, preventing the deactivation of the T-cell. Future work will aim to characterize these variants as soluble proteins and to test their ability to reduce tumor burdens *in vivo*.

Effects of Dairy Intake on Insulin Resistance: A Systematic Review & Meta-analysis

Edeline Sanchez, Pardeep Kaur, MBBS, Michal Melamed, MD

Albert Einstein College of Medicine, Department of Nephrology, Department of Epidemiology and Population Health
Summer Undergraduate Research Program, Diversity Student Summer Research Opportunity Program

The incidence of obesity and type 2 Diabetes Mellitus has increased in the U.S over the last few years. The consumption of low-fat dairy foods has been linked with decreasing the risk of type 2 DM but research studies have yet to show a clear correlation. The purpose of this study was to conduct a systematic review and meta-analysis of all randomized clinical trials evaluating the effects of dairy on waist circumference, weight and insulin resistance.

With the help of a medical librarian, we identified articles in the MEDLINE and Embase databases with keywords *dairy products, low fat dairy, insulin resistance, weight loss, body mass index, diet and reducing.* We identified 2559 potentially relevant studies and after removing duplicates and articles with inappropriate study designs, study populations or outcomes of interest, we abstracted 51 articles. 26 **-** articles were included in our meta-analysis. Using the Comprehensive Meta-Analysis Software Version 2.0, we analyzed the pooled standardized mean differences for our data comparing the dairy intake group to the control group. Combining all studies for HOMA-IR, there were 17 articles including 965 individuals used for the meta-analysis. The pooled standardized mean difference between the dairy and placebo group was -0.33 with a p-value of <0. 001. Using 15 articles that included 839 participants, we also analyzed the effects of dairy intake on waist circumference. The pooled standardized mean difference was -0.53 cms (-0.63, -0.42) between the dairy intake and the control groups (p-value <0. 001). Finally, we included 24 articles with 2,029 individuals to analyze the effect of dairy intake on weight and found the dairy intake intervention group was 0.66 kgs (-0.9, -0.41) lighter than the control groups (p-value of< 0.001).

Our findings suggest that dairy intake, especially low-fat fortified dairy products, has a beneficial effect on HOMA-IR, waist circumference and weight. HOMA-IR is a reliable marker for insulin resistance, and a decrease in HOMA-IR can potentially mean a decrease in the risk for type 2 DM along with other diseases including cardiovascular, peripheral artery disease and high blood pressure. These findings can be valuable to not only individuals trying to manage their weight but also healthcare providers advising their patients.

Low Intensity Focused Ultrasound (LOFU) combined with Radiation Therapy increases Immunogenic Susceptibility in Melanoma

Abdul Sanni-Adam, Saurabh Singh, Indranil Basu, Karin Skalina, Wade Koba, Stephen Barry and Chandan Guha

Department of Radiation Oncology, Albert Einstein College of Medicine, Bronx, New York, USA

Tumors have intricate mechanisms for evading detection of the immune system, making immunotherapy treatment ineffective. High intensity focused ultrasound (HIFU) is promising non-invasive ablative approach for local tumor control but has limited ability in management of tumor recurrence and metastatic spread outside of the primary tumor site. Non-ablative, low intensity focused ultrasound (LOFU) coupled thermal heating has been shown to induce a sonic stress in tumors, enhancing tumor immunogenicity by increasing intracellular HSP70 and decreasing STAT3 activity at 2-6 hours post thermal heating. Tumors pre-treated with LOFU prior to radiation results in significant tumor growth retardation, and prevention of tumor recurrence and metastases. We have investigated the utility of combining LOFU and hypo-fractionated radiation therapy (RT) in a clinically relevant model of spontaneously metastatic melanoma. LOFU+RT treated murine B16 melanoma cells were analyzed for surface membrane expression of stress and immunogenic cell death markers using flow cytometry. The results showed that the combination treatment increased the expression of stress and immunomodulatory markers like CRT, HSP70, GRP78, MHC-I, CD40, CD47, CD86 and FasL that play a major role in immunogenic cell death. This was further supported by increased apoptosis in LOFU treated B16 cells. Further, in-vivo efficacy of LOFU+RT is presently being studied in B16 tumor model in C57BL/6 mice. The immune priming effect of LOFU and its efficacy as a radiosensitizer will provide important preclinical data for future tumor vaccine trials for patients with metastatic disease. The preliminary results demonstrated here require further investigations tailored to augment tumor-specific immune responses to control recurrent and metastatic cancer.

