2014
ABSTRACT BOOK

Summer Undergraduate Research Program

Graduate Programs
in the
Biomedical Sciences

EINSTEIN
Albert Einstein College of Medicine
OF YESHIVA UNIVERSITY

Albert Einstein College of Medicine
OF YESHIVA UNIVERSITY

Graduate Division of Biomedical Sciences

SUMMER UNDERGRADUATE RESEARCH PROGRAM

2014

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

2014 Summer Undergraduate Research Program

Student Name	Undergraduate School	Mentor Name
Dillon Ade	North Carolina State University	**Jonathan Lai**
Lorita Agu	Converse College	**Jacqueline Achkar**
Julia Alba	University Of Wisconsin-Milwaukee	**Pariac Kenny**
Sonia Barakat	Fordham University	**Marion Schmidt**
Jacqueline Benayoun	Yeshiva University	**Johanna Daily**
Christie Black	Williams College	**David Fooksman**
Shacelles Bonner	College of New Rochelle	**Kelvin Davies**
Monica Castle	Rutgers, The State University of New Jersey	**Ekaterina Dadachova**
Carlton Christie	Franklin And Marshall College	**Pablo Castillo**
Adi Cohen	Yeshiva University	**Myles Akabas**
Charles Crouse	University Of Maryland- Baltimore County	**Scott Emmons**
Jessica Cysner	Lafayette College	**Kostantin Dobrenis**
Lilah Fones	Wesleyan University	**Derek Huffman**
Casey Garrett	University of Georgia	**Nicholas Baker**
Tanvi Goyal	Rutgers University	**Bridget Shafit-Zagardo**
Talia Greenstein	Rutgers University	**Bernice Morrow**
Hadassa Holzapfel	Stern College for Women	**Ljiljana Vasovic**
Shira Kaye	Yeshiva University	**Wei-li Liu**
Esther Kazlow	Yeshiva University	**Allan Wolkoff**
Vanessa Lehmann	Oberlin College	**Joan Berman**
Chanel Ligon	Swarthmore College	**William Jacobs**
Tamar Ariella Lunzer	Yeshiva University	**Louis Weiss**
Cara McDavitt	Fordham University	**Naum Shaparin**
Alina Mitina	California State University Channel Islands	**Ganjam Kalpana**
Roberto Morales Silva	University Of Puerto Rico-Ponce	**Steven Walkley**
Alec Musial	Fordham University	**David Spray**
Desiree Nieves	University of California- Davis	**Saleem Nicola**
Bracha Robinson	Yeshiva University	**Antonio Cristofano**
Natalia Rodriguez	University of Pennsylvania	**Thomas Leyh**
Sophia Salazar	University of Texas at El Paso	**Hernando Sosa**
Nadja Santana	University of Puerto Rico	**Elyse Sussman**
Aaron Schwartz	SUNY College At Purchase	**Harris Goldstein**
Philip Shamash	University Of California-Los Angeles	**John Foxe**
Alyssa Shearer	Clemson University	**Nicholas Sibinga**
Sara Shi	Wake Forest University	**Dianna Cox**
Maria Shibatsuji	Loyola Marymount University	**Saleem Nicola**
Zachary Shuler	Muhlenberg College	**Naum Shaparin**
Zoe Spieler	Smith College	**Matthew Scharff**
Valerie Sydnor	Brown University	**Sridhar Mani**
Nathaniel Tracer	Yeshiva University	**Ertugrul Ozbudak**
Justin Vercellino	Montclair State University	**Felipe Griffero**
Ryan von Kleeck	Franklin And Marshall College	**Vern Schramm**
Liat Weinstock	Stern College for Women	**Rebecca Madan**
Joshua Wetzler	Johns Hopkins University	**Tom Belbin**

Mapping Functional Epitopes of Dengue Virus E2 DIII for Binding to Broadly-Neutralizing Antibody 4E11

Dillon Ade, Julia Frei, Jonathan R. Lai
Department of Biochemistry, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx,NY

Dengue Virus (DENV) is a single stranded RNA virus that is a member of the flaviviridae family. It annually infects about 400 million people and is a leading cause of illness and death in the tropics and subtropics. There are four serotypes of DENV which co-circulate in endemic regions and they are all transmitted by the mosquito family Aedes. There are no approved vaccines or treatments available for DENV. The surface of the DENV virus is coated by the homodimer glycoprotein E, which is composed of three domains. The binding domain on the E2 subunit, DIII, is responsible for entry into host cells.

Murine antibody 4E11 binds to DIII and neutralizes the four serotypes of DENV (DENV1-4), albeit with weak binding affinity and potency against DENV-4. 4E11 is considered a broadly neutralizing antibody (bNAb). A bNAb's broad neutralizing characteristic originates from its ability to bind to multiple strains or serotypes of viruses, and thus rendering the virus biologically inactive (neutralization). Targeting DIII, the receptor-binding domain of DENV, a bNAb should prevent all four strains from being endocytosed and stop viral-host cell membrane fusion. bNA;s rely on focused and conserved residues for binding to DIII. Previous work shows that 4E11 recognizes DIII on all four serotypes through charge complementarity between the 4E11 paratope and the DIII epitope. We are interested in how bNAbs bind and neutralize diverse targets, and are therefore mapping the 4E11 functional epitopes of DIII in order to determine which residues are integral to binding energetics.

We are mapping the DIII functional epitopes of DENV using combinatorial shotgun scanning mutagenesis with phage display. This involves producing filamentous bacteriophage with N-terminally FLAG-tagged DIII attached to the minor coat protein, pIII of M13 bacteriophage. We use ELISAs to screen a library of DIII-mutants, in which the residues of the structural epitopes vary between alanine and wild-type for binding to 4E11. Libraries for DENV1 and DENV2 have been fully mapped, and the DENV3 library has been screened twice. We will be finishing the DENV3 screens and begin on DENV4 in the near future.

I would like to thank Julia and the rest of the Lai lab. They have been tremendously helpful this summer. They are a very welcoming and hardworking bunch, an overall great scientific group.

Protective antibodies against *M. tuberculosis* infection

Lorita Agu[1], Caroline Blanc[2], and Jacqueline M. Achkar[2]
[1] Converse college, [2]Department of Medicine, Division of Infectious Diseases, Albert Einstein College of Medicine, Bronx, NY.

Background. Successful tuberculosis vaccine development is critically dependent on identifying host factors that protect against the development of active tuberculosis (TB). The immunologic components that prevent disease development are still not completely understood. Cell-mediated immunity (CMI) plays a pivotal role, but controversy remains about the additional role of antibodies (Abs) in the defense against *M. tuberculosis* (Mtb) infection. There is now increasing evidence that Abs contribute to the protection against TB (reviewed in (1)). The heparin-binding haemagglutinin (HBHA) of Mtb is required for extrapulmonary dissemination, and a monoclonal Ab (mAb) against HBHA has shown to reduce Mtb dissemination in mice (2). However, the role of Abs against HBHA has not been investigated in humans. **Objective.** The objective of this study is to investigate the levels of Abs against HBHA in TB patients, and compare Ab levels in patients with localized pulmonary disease to those with disseminated disease. **Methods.** Enzyme-Linked Immunoasbsobent Assay (ELISA) was used to analyze human samples, and a monoclonal anti-HBHA antibody served as the positive control. **Results.** Ab detection assays are still being optimized and results are pending. **Conclusions.** At this point no conclusions can be drawn, as experiments have not been completed. However, we hypothesize that Abs responses against HBHA will be higher in TB patients with localized pulmonary TB compared to those with disseminated TB.

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Abstract

Characterization of the EGFR signaling in acquired endocrine resistance using an MCF7 *in vitro* model

Julia L. Alba, Esther A. Peterson, Paraic A. Kenny

Department of Developmental and Molecular Biology, Albert Einstein College of Medicine, Bronx, NY 10461

The ERBB family of receptors are transmembrane tyrosine kinases involved in the activation of complex signaling pathways that controls a variety of functions, including proliferation, adhesion, migration and differentiation. When there is an abnormality somewhere in this pathway it can promote tumorigenesis. Several ligands are responsible for the activation of the receptors including EGF, TGF-alpha, Betacellulin, Epiregulin, Neuregulin and Amphiregulin (AREG). There is evidence that after initially responding to endocrine therapy, many ER+ tumors progress to resistance to endocrine therapies and are frequently characterized by higher levels of EGFR , although the identity of the ligand(s) responsible remains unclear.

For this study I am using sublines of an estrogen receptor (ER) positive breast cancer cell line, MCF7, which are resistant to either Fulvestrant (FulvR) or Tamoxifen (TamR). My goals are: (1) To test the expression of the EGFR ligands in each endocrine resistant line in comparison to parental MCF7 cells. (2) To assess the activation of the EGFR signaling pathway by testing the phosphorylation levels of EGFR and downstream effectors of the pathway (e.g. ERK1/2, AKT). The first approach used to test expression of the ligands was qRT-PCR. The data shows that FulvR line has higher Betacellulin expression compared to the parental control, while TamR has lower expression of all ligands tested when compared to the parental control.

We used western blotting to determine the expression and/or activation of effectors of the EGFR signaling pathway in the endocrine resistant lines. The expression and/or phosphorylation status of ERα, EGFR, ERK1/2 and AKT in MCF7 parental, FulvR and TamR was determined in starved, steady-state conditions (complete growth media) and upon treatment with endocrine inhibitors, Fulvestrant (ICI) and Tamoxifen (OHT). In all cases, GAPDH was used as a loading control. Analysis of blots shows higher activation of EGFR signaling in FulvR cell lines under starved and steady-state conditions.

Future experiments will include the use of siRNA techniques to knockdown the expression of the ligands expressed in the resistant lines. This will allow us to test if the resistant lines are dependent on specific ligands for survival and/or proliferation. In addition, targeting the EGFR signaling pathway using inhibitors will be performed to see if the endocrine resistant cells could potentially be resensitized to endocrine therapy.

In summary, understanding the role of EGFR signaling in endocrine resistant lines could potentially help to develop alternative therapies for ER-alpha positive breast cancers that become resistant to endocrine therapy.

Snf1-Regulated Transcription Factors are Required for Proteasome Mediated Lifespan Extension in *Saccharomyces cerevisiae*

Sonia Barakat, Yanhua Yao, Marion Schmidt
Department of Biochemistry at Albert Einstein College of Medicine
Bronx, New York

The AMPK pathway is highly conserved and plays a pivotal role in longevity and cellular response to caloric restriction. Our data demonstrates that proteasome mediated lifespan extension results in the deregulation of the AMPK/Snf1 signaling pathway causing an increase in respiratory capacity. We further propose an interconnected network containing the proteasome, Sir2, and AMPK/Hxk2 signaling that regulates lifespan in *Saccharomyces cerevisiae.*The switch from fermentation to respiration is controlled by AMPK/Snf1 signaling, Transcriptional regulators downstream of AMPK repress/activate genes in response to carbon source availability. A deregulation of the pathway leads to premature induction of respiration, increasing levels of ROS and affects aging.

Increased respiration and oxidative stress induces the activity of proteolytic systems responsible for preventing the accumulation of aggregates that can lead to cell death. In aging cells, however, we find that the activity of the proteasome declines due to increased oxidative damage. We have shown previously that upregulation of proteasome activity in aging yeast cells has a beneficial effect on lifespan. This beneficial factor can be due to increased protein homestasis or caused by increased degradation of a negative regulator of lifespan downstream of AMP-kinase, namely Mig1 and Hxk2. In this project we test the hypothesis that increased degradation of Mig1 might contribute to proteasome mediated effects on lifespan.

Mutant strains were constructed and allowed to grow on various media inducing different proteotoxic stresses and incubated at 30°C.Expression changes in response to proteotoxic stress prove that there is an interaction between these genes. Increased levels of proteotoxic stress, however, do not necessarily pose strictly harm to the cells. As you can see, cells were still able to grow even after being treated with different stresses. This helps to indicate that some doses of stress can be of valuable use to the cell as they activate different stress responses and pathways.

Rpn4 is a transcription factor that stimulates the expression of proteasome genes and is transcriptionally regulated by various stress responses. Ubr2 is a cytoplasmic ubiquitin-protein ligase required for the ubiquitylation of Rpn4p. In *ubr2*Δ cells, Mig1 levels were lower when compared to *rpn4*Δ cells that have reduced proteasome activity. Based on these observations, it would suggest that Mig1 is targeted and degraded by the proteasome, necessary for proteasome-mediated lifespan extension, and is incorrectly processed under repressive conditions in cells with increased proteasome abundance. Increased degradation of Mig1 leads to the inactivation of Mig1 and premature activation of the AMPK/Snf1 pathway.

Mig1 was further deleted in two other strains (Sir2 and Hxk2) that allow for lifespan extension. Sir2 is a conserved NAD^+ dependent histone deacetylase involved in negatively regulating the initiation of DNA replication. Overexpression of Sir2 allows for increased longevity. Hxk2 directly regulates Mig1 and deletion of Hxk2, which acts as an activator for Mig1, leads to increased respiration and extends replicative and chronological lifespan in yeast. Because Hxk2 is upstream from Mig1, loss of MIG1 would not have any consequences on lifespan extension in Hxk2 cells.

Having established that increased proteasome activity leads to a deregulation of the AMPK/Snf1 pathway, we investigated that the proteasome activity also affects Sir2 and Hxk2 mediated lifespan extension. In both strains, it was found that increased proteasome activity concurred an additive effect, while on the other hand, decreasing levels of proteasome activity completely blocked lifespan extension for both strains.

Increased proteasome capacity improves viability under proteotoxic stress and elevated proteasome activity reduces the aggregation of misfolding proteins in aging cells. Elevated proteasome capacity also results in reduced abundance and nuclear localization of AMPK/Snf1-regulated Mig1, increasing respiratory activity, and increased oxidative stress response. Reduced or impaired proteasome activity limits lifespan extension in cells overexpressing SIR2 or in cells lacking Hxk2. We can therefore propose that a network between the proteasome, Sir2, and AMPK/Hxk2 signaling exists and impacts the longevity in *Saccharomyces cerevisiae*.

Allelic Diversity of IFNAR-1 in African Patients with Malaria

Jacqueline Benayoun, Lucas R. Cusumano,Seungjin Ryu,
Catherine Manix Feintuch, Daouda Ndiaye, Esther Gondwe, Karl Seydel, Terrie Taylor, Yousin Suh, Johanna P. Daily

Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, N.Y.

Malaria is a serious and sometimes fatal disease that caused 207 million clinical episodes and 627,000 deaths in 2012 alone (World Health Organization). Genetic predispositions can modulate the risk for severe malaria. Prior studies have found the association of mutations within the Type I Interferon Receptor (IFNAR1) and disease outcomes in *Plasmodium falciparum* malaria. To further explore the role of mutations in IFNAR1 we carried out an in-depth Single Nuclear Polymorphism (n=21 SNPs) analysis at the IFNAR1 locus. We examined genomic DNA from children with cerebral malaria from Malawi and tested associations with disease outcomes. In addition we examined the IFNAR1 allelic diversity between West Africa (Senegal) and East Africa (Malawi).

Type I IFNs are involved in host response to malaria. Type I IFNs prime macrophage pro-inflammatory responses, enhance intracellular killing, dendritic cell (DC) maturation and T helper 1 cell responses; and promote lymphocyte activity. Type 1 IFN has been associated with modulating malaria infection outcomes in the animal model of cerebral malaria and in human malarial disease.

Type I IFN receptor (IFNAR1) polymorphisms are associated with disease outcomes in malaria. This will be tested through an association study of IFNAR1 alleles in patients with mild versus severe malaria.

Genomic DNA was extracted from dry blood spots of African Patients using a DNeasy extraction kit. The samples were quantified by nanodrop and amplified using PCR. Gel electrophoresis was run to confirm the presence of the DNA. Then, Iplex was performed to determine the alleles present in the African cohorts in 21 SNPs. Finally, a Typer program was used to generate the patient spectra and allele call rate.

After testing 21 SNPs, a 94% call rate from 20 SNPs was achieved. Out of the 20 primers that worked, 7 primers were monomorphic. From the remaining 13 primers, 4 primers showed no significant differences between the acute and mild cohorts, and. 3 were statistically significant.

Significant differences are present in the IFNAR1 locus between severe and mild malaria cohort. Further studies should be repeated using a mild malaria cohort from Malawi to match population background. Additionally, the functional significance of this allelic diversity must be defined and linked to additional disease outcome (such as anemia and survival rate).

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The Use of Intravital Two-Photon Imaging to Capture the Egress of Blimp-1-YFP^{+}B1.8$^{hi+/-}$ Plasma Cells from the Popliteal Lymph Node

By Christie Black and Dr. David R. Fooksman

Abstract

Plasma cells (PC) are antibody-secreting cells that develop from naive B-cells (1). B cells undergo a series of activation steps that result in highly specific, long-lived plasma cells that egress from the lymph node (LN) and relocate to the bone marrow (BM) (2). The ability of PCs to remain within the BM, often for extended periods of time, and secrete antibody is integral to the success of the adaptive immune response and most current vaccines (2). While previous studies have shown that chemotactic signals play a large role in the homing of plasma cells to the BM (3), the exact path that plasma cells use from the LN to the BM is not completely understood. Findings by Pabst et al. (4) demonstrate that LFA-1 (one of the B_2-integrin heterodimeric complexes) deficient PCs fail to exit but it is known that other lymphocytes do not require it for exit (5).

It is assumed that PCs exit the LN through the lymphatics but it is also possible that they use blood vessels, which may require LFA-1. The aim of this study is to explore exactly how PCs physically depart the LN's medullary cords and migrate to the BM.

One method of visualizing the physical departure of PCs from the LN is through intravital two-photon imaging. Unlike conventional confocal microscopy, two-photon microscopy allows for the observation of living cell behavior deep within tissues and organs without killing the cells through fixation and thin sectioning (6). Thus, this tool makes it possible to visualize fluorescently labeled plasma cells and their dynamic behavior within the LN of a living mouse.

During imaging, we observed PCs arrested and moving around blood and lymphatic vessels. We could observe other lymphocytes traveling within the blood and lymphatic vessels in the LN. In contrast, we did not detect PCs entering either vessel or exiting the LN. With further imaging experiments, we could eventually observe PC exit from the lymph nodes through blood vessels, lymphatics, or otherwise.

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Shacelles Bonner

Mentor: Dr. Kelvin Davies

DSSORP/SURP Abstract

Gene transfer of h-Maxi-K Plasmid with use of Nanoparticle

Several diseases, such as diabetes, partial urethral obstruction and aging, result in reduced activity of the MaxiK potassium channel in urogenital smooth muscle tissue. Activity of the MaxiK potassium channel is an important regulator of smooth muscle tone and reduced expression levels are a factor in the development of over-active bladder and erectile dysfunction. The hMaxi-plasmid is a gene transfer product that was developed by Ion Channel Innovations to increase expression of MaxiK channels in smooth muscle. At present this vector is used in "naked gene therapy". Naked gene therapy with non-replicative plasmid is considered one of the safest forms of gene therapy, but has the drawback that relatively few cells take up and express the plasmid. In the current project we hypothesized and tested if the efficiency of cellular uptake could be enhanced by using a nanoparticle (np) delivery system, developed in the laboratory of Dr. Joel Friedman. In a first set of experiments we applied different doses of MaxiK-np to HEK 293 cells and demonstrated significant increases in expression of MaxiK using quantitative RT-PCR. Even the lowest doses of MaxiK gave significant increases in expression after 24 hours. In a second set of experiments we labeled nanoparticles with FITC and conjugated to a florescent marker plasmid p-mCherry in order to determine both the location of the nanoparticles and the transfection efficiency. Fluorescence from the mCherry was used to demonstrate high transfection efficiency, as well as a rapid onset of transcription (after approximately 4h) which reached a peak at 22h, and persisted to at least 44h. Overall these results suggest that using MaxiK-np is an efficient delivery system for the MaxiK gene, which potentially could increase the efficacy of hMaxi therapy in treating urogenital smooth muscle pathology.

Monica Castle Mentor: Dr. Ekaterina Dadachova

Radioimmunotherapy As A Strategy for Targeting Latently Infected Cells

M. Castle[1], D. Tsukrov[2], R. Bryan[2], O. Kutch[3] E. Dadachova[2]

[1]Rutgers University, New Brunswick, NJ; [2]Albert Einstein College of Medicine, Bronx, NY; [3]University of Alabama, Birmingham, AL.

ABSTRACT:

Background: Despite successfully suppressing viral loads in HIV-infected patients, anti-retroviral therapy (ART) alone cannot cure HIV because it fails to kill HIV-infected cells. Contributing to viral persistence in ART patients is the existence of latently infected cellular reservoirs. Curative strategy for HIV must therefore include a method that targets and kills both latently and actively infected cells. Radioimmunotherapy (RIT) is an established clinical method that pairs a radioactive moiety to a monoclonal antibody to specifically deliver cytocidal doses of radiation to targeted cells. Previous studies have successfully used RIT to target the HIV gp41 surface glycoprotein and kill actively infected cells. Cells in latent reservoirs however, show an attenuated gp41 expression. We investigated the efficiency of RIT in delivering cytocidal radiation to and killing this cell population. **Methods:** We used cell lines that were infected with the dual-tropic 89.6 HIV strain and were engineered to express enhanced green fluorescent protein (EGFP) as a marker for viral expression. The THP89GFP and the J89GFP cell lines model latently infected monocytes/macrophages (THP-1) and T-cells (Jurkat), respectively. The respective uninfected parental cell lines THP-1 and JLTRG were used as negative controls. Latent and TNF-α (5ng/ml) activated cell populations were treated with the gp41-targeting monoclonal antibody 2556 conjugated to radionuclide Bismuth-213. Post-treatment, cell survival was assessed by Trypan blue exclusion and gp41 surface and p24 capsid HIV protein expression was verified concurrently through fluorescence microscopy and flow cytometry. **Results:** P24 fluorescence was observed throughout the cytoplasm of latently infected THP89-GFP cells. Both latent and activated cells exhibited a dose-response to increasing RIT treatment levels. An 8 μCi dose of ^{213}Bi-2556 successfully killed over 50% of infected cells ($p<0.05$). **Conclusion:** Although attenuated in latent cell lines, surface gp41 expression was sufficient for ^{213}Bi-2556 to specifically bind and kill both latent and activated cells in a dose-dependent fashion. Results suggest that latently infected cells can be identified by p24 expression and that RIT in combination with current viral load-reducing treatment methods can potentially eradicate latent HIV reservoirs. **Acknowledgements:** Monoclonal antibody to HIV-1 p24 (AG3.0) was obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH, from Jonathan Allan.

The Role of Presynaptic Protein Synthesis in Endocannabinoid Mediated Long-Term Plasticity

Carlton W. Christie, Thomas J. Younts, Ph.D., Pablo E. Castilo M.D., Ph.D.

Dominick P. Purpura Department of Neuroscience
Albert Einstein College of Medicine, Bronx, NY

Endocannabinoids are retrograde lipid messengers that mediate short and long-term suppression of neurotransmitter release throughout the nervous system by activating presynaptic type 1 cannabinoid receptors (CB_1Rs). After postsynaptic production of either AEA or 2-AG, the CB_1R agonist travels back across the synapse, binding type 1 cannabinoid receptors, which through a subsequent signaling cascade inhibits protein kinase A (PKA) and activates calcineurin to favor the dephosphorylation of some target protein, causing the suppression of neurotransmitter release. Previous studies done in the peripheral nervous system and in invertebrates indicate that presynaptic protein synthesis is required for types of long-term plasticity. However, it remains unclear whether protein synthesis plays an important role in the inhibition of long-term depression mediated by endocannabinoids in the central nervous system (CNS) of mammals.