Information Relay Cascade in the Zebrafish Segmentation Network

Nicolas Selemon, Sevdenur Keskin, Stuart Levitin, and Ertugrul Özbudak

Genetics Department
Albert Einstein College of Medicine
Summer Undergraduate Research Program

Segmentation of the paraxial mesoderm into somites commences during embryonic development and is well conserved across vertebrates. Perturbations to somitogenesis signaling pathways have been implicated in human spinal malformations from scoliosis to Klippel-Feil syndrome. In zebrafish (*Danio rerio*), oscillating spatiotemporal expression of Her proteins regulates the timing of somitogenesis, while Delta-Notch signaling synchronizes segmentation. In mice, the bHLH domain of Mesp2 is an identified transcriptional regulator of Notch. Zebrafish contain 4 mesp genes, mesp-a and mesp-b, each with duplicate derivatives. The current study utilizes heat-inducible mesp overexpression to investigate mesp regulators. Crosses of transgenic heterozygous with wildtype zebrafish yielded samples containing an inner control and 1:1 expected transgenic:wildtype ratio. Thirty-minute heatshocks (37 °C) were given to embryos at 4, 8, 12, 16, and 20 somite stages to initiate mesp overexpression. Embryos were fixed at 36hpf. Xirp in-situ hybridization was performed to visualize somite boundaries. Significant delays in the onset of mesp-induced somite disruptions were observed relative to the timing of overexpression. Short transcriptional/translational delays were expected, but the noted 2-3 somite shift implies a systematic cause. The presence of a Mesp determination front predicts such delays – several somites contain predetermined mesp expression stamps when heatshock is administered. While further mesp studies are needed, the current study classifies mesp-b as an important intermediate regulator of zebrafish paraxial development and supports the existence of a Mesp determination front. Acknowledgements to Dr. Özbudak for the research opportunity, SK for her mentorship and aid, and the Einstein SURP and NIH for their generous funding.

Effect of Cancer associated mutations in the N-terminal domain of INI1/hSNF5 on subcellular localization

Cylena Stewart, Estah Blumen, Annalena LaPorte and Ganjam V. Kalpana
Albert Einstein College of Medicine- Bronx, New York
Department of Genetics
Summer Undergraduate Research Program

Integrase Interactor 1(INI1)/hSNF5 is a core subunit of the SWI/SNF complex, a multiprotein complex involved in chromatin remodeling. *INI1* is a tumor suppressor gene mutated in a large number of human cancers including rhabdoid tumors and schwannomatosis schwannoma. These cancer associated *INI1* mutations include deletions and missense mutations. The mechanisms by which the *INI1* mutations induce cancer are not well understood. There are two isoforms of INI1, a and b, which differ by 9 amino acids and both have multiple domains. Several cancer associated mutations have been identified in INI1 N-terminal domain (amino acids 10-110) including P48S. The functional consequence of the cancer associated mutations of INI1 is unknown.

The goal of my project was to determine the effect of the P48S mutant and other cancer associated mutations on subcellular localization of INI1 isoforms in both the full length and 1-110 amino acid context. Specific subcellular localization is important for function of proteins and the mutations may influence various aspects of the protein function and localization. Therefore, we hypothesized that cancer associated mutations may influence INI1 function, or its biochemical properties, protein-protein interactions or subcellular localization. To test this hypothesis, 293T cells were transfected with full length INI1a, INI1b, INI1-P48S and the two INI1(1-110) fragment isoforms with and without P48S substitution, by using immuno-fluorescence analysis.

We found that INI1a and INI1b localized in the nucleus and exhibited nuclear speckles. The presence of P48S mutations reduced the nuclear speckles. Interestingly, INI1(1-110) domain localized in the nuclear and/or cellular periphery. Furthermore, presence of P48S mutation resulted in diffuse nuclear and cytoplasmic localization.

These studies indicate that cancer associated mutations of INI1 such as P48S may affect subcellular localization and hence influence the function of INI1.

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Using Specialized Transduction to Generate a Mannose Auxotroph in *Mycobacterium smegmatis*

Robert W Usab; Tracy L Keiser PhD; Paras Jain PhD ; William Jacobs Jr. PhD

Microbiology and Immunology, Albert Einstein School of Medicine, Bronx, New York

Mycobacterium tuberculosis (MTB) is one of the most successful infectious disease killers in human history and at present day, the WHO estimates that approximately one third of the world's population has been infected. Due to the long course of and accessibility to treatment, there has been a steady increase in multi-resistant, extremely resistant and finally totally resistant MTB strains reported. Therefore, the need for new treatments is at a critical level and research on the pathogenesis of MTB will give insight into new possible targets. MTB possesses a compositionally unique cell wall containing long chain mycolic acids topped with mannosylated lipoglycans. These mannosylated lipoglycans have been shown to be important in entry into the host cell macrophage as well as being important immunomodulatory molecules within the host cell phagosome. This project will focus on a pathway important in the generation of mannose donor molecules, GDP-mannose and Polyprenyl-phosphate mannose, which serve as the substrate for mannosylation of the lipoglycans. The deletion of a homologue to the MTB manA in M. smegmatis (MSMEG-1836, manA) will result in mannose auxotrophy and allow us to study the cell wall defects in a faster growing surrogate organism.