Working within this signaling framework, protein synthesis either local or somatic may affect the induction or maintenance of the long-term depression of inhibition (iLTD) caused by endocannabinoid signaling. Recording field potentials from CA1 pyramidal cells receiving inputs from GABAergic inhibitory interneurons near and around the striatum pyramidale of the murine hippocampus, protein synthesis inhibitors cycloheximide, ansiomycin, and ISRIB, which is an inhibitor of eIF2α, were administered to assess necessary protein synthesis on chemically induced iLTD. Studies characterizing eIF2α suggest that it acts to direct the binding of methionyl-tRNAi to 40 S subunits in a codon-specific manner.

The general inhibitors of protein synthesis cycloheximide and ansiomycin showed a block of WIN (a CB_1R agonist) mediated iLTD and the ISRIB showed a partial block of iLTD. Additionally, the block in the WIN-induced increased paired pulse ratio with the addition of protein synthesis inhibitors suggests that presynaptic protein synthesis is necessary for WIN mediated iLTD. Future studies should probe the nature of the ISRIB inhibition with whole cell and paired cell recordings. I would like to express my sincere gratitude for the direction, help, guidance, and teaching of every Castillo lab member. Especially, Karina Alvina and Tommy Younts for helping me to achieve stable baselines and for answering an interminable series of questions about electrophysiology.

Characterization of *Plasmodium vivax* ENT1 Single Nucleotide Polymorphisms

Adi Cohen, Roman Deniskin, Myles Akabas

Department of Physiology and Biophysics

Albert Einstein College of Medicine of Yeshiva University, Bronx, NY

Malaria is a life threatening disease caused by parasitic protists of the genus *Plasmodium*. Every year, ~3 billion people are at risk of getting malaria and about half a million people die from malarial infections. Two *Plasmodium* species contribute to >80% of the disease mortality and morbidity: *Plasmodium falciparum* and *vivax*. Rapid antimalarial drug resistance has developed over the past two decades, which underscores the importance of identifying new drug scaffolds with unique targets. Since *Plasmodium* spp. are purine auxotrophic organisms and require the salvage of purines from their host, the parasite purine salvage pathway is a great target for drug development. In *Plasmodium*, the import of purines into the parasite is mediated by equilibrative nucleoside transporters (ENTs). In *P. falciparum*, PfENT1 is the principal purine transporter. While much is known about PfENT1, little is known about PvENT1 and the three single nucleotide polymorphisms (SNPs: M99I/Q367K, N329S) of the gene. In this body of work, we used purine auxotrophic *Saccharomyces cerevisiae* yeast cells to heterologously express wild-type protein and the PvENT1 SNPs. We evaluated the ability of these strains to take up purine and grow on either solid media or in liquid culture. All strains grew equally in media where adenine was the sole purine source (EC_{50}: 20-40 μM). However, in the presence of adenosine, the N329S mutant failed to proliferate; the remaining SNPs had similar EC_{50} values (~0.3-1 mM). Next we evaluated whether the SNPs altered the binding profile of various purine substrates by measuring the uptake of [^{3}H]adenosine in the presence of increasing concentrations of inosine and hypoxanthine (HX). EC_{50} values for the inhibition of radiolabel was the same for all the SNPs (EC_{50} inosine: ~6-15 μM; EC_{50} HX: ~0.6-1 mM). We also demonstrated that adenosine uptake is pH dependent: uptake was higher in acidic pH compared to more alkaline conditions. We are currently characterizing the N329S SNP as it is the only mutant that has inconsistent results across the different yeast assays.

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Dr. Scott Emmons

Department of Genetics, Dominick D. Purpura Department of Neuroscience

Albert Einstein College of Medicine

Emmons Lab Abstract

In 1986, John White and colleagues published “The Mind of a Worm”, a culmination of 10 years' research into the wiring and connectivity of the *C. elegans* nervous system. This was an enormous task, requiring manual annotations of neurons and synapses using markers on individual magnified electron micrographs. Due to the project's scale, it was not reattempted for many years; however, improvements in computing since that time now allow for this project to be completed again.

The Emmons lab aims to map the various neurons and synapses of the *C. elegans* nervous system in order to better understand synapse formation and the genetic basis of behavior. Using “Elegance”, a program developed within the lab, users are able to mark individual cells on series of electron micrographs with a unique overlay, called a “node”. Nodes meet together at “edges”, where one cell synapses onto a neighbor, and the size and number of edges between two nodes is the “edge weight”, an indicator of general synaptic strength. Researchers can then use Elegance to generate a list of all synaptic contacts for a particular cell, compare the weights of varying edges, or develop 2D or 3D models of neuron morphology. The resulting data sets can then be compared, allowing users to examine differences in edge weight or neuron shape between different areas of the body, different worms, or different developmental stages.

This project compared three datasets containing sections of *C. elegans* pharynx in order to determine variation between these sets. The JSA and N2W datasets were taken from adult hermaphrodite worms, and the N1126F dataset was taken from an L1 larval worm. After reconstruction, it was found that the JSA worm had many more synaptic contacts than the N2W worm. The JSA worm had almost twice as many unique edges resulting in a total edge weight nearly four times as great as that of the N2W worm. The weight of the JSA worm's unique edges was found to be four times that of the N2W worm. In a comparison of the unique edges of both data sets, different nodes formed these unique edges with each other. The L1 worm had even fewer synaptic contacts than N2W, and, in addition, was found to have significant differences in neuron morphology. The L1 sections had fewer cells in both ventral nerve regions in the pharynx, but had more in the dorsal region. An examination of edges of cells with left-right homologues revealed that these homologues made contacts with the same cells or matching cell homologues in, at best, 50% of the time.

The data gathered during this project leaves several avenues for future inquiry. More robust comparisons between different animals and developmental stages can now be performed, including examination of the differences between neuron maps between larval and adult forms. It also further refines models of the *C. elegans* pharyngeal connectome in order to create a model of the wild-type connectome. Mutagenesis studies can now be conducted, allowing researchers to elucidate the genetic factors behind neuron and synapse formation.

Neuronal Therapeutic Approaches to Lysosomal Storage Disorders

Jessica Cysner, Shannon Odell, Ben Papapietro, Kostantin Dobrenis.
Dominick P. Purpura Dept. of Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461

Lysosomal storage disorders (LSDs) are diseases marked by the inability of cells to correctly degrade or mobilize specific substrates in the lysosomal system. Genetic deficiency in lysosomal system proteins, often catabolic enzymes, results in the accumulation of these substrates within cells, and leads to various disease symptoms. Many LSDs, like Tay-Sachs, Sandhoff and Niemann pick type C (NPC) diseases affect the central nervous system (CNS) with severe intellectual disabilities and early death. Overall, there has been little progress in developing treatments to successfully target the brain. Although several promising therapies exist, the relative impermeability of the blood-brain barrier (BBB) and specific neuronal properties impeding protein or gene replacement present significant challenges to their effectiveness.

In Tay-Sachs and Sandoff diseases, G_{M2} ganglioside (G_{M2}) is stored due to deficiency of β-Hexosaminidase (Hex) which normally cleaves it. G_{M2} accumulation is also a prominent part of neuropathology seen in numerous other LSDs. N-butyldeoxynojirimycin (NB-DNJ) or "miglustat" is a drug that inhibits production of a precursor of $G_{M2,}$ therefore, inhibiting G_{M2} synthesis. In past studies, the ability of NB-DNJ to show positive effects on the brain has been extremely limited. Our research seeks to assess the effectiveness of miglustat in helping to correct storage in Sandhoff neurons by administering NB-DNJ in conjunction with enzyme (Hex) therapy. Overall, miglustat showed very little effect on the reduction of G_{M2} storage within Sandhoff model neuronal cultures. Treatment with the Hex enzyme, even at a relatively low dose of 3000U/mL, demonstrated a greater degree of storage improvement than did administration of miglustat alone or in conjunction with the Hex enzyme.

Towards effective gene therapy of the brain, several Naldini lentiviral plasmids were purified and packaged into VSV-G pseudotyped lentiviral vectors. These expressed a Hex transgene or one of three different hybrid constructs of Hex with a downstream linker sequence followed by sequences encoding shortened portions of he atoxic C-fragment (H_c) of tetanus toxin. Two versions also contained a bicistronic GFP. Based on previous experiments by this lab, these peptides could allow CNS entry, efficiently enhance neuronal uptake of enzyme and permit retrograde trans-synaptic transport. Sandhoff neuronal cultures were infected with these constructs to determine whether these enzymes when produced within neurons would still be delivered to their lysosomes. No toxic side effects were evident. Secondly neurons were transduced with bicistronic GFP versions to determine if these modified enzymes could also be effectively delivered from one neuron to a secondary non-transduced neuron. This ability to "cross-correct" would help towards overcoming limits in efficiency of gene therapy and widespread delivery of vectors particularly when having to deal with the volume of the human brain. Results on these experiments are pending.

Peripheral injection of Cyclodextrin (CD), a common excipient used by the pharmaceutical industry, in mouse models of NPC disease has demonstrated significant reduction of the lysosomal unesterified cholesterol storage in neurons characteristic of this disease. CD is now being used in small Phase I NIH clinical trial on NPC affected children. However, the mechanism by which CD acts to reduce neuronal storage is unknown. Additionally, studies suggest that little CD enters the CNS and show no evidence of active transport or neuronal uptake. Here, we tested the idea that CD acts indirectly to decrease neuronal storage by sequestering exogenous loads of cholesterol thereby minimizing additional accumulation in neurons. For this to be consistent with in vivo observations suggesting reversal of preexisting storage by CD, neurons would have to have a mechanism that can remove cholesterol from lysosomes in the absence of the NPC proteins that normally allow cholesterol to exit the lysosome. Purified cortical neuronal cultures from NPC disease mice maintained in the absence of serum, were exposed for 24hrs to 13μM cholesterol (equivalent to levels in human cerebrospinal fluid), washed and maintained another 6 days. These were compared with disease cultures not exposed to cholesterol and wild type cultures that were similarly treated. Based on histochemical staining of unesterified cholesterol storage using fluorescent filipin, NPC cultures loaded with cholesterol evidenced a greater degree of storage than wild type cultures. NPC cells not treated with cholesterol resembled wild type cultures. Conclusions of this experiment are pending quantitative analysis.

Ultimately, these studies help us to further understand the mechanisms related to these diseases and bring us closer to viable treatment options for diseases of the CNS. By incorporating these treatment options into the therapy provided for patients with LSDs, the unfortunate outcome of these diseases can ideally be reduced.

Suppression of Glucose Production by Central Insulin or IGF-1 is Associated with Paradoxical Shifts in Hepatic Gene Expression with Aging

Lilah Fones[1,2,3], Ardijana Novaj[1,2,3], Zunju Hu[1,2,3], Kai Mao[1,2,3], Donghai Wang[1,2,3], Gabriela Farias Quipildor[1,2,3], and Derek M. Huffman1,2,3

[1]Departments of Medicine and [2]Molecular Pharmacology, and [3]Institute for Aging Research, Albert Einstein College of Medicine, Bronx, NY 10461

IGF-1 is well known for its role in growth, including brain development, but has been recently shown to modulate whole-body glucose metabolism, similar to insulin, via central mechanisms. Aging is characterized by hyperinsulinemia and central insulin resistance, with a concomitant decline in the GH/IGF-1 axis. Given these characteristic changes in insulin and IGF-1 with aging, we hypothesized that IGF-1 may have superior therapeutic potential to insulin for restoring peripheral insulin action in aging. In order to determine the metabolic effects of central insulin and IGF-1, we performed hyperinsulinemic-euglycemic clamps with intracerebroventricular infusions in 4 and 24 month old FBN rats. We also evaluated gene expression in liver for glucose-6-phosphatase (G6Pase), phosphoenolpyruvate carboxykinase 1 (PEPCK1), glycogen phosphorylase (Pygl), and plasminogen activator inhibitor-1 (PAI-1) and in mediobasal hypothalamus (MBH) for neuropeptide Y (NPY), agouti-related peptide (AgRP), and pro-opiomelanocortin (POMC). Using central doses that elicited similar results in young rats, clamp studies in old rats revealed that both central IGF-1 and insulin resulted in comparable improvements in insulin sensitivity, as compared to controls ($P<0.05$), mainly by suppressing hepatic glucose production. However, unlike young animals, central IGF-1 and insulin infusion in old animals resulted in a paradoxical increase in PEPCK1 and Pygl expression in liver ($P<0.05$). In the MBH, POMC expression was significantly lower in old animals ($P<0.05$), but no effect of treatment was observed on neuropeptide transcripts. In summary, both central IGF-1 and insulin restored peripheral insulin sensitivity to levels observed in young controls, which was accompanied by paradoxical shifts in liver transcripts important for glucose metabolism. The functional relevance, if any, of the differential response to central IGF-1 and insulin in liver expression with aging will require further study.

Molecular Characterization of Cell Competition in Drosophila

Casey Garrett, Chang Hyun Lee, Nicholas Baker

Department of Genetics, Albert Einstein College of Medicine

1300 Morris Park Avenue Bronx, NY 1046

Cell competition is a phenomenon that occurs when a cell's survival is dependent on its relative fitness compared to the adjacent cells. The more fit cells (winner cells) induce apoptosis in the loser cells. The winner cells then take over the tissue. This leads to tissue where one genotype outcompetes the other. Cell competition was first characterized in drosophila. Cells heterozygous for a ribosomal protein, which are deemed *minute* cells, are outcompeted by wildtype cells. This occurs even though the *minute* cells are completely viable and mature to a normal size as the wildtype cells. It's only when the two genotypes are adjacent to each other that cell competition is observed. Though cell competition has been shown to be conserved in drosophila and in mice no clear consensus has been reached on the cellular mechanisms responsible for it. Many studies have shown genes that when induced can affect the winner/loser status of the cell but no 'cell competition genes' have been identified as of yet. It is likely that there is no gene responsible for solely cell competition but rather a series of genes involved in various complex mechanism. Our studies show drosophila lines mutated by EMS where no cell competition phenotype has been observed. This proves as a powerful tool because the mutated genome can be sequenced and potential genes responsible for cell competition can be identified. One of the genes found to be mutated was XRP1, which we hypothesize is the gene that is responsible for the loss of function phenotype. It has been suggested that XRP1 acts in a P53 response pathway. P53 is a tumor suppressing gene that is involved in many different cellular pathways such as apoptosis, cell cycle arrest, etc. It has also been found that XRP1 overexpression induces antennal duplication and has an effect on eye disc cell proliferation. To test the hypothesis we first verified the mutation by sequencing. The CRISPR system was then used to develop flies that expressed the XRP1 mutation. We used restriction enzyme digest to test whether the mutation was successfully incorporated into the genome. Unfortunately, we have not had a positive result yet. There are many steps where something may have gone wrong. In the future, the CRISPR system we be revisited and corrected if a problem is found. Once a XRP1 mutant fly is found, the next step is to verify whether cell competition is occurring or not. Cell competition has been implications from organ growth and size determination to cancer cell proliferation in a tumor. Further understanding of the pathways responsible for cell competition can lead to a better understanding of organogenesis and tumorigenesis. I'd like to thank the entire Baker lab for their support, especially Dr. Baker for giving me the opportunity to work in his lab and Chang for his continued guidance and encouragement.

Characterization of Gas6$^{-/-}$ Mice in Health and Disease

Tanvi Goyal[1, 2], Ross Gruber[1], Alex Ray[1] and Bridget Shafit-Zagardo[1]
[1]Department of Pathology, Albert Einstein College of Medicine, Yeshiva University, Jack and Pearl Resnick Campus, Forcheimer Building, Room 524, 1300 Morris Park Avenue, Bronx, NY 10461. [2]Rutgers University, The State University of New Jersey, New Brunswick, NJ.

Multiple sclerosis (MS) is an autoimmune disease affecting the brain and spinal cord. Onset occurs in young adults. Symptoms include optic neuritis, vision deterioration, paralysis, fatigue, and cognitive decline. Genetic susceptibility and environmental factors can contribute to disease progression. According to the National Multiple Sclerosis Society, more than 2.3 million people are afflicted with the disease worldwide. The prevalence of MS is approximately twice for women as for men. A cure for MS has not yet been found. The exact causes of the inflammation in MS are unknown; however, the body's immune system attacks the central nervous system (CNS) destroying the brain's myelin. Myelin is the protective coating that insulates axons, increasing the speed of neural information that travels along nerves. Clinical and pathologic analysis of the CNS during MS and a MS mouse model, myelin-oligodendrocyte glycoprotein (MOG)-induced experimental autoimmune encephalomyelitis (EAE), show an influx of inflammatory cells from the periphery and glial proliferation as the primary causes of the ensuing demyelination, axonal damage and neuronal loss. This eventually leads to the degeneration of neurons and loss of the myelin-synthesizing cells known as oligodendrocytes.

Growth arrest-specific protein 6 (Gas6) is one of the most abundant Vitamin K-dependent, gamma-carboxylated growth factors found in the CNS. Gas6 is a soluble agonist for the Tyro3, Axl, and Mer (TAM) family of receptor tyrosine kinases. Gas6 is the sole ligand for Axl. Axl receptors have the highest relative affinity for Gas6 (Axl>Tyro3>Mer). Tyro3 and Mer are also activated by the serum protein ProS1. Deletion of ProS1 results in embryonic lethality due to its role in blood coagulation independent of TAM signaling, whereas Gas6 knockout mice are viable. In MS lesions, there is a greater level of soluble Axl and Mer relative to normal appearing white matter from the same patients. Soluble Axl and Mer prevent Gas6 from binding to membrane bound receptors, inhibit Gas6 signaling, and contribute to the severity of disease in mouse models of MS. Studies by our laboratory show that dysregulation of Gas6 receptor signaling may prolong lesion activity indicating an important role for Gas6 and TAM signaling in the CNS. TAM signaling reduces proinflammatory cytokine production thus decreasing inflammation.

This research study examined the role of Gas6 in the CNS. We used quantitative real time RT-PCR (qRT-PCR) to quantify the level of expression of various regulatory genes in naïve WT and Gas6$^{-/-}$ mice and during EAE. We hypothesized that naïve Gas6$^{-/-}$ mice and WT mice would show no significant difference in gene expression because naïve Gas6$^{-/-}$ mice show no overt phenotype. We found that relative to naïve WT mice, naïve Gas6$^{-/-}$ mice have equivalent expression of TAM receptors and ProS1, but increased mRNA expression of Sox10 transcription factor, the chemokine receptor CCR2, suppressor of cytokine signaling 3 (Socs3), and interferon gamma, $p<0.05$. Clinical scoring, immunostaining, and qRT-PCR along with statistical analysis showed that Gas6$^{-/-}$ mice have a worse clinical course with significantly more demyelination and axonal damage, and increased proinflammatory cytokine expression of IL-17, TNFα, IL-6, and TGF-β, $p<0.05$.

Through immunostaining for myelin basic protein (MBP), we confirmed there are no differences in CNS architecture or myelination in naïve Gas6$^{-/-}$ Axl$^{-/-}$ double knock-out mice compared to naïve WT mice. In the future, we will examine the levels of gene expression in the naïve Gas6$^{-/-}$ Axl$^{-/-}$ double knock-out compared to naïve WT mice. It is important to study the differences between naïve WT and the single and double knock-outs to see whether the knock-out mice are compensating for the deletions of the Gas6 and/or Axl genes and if knocking out those genes in naïve mice results in changes in phenotype. This study establishes the important protective role of Gas6 in the CNS and encourages further research studying the potential of Gas6 as a therapeutic agent.

Title: The role of *Jag1* and *Tbx1* in neurogenic and sensorigenic pathways in the inner ear

Talia Greenstein, Stephania Macchiarulo, Bernice Morrow

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Albert Einstein College of Medicine
Summer Undergraduate Research Program

Abstract:

Proper genetic function is crucial to neurogenic and sensorigenic morphogenesis of the inner ear. These pathways give rise to the neural, sensory, and nonsensory cells that function in the auditory and vestibular systems in the inner ear. Many genes are utilized in both pathways, such as *Sox2*, *Tbx1*, and the Notch signaling pathway, including *Jag1* and *Dll1*. The precise pathways are still unclear. We compared otic vesicle and cochleo-vestibular ganglia sizes and Notch1 expression in various *Jag1* mutant and wild-type mouse embryos to further elucidate the mechanisms of neurogenesis and neurogenic repression in the inner ear. Decreased otic vesicle size, hypoplastic cochleo-vestibular ganglia, and down-regulated Notch1 expression in *Jag1* mutants demonstrate that *Jag1* plays a significant role in the overall development of the inner ear and a greater role in neurogenesis than previously shown.

Standardization of Trypan Blue Viability Assay for Cryopreserved-Thawed Hematopoietic Progenitor Cell Products

Hadassa Holzapfel[1], Carlo Palesi[2], Domenica Imperato[2], Anesa Mirza[2], Ljiljana V. Vasovic[2,3]

[1] Department of Biochemistry, Stern College for Women, Yeshiva University, New York, NY, [2] Department of Pathology, Montefiore Medical Center, Bronx, NY, [3] Albert Einstein College of Medicine, Bronx, NY

Hematopoietic progenitor cells (HPCs) can differentiate to various specialized blood cells and form the basis of cellular therapies for patients with cancers and other disorders of the blood and immune systems. The cellular therapy product (CTP) containing HPCs can be obtained directly from the bone marrow or can be collected by an apheresis procedure after HPCs are mobilized into circulating blood. Collected CTPs inevitably also contain variety of other blood cells including maturing white blood cells (WBC), red blood cells (RBC) and platelets. Cryopreservation then allows CTP to be stored at -80°C to -196°C until the transplant is medically indicated. The cell viability is reduced after CTP cryopreservation and cryogenic storage. Nowadays, laboratory determination of the CTP viability is a measure of product quality and is traditionally based on:

1. Trypan blue (TB) assay for total cellular viability. HPCs appear *in vitro* as ordinary white blood cells and it is not possible to identify them morphologically by traditional staining methods, including TB.
2. 7AAD in combination with CD34, an immature cell surface protein marker, can specifically estimate viability of HPCs via flow cytometry.

Both methods are standardized and optimized for staining of fresh CTPs post-collection.
Trypan blue (TB) has a high molecular weight which makes it relatively impermeable to the highly selective membranes of live cells. However, dead cells have damaged membranes which allow trypan blue to permeate, showing a distinctive blue color of the nuclei under the microscope.

The viability determination of cryopreserved CTP after thawing, immediately prior to the transplant, is challenging and difficult to standardize as traditional cryoprotectant containing DMSO is cytotoxic in time related manner and DMSO also interferes with a viability determination. This is seen with both viability methods but especially flow cytometry technique, necessitating use of TB for post-thaw samples. In addition, the TB assay is usually conducted by counting cells with a hemocytometer. Technicians vary in their viability counting due to counting speed and challenge of cell identification. This is a critical parameter for the assay standardization. Therefore, a standardized and reproducible viability method is needed.
Our goals were to improve trypan blue assay sensitivity and accuracy to determine viability in cryopreserved, thawed samples, standardize interobserver variability, improve test result documentation and, ultimately, use the improved assay to optimize the post-thaw process and improve viability of HPC-CTP.

Fresh HPC, apheresis CTPs collected from peripheral blood were processed immediately. A sample was taken pre-manipulation and post-centrifugation after addition of a cryopreservative containing 10% DMSO.
Frozen CTP bags were thawed in a 37°C water bath and split into two aliquots:

1. Unmanipulated, undiluted CTP
2. 10% Dextran 40 solution in saline gently diluted 1:2

Cell viability was counted by trypan blue neat or first diluting each sample 1:10 with either saline or Dextran 40 and then staining with trypan blue 1:2.
Microphotographs were documented by using the Nikon Eclipse Ti microscope and NIS Elements D software.