Generating Antigens for New TB Vaccines from Mycobacterial Ribosome

Amy Wang, Steven Kennedy, Steven Porcelli
Department of Microbiology and Immunology, Albert Einstein College of Medicine (NY, NY)

Tuberculosis is a potentially deadly infection affecting over two billion people. The only approved vaccine, BCG, has limited efficacy. Developing an improved vaccine would assist long-term efforts to control and eradicate TB. Unpublished data from our lab show that mice primed with genetically altered *Mycobacterium smegmatis* can better resist *Mycobacterium tuberculosis* (Mtb) infection. Our lab subsequently discovered these mice have memory T-cells that respond strongly to structural mycobacterial ribosome proteins, though few specific epitopes have been identified. The purpose of this project is to create a library of plasmids encoding ribosomal proteins. Ribosomal protein genes were amplified by PCR and introduced into pcDNA3 GFP LIC cloning vector (6D) through ligation independent cloning. Constructs were transformed into *E. coli* and transfected into HEK293 cells. Western blotting, microscopy, and DNA sequencing were used to confirm successful constructs. Thus far, 24 genes have been successfully cloned into vector 6D, as demonstrated by Western blots and plasmid sequencing. Western blots of some transfectants showed multiple bands likely due to minor contaminants and protein aggregation. The remaining genes are being further pursued by altering PCR and transformation conditions. Once completed, this library will enable production of individual ribosomal proteins to probe responses of T-cells reactive to whole Mtb ribosomes. This will allow immunogenic proteins to be identified and used to develop an improved vaccine.

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The Effects of T232P Cancer Mutation in BAF180 on its Association with Chromatin

Sara L. Wiener, Charles Kenworthy, Wei-Li Liu and Robert A. Coleman
Department of Anatomy and Structural Biology, Albert Einstein College of Medicine, Bronx NY

The chromatin remodeling complex PBAF is a multi-subunit transcription factor found in mammalian cells. PBAF remodels nucleosomes to maintain cellular homeostasis and regulate transcription of the genome. This chromatin-remodeling complex is highly mutated in many cancers, including clear cell Renal Cell Carcinoma (ccRCC). BAF180 is a chromatin-targeting protein encoded by the *PBRM1* gene. BAF180 is a subunit within the PBAF complex and acts by binding to chromatin to promote centromere cohesion and genomic stability. BAF180 has six bromodomains, which bind to acetylated lysine residues and are thought to localize the PBAF complex to specific chromatin sites.

Our research will investigate the T232P cancer-derived missense mutation in the second bromodomain of BAF180. The T232P mutation is hypothesized to inactivate BAF180's second bromodomain, which may compromise DNA damage responses and lead to genomic instability. The mutation inserts a proline amino acid instead of threonine, causing an unfolding of the second bromodomain's alpha helix structure revealing potential protease cleavage sites. When we express T232P mutated BAF180 in U2-OS cells, the T232P mutation results in a truncation in BAF180 following the third bromodomain. We also found that this mutant did not associate with the rest of the PBAF complex. Specifically, the truncated protein product fails to interact with the BRG1 motor ATPase subunit within the PBAF complex, while still maintaining functional bromodomains one and three that can interact with chromatin.

We hypothesized that this truncated mutant T232P BAF180 would have altered interactions with chromatin in vivo. Surprisingly our Single-Particle Tracking (SPT) imaging assays showed that the BAF180-T232P mutant interacted with chromatin longer than wild-type BAF180. The extended interaction with chromatin leads us to question whether the T232P mutation or the BAF180-T232P mediated truncation is relevant to the alteration of chromatin binding. To test these theories, we wanted to generate artificially truncated wild-type and T232P mutant BAF180 expression vectors and perform SPT imaging.

We succeeded in creating a truncated WT BAF180 protein through PCR and cloning techniques. SPT imaging indicated that the truncated BAF180 protein still binds to chromatin with residence times similar to the WT BAF180. This suggests that the longer residence time of the T232P cancer mutant BAF180 protein may be related to the mutation on bromodomain 2, and not due to the truncation itself. We then genetically truncated the T232P mutant BAF180 protein and found that its residence times were shorter than the T232P protein that was not artificially truncated. This may suggest that the C-terminus of the T232P mutant BAF180 protein is necessary for its enhanced stable binding to chromatin. In the future, we will mutate the proposed cleavage site on BAF180 protein to confirm the truncation site and determine the chromatin binding activity of full length T232P BAF180. Investigating the effects of the T232P mutation on BAF180 binding to chromatin may lead to further discoveries in the field of tumorigenesis.