The cell viability of a fresh, pre-manipulation CTP was not affected by sample dilution method. The viability of the fresh cells decreased after the addition of DMSO-cryopreservative. The sample dilution with saline decreased viability of the cells more than the dilution with Dextran 40.
The cell viability of thawed products was most preserved when first diluting the sample with Dextran 40, then trypan blue 1:2. Furthermore, thawed, undiluted CTP samples had lower viabilities than samples diluted 1:2 with Dextran 40.

In conclusion, the viability of fresh, unmanipulated cells can be accurately determined using a standard trypan blue staining protocol. Cell dilution with saline to optimize sample concentration does not affect viability results. The viability result of fresh cells in 10% DMSO-based cryoprotectant is directly affected by a diluent. Saline dilution significantly decreases viability results while Dextran 40 dilution maintains accuracy of the assay. The viability of cryopreserved, thawed CTP is significantly decreased by diluting in trypan blue neat or saline. Dextran 40 dilution maintains high viability of cells for an extended period of time. All in all, we optimized the trypan blue viability assay using photomicroscopy to reduce variability of results amongst technologists.

Investigation of the Interaction between the p53 Tumor Suppressor and RNA Polymerase II Using Biochemical Analysis and Single Particle Cryo-Electron Microscopy

Shira Kaye, Sameer Singh, Lihua Song, Wei-Li Liu

In response to cellular stress, the p53 tumor-suppressor protein, or "guardian of the genome," becomes activated. Along with many other regulatory functions, p53 induces cell cycle arrest, repairs DNA, and even promotes apoptosis (programmed cell death) in the event of DNA damage by activating transcription of certain target genes. p53 activates transcription by binding to its response element, a short specific sequence of DNA in the promoter region. In over 50% of cancers, however, p53 is mutated and therefore unable to carry out its regulatory functions. There are certain hot-spot mutations in the DNA-binding domain of p53 prevalent in human cancer, such as R248Q.

p53 has been shown to recruit components of the pre-initiation complex (PIC), a large group of proteins that assembles immediately upstream of the transcription start site. Components of the pre-initiation complex include the general transcription factors TFIIA, TFIIB, TFIID, TFIIF, TFIIE, TFIIH, and RNA Polymerase II (Pol. II). Once Pol. II joins the PIC, it begins the process of transcription by moving along the DNA strand downstream, making mRNA. This mRNA will undergo translation into the necessary proteins.

p53 has been shown to bind and recruit TFIIA, TBP, and TFIIF to the PIC. This project will investigate whether p53 recruits Pol. II in a similar fashion. The first aim will thus be to determine if p53 directly interacts with Pol. II, using co-immunoprecipitation assays. In the event that p53 does bind Pol. II, cryo-electron microscopy will be used to determine the high-resolution three-dimensional (3D) structure of this co-complex.

Co-immunoprecipitation involves adding a specific anti-Pol. II antibody to a Pol. II/p53 solution. We then added Protein-G beads in order to capture the anti-Pol. II antibody and its precipitates. To determine whether or not p53 binds to Pol. II, immunoprecipitates were analyzed using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and western blot analysis via mouse α-p53 and mouse α-Pol. II. Both wild-type and R248Q p53 were visible on the membrane at a band length of 53 kDa, indicating that p53 binds Pol. II in the absence of DNA.

Once we established that p53 binds Pol. II in the absence of DNA, we proceeded to determine the 3D structure of the co-complex using single particle cryo-electron microscopy. High magnification images of the Pol. II/p53 co-complex were recorded using a transmission electron microscope. The complexes were then visually selected in a process known as particle picking, and we generated 2-D class averages using the EMAN program. The structure of Pol. II was clearly visible in the 2-D averages. p53 might also be discernable within the cleft of Pol. II, but a 3-D structure using many more particles must be generated in order to reveal where p53 binds on Pol. II.

p53 binding Pol. II reveals critical information regarding the assembly of the pre-initiation complex. Pol. II is necessary for transcription to begin, and if p53 recruits Pol. II, then p53 must be functioning properly in order for the genes to be transcribed. Interestingly, mutated p53 also binds Pol. II, but perhaps the binding is not effective. The stability of the interaction between R248Q p53 and Pol. II needs to be further examined within transcription. Furthermore, future label transfer assays will be conducted to map the binding interface of the Pol. II/p53 interaction. Additionally, our 2-D class averages revealed apparent structural differences between Pol. II and Pol. II bound to p53. An eventual 3-D structure will uncover whether there are local or global conformational changes in Pol. II when p53 binds, and how these changes are similar or different in mutated p53.

Trafficking Mechanisms of Organic Anion Transport Proteins in Hepatocytes

Esther Kazlow, Pijun Wang, John W. Murray, Allan W. Wolkoff

Marion Bessin Liver Research Center:
Albert Einstein College of Medicine Bronx, NY

A major function of the liver is uptake and metabolism of organic anionic compounds, including drugs. Much of this uptake function is mediated by members of the organic anion transport protein (oatp) family, twelve transmembrane domain glycoproteins that localize to the basolateral plasma membrane of hepatocytes. These proteins are also present in intracellular endocytic vesicles and traffic to and from the plasma membrane. Only transporters on the cell surface can take up ligand from the circulation. Our previous studies on rat oatp1a1, showed that it has a PDZ consensus sequence at its C-terminus and binds to PDZK1, a protein required for its trafficking to the plasma membrane. Oatp1a4 is also found on the plasma membrane, but does not have a PDZ binding sequence. We hypothesized that oatp1a1 co-localizes in vesicles with oatp1a4, determining its subcellular localization by co-trafficking. In this project, plasmid encoding sfGFP-oatp1a1 and RFP-oatp1a4 were prepared and characterized. pcDNA 3.1 plasmids encoding oatp1a1 and oatp1a4 were used to prepare cDNAs by PCR amplification and products were inserted into sfGFP (oatp1a1) and RFP (oatp1a4) expression plasmids using appropriate restriction enzymes. Identity was confirmed by nucleotide sequencing. Plasmids were transfected into HEK 293T cells and expression was examined by immunofluorescence and Western Blotting. The results showed the expression of these proteins in the lysates of the transfected cells. Oatp1a1 transfected cells showed the localization of protein intracellularly and on the plasma membrane. Oatp1a4 transfected cells showed localization of protein intracellulary and on the plasma membrane as well. A Western Blot was performed and confirmed the results seen with immunofluorescence; expression of the proteins oatp1a1 and oatp1a4 that were transfected into the HEK293T cells. In conclusion, we have prepared fluorescent tagged forms of oatp1a1 and oatp1a4 that will permit future studies of their subcellular trafficking.

Abstract: Characterizing opioid receptor expression on mature human monocytes

Names: Emma Lehmann, Matias Jaureguiberry, Dr. Dionna Williams, Dr. Loreto Carvallo-Torres, Rebecca Wilson
Mentor: Dr. Joan W. Berman
Albert Einstein College of Medicine

HIV-1 infection has many possible outcomes in the long term. One unfortunate effect of the virus is a set of neurological issues, collectively termed HAND (HIV-associated neurological disorders). 40-70% of people living with HIV also suffer from HAND. Treatment with cART enables HIV+ individuals to maintain a low to undetectable viral load, but some reservoirs of the virus are still present in the CNS. HIV+ individuals are living longer as a result of cART, but their quality of life can be impacted by serious neurological symptoms. Drug abuse (particularly intravenous drugs such as heroin) is a major risk factor for HIV-1. People in treatment for opioid abuse typically receive methadone or buprenorphine. We are interested in the effects of buprenorphine on HAND symptoms. HAND is, in part, mediated by monocyte transmigration across the blood-brain barrier. Research suggests that opioid abuse worsens HAND symptoms. Buprenorphine is an opioid that acts as a partial agonist at the μ opioid receptor (MOR) and an antagonist at the K opioid receptor (KOR). We are ultimately interested in the effects of buprenorphine on monocyte transmigration across the blood-brain barrier. In this study, we examine a mature subpopulation of monocytes (expressing both CD14 and CD16) by FACS and qPCR to determine relative levels of opioid receptor expression. In our experiments, we cultured monocytes with M-CSF to obtain a mature population. We found that all populations studied expressed μ and K opioid receptors. Monocytes exposed to macrophage colony stimulating factor (M-CSF), had more surface expression of opioid receptors than other groups. M-CSF is increased in the serum of HIV+ individuals, so we propose that our culture system (which includes M-CSF) may be a model of the neuroinflammation seen in the HIV+ brain. Additionally, M-CSF may regulate monocyte surface opioid receptor expression.

LacZ Reporter Mycobacteriophage for Diagnosing Mycobacterium Tuberculosis

Chanel Ligon, William R. Jacobs PhD
Department of Microbiology & Immunology, Albert Einstein College of Medicine, Bronx, NY 10461
Howard Hughes Medical Institute, Exceptional Opportunities Program

Mycobacterium tuberculosis (Mtb) is the causative agent of tuberculosis , an infectious disease responsible for infecting nearly 1/3 of the worlds population and causing nearly two million deaths annually [1]. An effective treatment for a Tuberculosis infection involves a combination of four antibiotics for a duration of 6 months. However, with the emergence of multi-drug resistant strains of Mycobacterium tuberculosis (MDR-TB), treatment becomes more complex, longer in duration, higher in cost, and more severe in side effects. With the increase MDR-TB cases, it is increasingly important to be able to prescribe an effective combination of antibiotics to minimize further emergence of drug resistance and to find the most successful treatment for each patient. Current detection and drug-susceptibility testing consist of sputum smear microscopy to detect the presence of Mtb and automated fluorescent methods. [2]. While culturing is the gold standard of diagnosis and drug susceptibility, it is both time consuming and work intensive (taking >1 month). Fluorescent methods require specialized equipment and training to be properly utilized. [2]. The lack of a rapid and simple diagnostic tool requiring no special equipment for detecting TB is currently a large obstacle in controlling and eventually terminating Mtb.

In 2009, a new approach for diagnosis and drug susceptibility was developed utilizing mycobacteriophages, viruses that are able to infect and replicate within mycobacteria. A genetically engineered TM4 mycobacteriophage was created that carried the gene for green fluorescent protein. Upon infection, Mtb would glow green. When grown in the presence of antibiotics, the bacilli were examined under a fluorescence microscope for the presence of fluorescent bacteria indicating resistance [3]. This approach is very promising as it solves many of the problems that arose with previous diagnostic tools (namely cost and ease of use), however, fluorescent bacteria are not always easy to see and the equipment for detecting fluorescence can be costly. Modifying the phage to have a more pronounced visual cue for infection and requiring no specialized equipment to examine it with would greatly step forward phages as a source of diagnosis.

The addition of a specific gene, lacZ, which encodes for the enzyme beta-galactosidase, was used to create a novel reporter phage. Instead of fluorescencing, this encoded enzyme cleaves the substrate 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal), creating a blue pigment. When grown on a plate, viable bacteria infected by the phage will appear blue. When grown in the presence of antibiotics, only resistant bacilli will grow and become phage infected. Using a visual cue of blue on a plate allows for rapid detection and determination of antibiotic resistance of the Mtb. In addition to ease of use, no specialized equipment is required and no specialized training is needed reducing the cost for diagnosis and reducing the likelihood of mistaken interpretation.

When plated as a lawn of Mtb, plaques will form with blue borders. These plaques will be visible to the naked eye. However, our current strategy has not yet been optimized. While the pYUB328 is the standard cloning vector for generating reporter phages, other vectors are also being utilized to optimize blue phage creation. Additionally, the coloring of the plaques will also be optimized by utilizing different gene/enzyme/colored substrate combinations (phoA/Alkaline phosphatase/BCIP, NagZ/β-N-Acetylglucosaminidase/PNP-GluNAc, and GusA/β-Glucuronidase/Salmon-Gluc). In the end, we hope to have created a phage that easily and specifically infects Mtb and creates a visually clear plaque that can be used to determine drug susceptibility.

Coming up with a clear, easy, and inexpensive diagnostic tool for Mtb is of utmost importance. Current methods are either expensive, time consuming, or complicated requiring either extensive equipment or training. The development of fluorophages opened the door to new, easier ways of diagnosing antibiotic resistance, but still had the problem of requiring specialized equipment and had issues with clarity. Our work to create a modified blue reporter phage has allowed for the development of a novel, inexpensive diagnostic that is clear to read and requires no specialized equipment to do so. Upon optimization, these phages could hold the potential for a field diagnostic to better aid in getting quick and accurate antibiotic resistance profiles of patients' Mtb infections, and aid in getting them the medicine they need as quickly as possible. To better optimize the colored reporter phage plaquing, other are also being utilized.

I would like to thank Dr. William R. Jacobs, Oren Mayers and Torin Weisbrod from the Jacobs Lab for assisting me with my project. I would also like to thank Christy Schultz and Andrew Quon from HHMI for giving me the opportunity to work in Dr. Jacob's lab.

The Role of Cholesterol in Chagas Disease

Tamar Ariella Lunzer,[1] Vanessa Almonte,[3] Calvin Law,[2] Herbert Tanowitz,[3] Louis M. Weiss[3] and Fnu Nagajyothi[3]
[1]Yeshiva University Stern College for Women New York, NY, [2]The Bronx High School of Science, NY, [3]Department of Pathology, Albert Einstein College of Medicine, Bronx, NY
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Chronic Chagas disease, caused by the parasite *Trypanosoma cruzi,* is a leading cause of cardiomyopathy. Although endemic to Latin America, immigration has lead to the globalization of this disease. Studies have shown that *T. cruzi* has a high affinity for lipoproteins and cholesterol and that their cell surface receptors play a large role in the parasitic invasion of cells. To investigate the role of serum cholesterol on the pathogenesis of Chagas disease during acute infection, we employed *T. cruzi* infected PDZ Domain Containing 1 knock-out (k/o) mice for our experimental model. There was a very large increase in mortality rate, parasite load, and parasitemia in PDZK1 k/o mice as compared to the wild type. The was also a significant increase in vasculitis, inflammation, fibrosis, and amount of adipocytes, as well as the presence of amastigote nests in the heart tissue of infected PDZK1 k/o mice relative to wild-type. PDZK1 k/o heart mRNA levels of the genes involved in lipid internalization and lipogenesis followed a drastically different pattern than those of the control mice. The higher levels of cholesterol and LDL receptors in the PDZK1 k/o mice exacerbated the symptoms of infection and pathogenesis of disease and may also be responsible for the dramatically higher mortality rate. This is additional evidence that LDL receptors and cholesterol play a major part in *T. cruzi* infection and pathogenesis.

Prospective Observational Study of Pain Experience in Children During Propofol Administration

Principal Investigator: Terry-Ann Chambers, MD Assistant Professor Anesthesiology
Co-Investigator: Lewis Singer, MD, Professor of Clinical Pediatrics, Chief, Division of Pediatric Critical Care
Director of Research: Singh Nair, MD, Instructor, Department of Anesthesiology
SURP Mentor: Naum Shaparin, MD, Department of Anesthesiology, Hospice & Palliative Medicine, Pain Medicine
Clinical Observers: Cara McDavitt, SURP Class 2014 | Zachary Shuler, SURP Class 2014

Introduction: Propofol is an intravenous sedative-hypnotic agent that is commonly used for the induction and maintenance of general anesthesia. Pain and discomfort experienced by adults and children during the initial period of propofol administration is well documented. While a number of formulations and techniques are used to minimize pain upon injection of propofol, the practices vary by practitioner and institution. At Montefiore Medical Center, practitioners primarily use lidocaine in different formulations to minimize pain upon injection of propofol. We hypothesize that a propofol and lidocaine mixture in a 1:1 ratio will reduce pain caused by the injection of propofol in children.

Purpose: The primary purpose of this study is to evaluate the pain experienced by pediatric patients upon intravenous injection of propofol using the approved FLACC pain scale. Secondary objectives include monitoring the duration of forearm movement upon injection, observing adverse local or systemic effects resulting from propofol injection, and monitoring post-operative behavioral characteristics of the sedative mixture.

Methods: This is a prospective, observational study evaluating the effectiveness of a specific way of propofol administration in reducing pain. Potential patients are identified from the OR schedule or the Pediatric Intensive Care Unit Sedation Team List and assessed for qualification based on inclusion and exclusion criteria. Research associates then explain the study to the patients and their families and obtain informed consent for observation. Two observers blinded to the concentration and mixture of propofol then observe propofol administration and assess the patient's pain experience using the approved FLACC pain scale. For patients ten and older, a post-operative survey is administered asking if any pain was experienced and what they remember before propofol administration.

Results and Discussion: Fifty children who received propofol injection were observed. Baseline demographics are reported in Table 1. Although the results are preliminary, the results of the study support the hypothesis that children receiving propofol and lidocaine in a 1:1 mixture had lower median FLACC scores compared to other formulations (0 versus 2, p value of 0.29). Future data must include a larger sample size of OR cases and/or a larger sample size of patients receiving propofol and lidocaine in a mixture other than 1:1. Our research also provides support for a future double blind, randomized control study.

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Title: AlphaAssay to detect protein-protein interactions of HIV-1 integrase with INI1/hSNF5

Authors: Alina Mitina, Menachem Spira, and Ganjam Kalpana

Departments of Genetics and Microbiology & Immunology, Albert Einstein College of Medicine, Bronx, NY

The acquired immunodeficiency virus (AIDS) originates from HIV-1. There is currently no cure for AIDS or HIV infection. Although antiretroviral treatment can suppress HIV and can delay AIDS-related illness for many years, it cannot clear the virus completely. HIV-1 is considered to be a retrovirus because it contains RNA that can transcribe itself into dsDNA and enter a host cells genome catalyzed by the enzyme of our studies interest, integrase (IN). The HIV-1 IN interacts with several cellular proteins, but especially INI1/hSNF5, a component of the SWI/SNF chromatin remodeling complex. This binding affects multiple stages of HIV-1 life cycle including integration and assembly and particle production. Studies from Dr. Kalpana laboratory have identified several IN mutants that are defective for interaction with INI1. These interaction defective IN mutants when present in the virus prevents viral replication. Through the purification of IN and INI-1 we were able to characterize the interaction of these proteins through the use of an AlphaAssay. The alpha assay is bead-based chemistry used to study biomolecular interactions in a microplate format. Our strategy is to use 6His-IN and GST-INI1 and Donor beads conjugated to Ni-NTA and acceptor beads conjugated to glutathione to carry out the Alpha Assay. The Assay showed that 6HIS-IN at 300nM and GST-INI-1 at 100nM had the optimal interaction with each other. Characterizing the most favorable concentrations for interactions between these two proteins showed us that these proteins do in fact cooperate with each other on a molecular basis and that only at specific levels do these proteins show the most favorable interaction. Through the purification of IN mutants W235A, W235E, W235F, and W235K we will be able to look at there differential interaction with INI-1 as compared to the wild-type IN.

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Characterization of Dendritogenesis in Cortical Pyramidal Layer V Neurons in Niemann-Pick Type C Disease Using Yellow Fluorescent Protein

*JOHN ALVARADO-TORRES[1,5], ROBERTO MORALES-SILVA[3,4], LAUREN C. BOUDEWYN[2,5], STEVEN U. WALKLEY[5]
[1]Post- Baccalaureate Research Education Program, Albert Einstein College of Medicine, Bronx, NY
[2]Graduate Program, Albert Einstein College of Medicine, Bronx, NY
[3]SURP Program, Albert Einstein College of Medicine, Bronx, NY, [4]RISE Program, University of Puerto Rico, Ponce, PR
[5]Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY

Niemann-Pick type C (NPC) disease is an autosomal recessive lysosomal storage disorder affecting mostly children, causing progressive neurological deterioration and death typically in the second decade. NPC disease is caused by a mutation in either the NPC1 or NPC2 gene, leading to a loss of functional NPC1 or NPC2 protein. Since these proteins play a coordinated role in lipid egress from late endosomes and lysosomes, a deficiency in either results in intracellular accumulation of unesterified cholesterol and gangliosides. Storage is especially prominent in neurons and leads to widespread cellular pathology, including growth of ectopic dendrites on pyramidal neurons. Such aberrant growth of dendrites following normal dendritogenesis has been documented in several lysosomal diseases characterized by ganglioside storage. Until recently, the usual technique employed to visualize this phenomenon was the Golgi method. New techniques, such as endogenous expression of fluorescent proteins, allow for a more in depth analysis of neuronal structure. To further investigate these dendritic abnormalities in a murine model of NPC disease relative to lysosomal storage, and to test our hypothesis that accumulation of gangliosides in NPC disease will cause an initial enhancement of dendritogenesis along with ectopic dendrite growth followed by a subsequent degeneration of dendritic trees later in disease, we have generated transgenic Npc1 mice in which layer V cortical pyramidal neurons express yellow fluorescent protein (YFP). Confocal imaging and Neurolucida Software are being used to acquire images of YFP+ pyramidal neurons which will be subsequently traced and analyzed for changes in dendritic size, complexity and pathological state.

Gap Junction Nexus Organization in Astrocytes

Alec G. Musial, Randy F. Stout Jr., and David C. Spray
Dominic P. Purpura Department of Neuroscience
Albert Einstein College of Medicine, Bronx NY 10461

Gap junction intercellular communication has become a noteworthy area of study for neuroscientists who recognize that their existence is essential for the nervous system to function. Gap junctions in astrocytes are comprised of connexin 43 (Cx43) and connexin 30 (Cx30), which are proteins that interact to form the channels. When the gap junction is created the channels interact and a plaque forms within the gap junction; it is the mobility of this plaque in astrocytes that is the focus of our research. From past research, we have discovered that Cx43 has less mobility and is more stable than Cx30, which can be determined by the C-terminus of Cx43. We also ascertained that the mobility of both connexin types is dramatically affected by the location where a fluorescent protein tag is placed. These findings led us to reexamine if the mobility and effect of fluorescent protein tag location affects the rearrangement of the two connexin types when co-expressed within astrocytes. We used transient transfections of connexins fused to fluorescent proteins with confocal microscopy to show that a mobile form of Cx30 mixes with Cx43 while an immobile form of Cx30 segregates within the same cellular plaque structure. This finding is counter to the currently accepted notions of how gap junctions are arranged in astrocytes. In order to further test if this newly recognized cellular action occurs endogenously, we utilized immunohistochemistry to discover the location of the two-connexin types in primary and immortalized mouse astrocytes. This will be important to ascertain since the C-terminus determines astrocyte morphology, which has recently been found to regulate learning and memory in mouse models. Future experiments can be conducted to test if similar cellular mechanisms occur in human tissues or human astrocytes in culture. Moreover, through immunostaining, we found that of Cx43 and IP3 Receptors co-localize in astrocytes. Future experiments can also be conducted to further explore this relationship. All of this information helps us to better understand how intercellular signaling in glia affects brain function. I would like to acknowledge Einstein College of Medicine for accepting me into the SURP program, everyone in the Spray lab especially Dr. Randy F. Stout Jr. for being a great mentor to me during this experience as well as Marcia Maldonado for showing me many of the cell techniques I utilized in the lab, and Fordham University for providing me housing and a job. Finally, plenty of credit has to go towards the Analytical Imaging Facility (AIF) here at Einstein and the NCI cancer center support grant (P30CA013330) that funds much of the work for our shared facilities.

Title: Activation of μ and δ opioid receptors in the nucleus accumbens facilitates conditioned approach to high calorie reward

Authors: Desiree Nieves, Kevin Caref, and Dr. Saleem M. Nicola

Department: Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY, 10461

In drug addicts, exposure to drug-associated stimuli leads to drug seeking. The nucleus accumbens (NAc), a ventral forebrain region, has been strongly implicated in these behaviors, though the neural mechanisms underlying them remain unclear. Previous data from our lab have demonstrated that cued reward-seeking depends on activation of μ opioid receptors in the NAc. The NAc is also well endowed with δ opioid receptors, but their contribution to reward-seeking is unclear. It is furthermore unclear whether μ and δ opioid receptors act synergistically or in opposition. We therefore studied how the non-selective opioid receptor-antagonist naltrexone, the selective δ opioid receptor-antagonist naltrindole, and the selective μ opioid receptor agonist (DAMGO) affect reward-seeking behavior when microinjected into the NAc. Specifically, we hypothesized that endogenous ligands of both the μ and δ opioid receptor in the NAc promote cued reward seeking behavior.

To test this hypothesis, we performed a microinjection experiment with the predictions that blockade of all NAc opioid receptors would reduce responding to reward-predictive cues, as would blockade of only δ opioid receptors. In contrast, stimulation of μ opioid receptors would increase responding. We trained 8 ad-libitum fed male rats on a conditioned stimulus (CS) task in which they were required to respond to an auditory cue (CS +) in order to obtain a heavy cream reward. The rats were then surgically implanted with bilateral guide cannulae aimed at the NAc core. On test days, each rat was microinjected bilaterally with either naltrexone- (6 or 30 μg/side), naltrindole (8 μg/side), DAMGO (0.25 μg/side) or vehicle prior to task performance. Preliminary results reveal a decreasing trend in CS+ evoked reward-seeking after naltrexone and naltrindole injection, whereas DAMGO injection caused an increase. These results suggest that both μ and δ receptor activation in the NAc facilitate cued reward-seeking. Future experiments include obtaining a dose-response curve for naltrindole and testing the effect of a δ opioid agonist on cue responding.

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Defining the Mechanism Through Which Obatoclax Kills Thyroid Cancer Cells

Bracha Robinson, Devora Champa, and Antonio Di Cristofano
Department of Developmental and Molecular Biology, Albert Einstein College of Medicine

Background: Poorly Differentiated Thyroid Carcinomas are aggressive tumors that are often untreatable and fatal. Obatoclax, an anti-cancer drug, suppresses these tumors, but its mechanism of action remains unclear. Since Obatoclax was designed as an inhibitor of anti-apoptotic Bcl2 family members, apoptosis is one possible route to cell death. This may be determined through Mcl1 inhibition, since Mcl1 is an anti-apoptotic protein consistently overexpressed in thyroid carcinomas. Alternatively, Obatoclax may cause cell death through modulation of autophagy. ATG5 is a protein that is necessary for autophagy to occur, and suppression of ATG5 may prevent Obatoclax from effecting tumor suppression. Additionally, Obatoclax may cause cell death by inducing lysosome degradation. By altering lysosomal acidity with Bafilomycin, we can determine whether Obatoclax acts through degradation of the lysosomes.

Methods: To silence target proteins (Mcl1 or ATG5), plasmid DNA is amplified and purified through maxi preparation. The DNA is packaged into lentiviruses, collected, and the viruses are utilized to infect D445 cancer cells. The DNA encodes for RNA that binds to the target gene of D445 cells, preventing the proteins (Mcl1 or ATG5) from being expressed. Western blots were performed to confirm that the gene was silenced. The modified cells that lack Mcl1 or ATG5 were treated with 500 nM Obatoclax, and the number of cells was quantified at 0 and 24 hours to determine the amount of cell death with and without Obatoclax. To determine the effects of Obatoclax on lysosome degradation, D445 cells were treated with 500 nM of Obatoclax, as well as pre-treatment with 100 nM Bafilomycin at one and four hours prior to treatment. Cells were counted at 0 and 18 hours to determine the effects of the lysosomes on cell death.

Results: Mcl1 was knocked down with a sh-Mcl1 plasmid. These modified cells were treated with Obatoclax (treated) or DMSO (untreated); the parent cell line and empty plasmid cell line were also tested as controls. The data show that there is no difference between sh-Mcl1 cells and the controls, meaning that Obatoclax is able to cause cell death even without Mcl1. Therefore, Mcl1 is not integral to Obatoclax's pathway. To test whether Obatoclax acts through lysosome degradation, D445 cancer cells were treated with both Bafilomycin and Obatoclax. The data show that there is a partial rescue from Obatoclax induced cell death when cells are treated with Bafilomycin, which indicates that lysosomal de-acidification hampers Obatoclax from killing the cancer cells. Additionally, the results of the experiment with ATG5 will be available at the poster session.

Conclusion: Obatoclax does not utilize the anti-apoptotic factor, Mcl1, to kill thyroid tumor cells. However, our data indicates that the lysosomes are involved in Obatoclax's pathway. This suggests that the lysosomes may be therapeutic targets for aggressive thyroid tumors and should be studied further.

Investigating Allosteric Inhibition in Sulfotransferases

Natalia Rodriguez, Ian Cook, Ting Wang, Thomas Leyh
Dept. Biological Basis of Behavior, University of Pennsylvania, 3720 Walnut Street, Philadelphia, PA, 19104
Dept. Microbiology & Immunology, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461

Human cytosolic sulfotransferases (SULTs) catalyze transfer of the sulfuryl group ($-SO_3$) from the universal donor, 3'-phosphoadenosine 5'- phosphosulfate (PAPS), to the hydroxyls and primary amines of acceptors, which include metabolites, drugs, and other xenobiotics. We know that non-steroidal anti-inflammatory drugs (NSAIDS), like acetylsalicylic acid (aspirin), allosterically inhibit SULT1A1, a sulfotransferase that is extensively involved in regulating metabolism and detoxification. Potential NSAID binding sites were identified using Molecular Dynamics (MD), and NMR distance measurements were used to corroborate MD predictions. Here, a candidate binding site is disrupted *via* mutagenesis and its ability to bind NSAIDs is tested. Four positions in the binding site were selected for mutation and two were tested. The mutants behaved similarly to wild type toward ligand binding, turnover and inhibition; hence, these residues do not appear to be located at the binding site. More refined NMR distance studies are expected to identify the location of the binding site location.

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Impact of Klp10A neck phosphorylation on microtubule depolymerization

Sophia I. Salazar, Matthieu P. Benoit, Ana B. Asenjo, Hernando Sosa

Albert Einstein College of Medicine of Yeshiva University, Department of Physiology and Biophysics

Kinesin is a superfamily of ATPase motor proteins that are generally known to transport vesicular cargo by walking along microtubules (MT). Unlike other kinesins, kinesin-13s diffuse along and depolymerize MTs. Kinesin-13 domain organization characterized with preceding neck region and an internal motor domain, whereas most other kinesins contain an N-terminal motor domain followed by the neck region. Although the motor domain alone is sufficient for depolymerization activity, previous studies indicate that the positively charged neck region increases the efficiency of MT depolymerization and impacts binding pattern of kinesin-13s on curved tubulin. Additionally, a study reports that a single phosphorylation site of the neck-linker region on full length MCAK kinesin-13 reduces depolymerization activity, both *in vivo* and *in vitro*. Despite these preliminary studies, the function of the neck region of kinesin-13s is still poorly understood.

The objectives of this study are to determine whether a single phosphorylation on the neck region of drosophila kinesin-13 KlP10A impacts a change in depolymerization activity and how phosphorylation affects Klp10A binding to curved tubulin. A conserved serine residue in all kinesin-13s, including MCAK and the *Drosophila melanogaster* kinesin-13 (KlP10A), is used as site directed mutagenesis of the neck region to change a serine (amino acid 210) to a negatively charged glutamic acid to mimic phosphorylation. We also use AMPPNP (a nonhydrolyzable ATP analogue) with kinesin-13 rather than ATP, with the intent to capture the intermediate step of curved tubulin bound kinesin-13. We found a possible slight decrease of activity between the WT and the phosopho-mimic S210E. Regarding tubulin decoration, by now the alignment of the curved tubulin particles appears identical for the WT, S210A, and S210E construct. However, it will be necessary to check that the rings are comprised of single filaments of tubulin and not of spirals, and that variability of kinesin binding (e.g. mixture of skipping and full tubulin decoration) does not bias this alignment.

Preliminary evidence for a neural correlate of pattern change detection

Nadja Santana[a], Caitlyn Linehan[b], Renee Symonds[c], and Elyse Sussman[c]
[a] Department of Chemistry, University of Puerto Rico, Mayagüez, PR [b] Preston High School, Bronx, NY [c] Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY

Abstract:

The ability to notice physical changes in our surroundings is dependent upon first adapting to what is ongoing in the environment. However, there is a question about whether change detection is solely due to adapting to a "norm" or also to higher-level processes of noticing this physical change through a violation of a sensory memory. To test this question, we recorded human electroencephalography (EEG) to understand how information is processed in the brain. EEG is a non-invasive technique used to measure brain scalp potentials in order to understand underlying cognitive processes. Specifically, we relied upon event-related potentials (ERPs), which are time-locked to the presentation of a stimulus. The primary objective of this study was to determine whether changes in the ERPs would reflect detected pattern violations that were independent of physical changes in the stimuli. The N2 component, a negative-going peak that occurs 200 ms after the onset of a stimulus, reflects detection of stimulus change. When a subject is constantly presented a standard stimulus, a population of neurons may become desensitized to this repeated presentation. When a deviant is introduced, a separate population of less adapted neurons is recruited which evokes a larger amplitude response. Thus, elicitation of the N2 component without a stimulus change would be indicative of higher level violation of expectation. Twelve subjects participated in the visual change detection task experiment, in which a standard temporal pattern of visual stimuli was three dark circles followed by a change to a light circle (OOOO). Participants were instructed to press a response key if the standard pattern was violated. Pattern violations were: when the stimulus change occurred too early, first deviant (D1), or too late, second deviant (D2). The N2 component was elicited, time-locked to the physical stimulus change when it occurred too early (D1). However it was also elicited by the expected position when it was too late (D2) when there was no physical stimulus change. These results provide preliminary evidence that change detection can be influenced by both sensory memory and neural adaptation. This research was supported by the Einstein SURP, Einstein-Montefiore High School Program, DSSROP, and Dr. Elyse Sussman. Special thanks to Renee Symonds and all other members of Dr. Sussman's laboratory.

Activation of Human NK Cells By *In Vivo* Treatment of Humanized Mice With An IL-15 Superagonist Inhibits Acute HIV-1 Infection

Aaron Schwartz, Kieran Seay[1], Jian Hua Zheng[2], Kathryn Deneroff[2], Christina Ochsenbauer[3], John C. Kappes[3,4], Bai Liu[5], Emily K. Jeng[5], Hing C. Wong[5] and Harris Goldstein[1,2]

Departments of [1]Microbiology & Immunology and [2]Pediatrics, Albert Einstein College of Medicine, Bronx, NY, 10461, U.S.A.
[3]Department of Medicine, University of Alabama at Birmingham, [4]Birmingham Veterans Affairs Medical Center, Research Service, Birmingham, AL 35294
[5]Altor BioScience Corporation, Miramar, FL 33025

Highly active antiretroviral therapy (HAART) potently reduces viral replication in HIV-1 infected individuals. However, while HAART can completely inhibit HIV-1 replication within HIV-1 infected individuals to undetectable levels, it is incapable of killing previously infected cells. Consequently even prolonged HAART cannot cure HIV-1 infection, and infection will rebound from reservoirs of latently infected cells if HAART is stopped. Cytotoxic immune cells kill virally infected cells enabling eradication of viral infection and thus should be crucial in eliminating HIV-1. IL-15 activates cytotoxic T cells and NK cells, and consequently can effectively treat some cancers in mice. We hypothesize that IL-15 should induce HIV-1 specific cytotoxic responses to eliminate reservoirs of infected cells within HIV-1 infected individuals. ALT-803, a molecularly engineered IL-15 superagonist which activates $CD8^+$ T cells and NK cells, displays more potent activation activity, soluble half life, and binding affinity than natural IL-15. ALT-803 treatment markedly inhibited HIV-1 infection in humanized NOD-SCID-IL2rγ-/- (NSG) mice intrasplenically injected with human PBMCs and treated with ALT-803 when administered 24 hours after infection with HIV-1. We demonstrated that NK cells, as opposed to $CD8^+$ T cells, are the primary mediators of HIV-1 inhibition induced by ALT-803. Through flow cytometry analysis for CD69 expression by transplanted human PBMCs we found that *in vivo* treatment with ALT-803 activates NK cells. We also evaluated the suppressive effect of ALT-803 using a complete mouse model for HIV-1 infection, transgenic mice that express human CD4, CCR5, and Cyclin T1. We demonstrate that ALT-803 inhibits systemic *in vivo* infection not only in the spleen but also in the gut of these mice. Taken together, our data supports our hypothesis that ALT-803-mediated activation of cytotoxic NK cells could prove to be an effective systemic treatment to reduce the number of infected cells in HIV-1 positive individuals in combination with the suppressive effects of HAART and contribute to the eradication of HIV-1 infection.

Neural Oscillations Predict Performance in a Duration-Expectation Task

Philip N. Shamash, Michel J. Gray, Jeremy W. Murphy, John J. Foxe
Cognitive Neurophysiology Laboratory, Department of Neuroscience, Albert Einstein Colleg e of Medicine

While paying attention to a rhythmic stimulus, the brain's attention network entrains ongoing neural oscillations to occur at the stimulus' frequency and its harmonics. It is unclear how an d to what extent these oscillations enhance perception of the attended stimulus. In our study, participants actively attended to one of two competing rhythmic stimuli and responded whene ver the duration of the attended stimulus temporarily increased. We measured neural activity during the 8 seconds prior to responses ('hits') and failures to respond ('misses') using high-de nsity electroencephalography (EEG). First, power in the alpha band (9-13Hz) differs between hits and misses, confirming that this task can be used to differentiate between brain activity c orresponding to effective versus ineffective deployment of attention. Next, we find that entrai ned oscillations preceding hits cluster more tightly around the average phase, indicating that o scillations need to hit neural populations at the right moment to be effective. Further supporti ng this claim, effective oscillations' peaks have greater amplitudes when they occur at this 'ri ght' moment but lower amplitudes when they occur out of phase with it. While these findings hold true for the attended stimulus' frequency and at least two of its harmonics, these three os cillations appear to be temporally and functionally independent, suggesting that each one sub serves a different physiological process. Altogether, our findings constitute a brick in the pro verbial brick road to understanding how neural oscillations mediate performance in a spatio-t emporal attention task. Still, further work is needed to untangle each oscillation's relationship with temporal expectation, selective attention, and visual perception. Such results could direct ly underpin translational research on the neurological basis of disorders such as schizophrenia and autism.

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The role of Fat1 in smooth muscle cell EMT-like processes

Alyssa M. Shearer, Charlene M. Dunaway, Prameladevi Chinnasamy, Dario F. Riascos Bernal, Lily Cao, Nicholas E.S. Sibinga

Department of Medicine/Cardiology, Department of Developmental and Molecular Biology, Wilf Family Cardiovascular Research Center, Albert Einstein College of Medicine of Yeshiva University, Bronx, NY

Atherosclerosis, the hardening of arteries due to the build-up of plaque, is one of the major health concerns in America. Vascular smooth muscles cells (VSMCs) contribute to atherosclerotic lesion expansion by undergoing dedifferentiation and migrating into the intima from the media. The atypical cadherin Fat1 is upregulated in atherosclerotic lesions, and VSMC-specific Fat1 knockout ($Fat1^{SMKO}$) mice exhibit larger atherosclerotic plaques compared to the control. After arterial injury, $Fat1^{SMKO}$ mice display VSMC hyperproliferation and impaired VSMC redifferentiation. A screen performed for Fat1 interacting proteins identified a novel interaction of Fat1 with vimetin, an intermediate filament protein. Like Fat1, vimentin is upregulated in arterial cells after injury and also is present in atherosclerotic lesions. Fat1 and vimentin interaction has been validated by co-immunoprecipitation, and Western Blot analysis showed that vimentin protein levels were higher in $Fat1^{SMKO}$ VSMCs. Interestingly, other studies have shown that Scribble, a protein involved in cell migration and polarity, interacts with both Fat1 and vimentin individually, yet whether all three proteins interact as a complex is not known. To test for this possible interaction, we cloned a Scribble sequence into a mammalian expression vector that will be used in co-immunoprecipitation assays along with Fat1 and vimentin. Since vimentin is a well-known marker of mesenchymal state, we want to further investigate whether Fat1 is playing a role in EMT-like process in VSMCs. For this, we performed a qPCR array looking at genes involved in EMT, comparing $Fat1^{SMKO}$ VSMCs with wild-type ones. The goal of this project is to further elucidate the mechanism of Fat1-mediated regulation of VSMCs during atherosclerosis.

Sara Shi, Pablo Rougerie, Dianne Cox – Department of Anatomy & Structural Biology

Abstract – SURP 2014

The Role of Myosins in the Formation of Phagocytic Cups During FcγR Mediated Phagocytosis

Phagocytosis, the process in which a cell engulfs apoptotic cells or foreign particles, is important in the human body for protection against dangerous invaders and maintenance of healthy tissues. It can be observed at the site of infections for pathogen removal and the uptake of dead cells. Phagocytosis is initiated by the binding of Fcγ receptors (FcγR) on the cell surface to the Fc domains of IgG molecules attached to the target, leading to the polymerization of F-actin and the formation of the phagocytic cup. Myosins are a family of actin-binding motor proteins whose importance in phagocytosis has been suggested but there has been no formal demonstration of their roles. To answer that question we aimed to set up a shRNA screen where expression of targeted myosins would be selectively silenced. Due to the better rate of transfection of shRNA in fibroblasts (MEF) compared to macrophages (RAW-LR5), our first step was to engineer a phagocytic MEF cell. Artificial FcγR constructs, 16:7:syk and FcRIIa, were expressed in MEF cell lines to induce phagocytosis in these non-phagocytic cells. Although the cells expressing FcγR bound opsonized particles, they were not internalized. Therefore, we attempted to use the less transfectable but more phagocytic macrophage cell line RAW-LR5. However, this proved difficult as the GFP marker of transfection was lost during the experiment. We solved this problem by bypassing the permeabilization step, the step where the GFP signal was lost, using already fluorescent beads. A pilot experiment using this protocol indicated that myosin II was required for phagocytosis, confirming previously published data using inhibitors. On the other hand myosin IXb seems dispensable. The immediate next steps will be to extend the screen to other myosins (X, V, Ic) and to confirm the shRNA-induced silencing of myosin expression.

Microinjection of dopamine antagonists into the NAc core reduce goal-directed behavior

by Maria Shibatsuji, Sara Morrison, and Saleem Nicola
Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461

Studies have suggested that animals conduct cost-benefit analyses in order to determine if an increased amount of work or risk is worth undertaking for a higher reward. Dopamine released in the core of nucleus accumbens (NAc) is thought to play a role in effort-based decision making by regulating the amount of effort invested for a reward. While it is established that NAc dopamine is needed to perform high effort tasks, the underlying neural mechanisms of effort-based decision-making are up for debate. One hypothesis is that the activation of dopamine receptors directly influences effort-related decision-making (Salamone et al., 2007): the activation of dopamine receptors allows animals to overcome response constraints that may prevent them from engaging in high effort tasks. However, other studies suggest that the effort exerted in order to obtain reward is not affected by NAc dopamine manipulation (e.g. Walton et al., 2009), implying that NAc dopamine might not play a specific role in supporting effort. Instead, we hypothesized that NAc might play a more general role in goal-directed behavior.

In order to test these hypotheses, rats (n = 8) were trained on a T-maze baited with a large reward (3 sucrose pellets) in one arm and a small reward (1 pellet) in the other. Half of the rats were presented with one barrier in the HR arm and no barrier in the LR arm ("one-barrier task"), while the other half of the rats were presented with two barriers in the HR arm and one barrier in the LR arm ("two-barrier task"). Both groups of rats preferred the high effort/large reward arm. Prior to being placed in the T-maze, rats were microinjected in the NAc core with either a dopamine D1 receptor antagonist (SCH 23390) or a D2 receptor antagonist (raclopride). Based on prior studies, we predicted that dopamine antagonists would cause rats to prefer the low effort/small reward arm in the one-barrier task. If NAc dopamine directly influences effort-based decision-making, then we would predict the same effect on the two-barrier task. However, if NAc dopamine plays a more general role in goal-directed behavior, we would predict that rats would show no particular bias towards the LR arm.

Our preliminary results suggest that microinjection of dopamine antagonists into the NAc core increased rats' choices of the low effort / low reward arm on both tasks.

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A search of novel post-translational modifications of activation-induced deaminase with functional impact on somatic hypermutation

Zoe Spieler

Mentors: Matthew D. Scharff, Xiaohua Wang

Albert Einstein College of Medicine

Somatic hypermutation (SHM) and class switch recombination (CSR) are mechanisms employed by B cells to generate antibody diversity beyond the germline-coded repertoire. Both processes are mediated by the enzyme activation-induced cytidine deaminase (AID), which deaminates deoxycytidine on genomic DNA to generate a G:U mismatch. While AID mainly targets immunoglobulin genes in B cells, off-targeting events of AID to non-Ig genes often leads to DNA damage that contributes to the development of B cell malignancies. The mechanisms responsible for preferential targeting of AID activity to Ig loci remain elusive. Post-translational modifications (PTM), such as phosphorylation on Serine 38, have been indicated to play a significant role in AID's ability to initiate CSR. With this precedent, our collaborator identified several novel PTMs of AID. Here, we planned to take advantage of the recently establish SHM detection platform based on the human Ramos B cell line to assess whether these newly identified PTMs, particularly phosphorylation, are important for SHM. We cloned eleven different mutations of murine AID containing a single amino acid substitution at different PTM sites into a lentiviral expression system. We confirmed the delivery of those expression vectors into Ramos cells through molecular beacon GFP, and further confirmed the successful expression of exogenous murine AID proteins through western blotting analysis. At the same time, we examined whether mouse AID would cause mutations in the human V region in the Ramos cells. Since human AID works in mouse cells, we were surprised to find that in preliminary SHM analysis, murine AID might not be able to operate in a human cell line. This suggests that some part of the murine AID molecule may dictate functional specificity between species.

Pregnane X Receptor Acetylation: Molecular and Functional Consequences

Valerie Sydnor, Danielle Pasquel, Sridhar Mani and the Mani Lab
Albert Einstein College of Medicine, Department of Genetics, Cancer Center – Bronx, NY

The Pregnane X Receptor (PXR), known for its ability to detect and enhance the metabolism of xenobiotics, belongs to the Nuclear Receptor (NR) Superfamily of ligand activated transcription factors. PXR has a uniquely large ligand binding pocket that enables it to bind a substantially wide-ranging set of low affinity ligands, including endogenous metabolites (e.g. steroids and bile salts) and exogenous chemicals and toxins (e.g. pesticides, pollutants and prescription drugs). Upon ligand binding, PXR is able to induce transcription of genes that produce metabolizing and detoxification enzymes (e.g. CYP3A4) and efflux pumps (e.g. SULTS).

In addition to its central role in detoxification, PXR is also involved in the regulation of hormone, lipid and steroid metabolism and in maintaining bone, endocrine and energy homeostasis. PXR's impact on such diverse biological processes is potentially explained by post translational modification (PTM) of the receptor, as PTMs are known to expand the functional capacities of many regulatory proteins and other NRs. Dysregulation of NR PTMs is linked to abnormal cell signaling and growth, tumorigenesis and metabolic dysfunction.

It has previously been determined that PXR is phosphorylated, ubiquitinated and SUMOylated. Our lab has recently demonstrated that PXR is also acetylated, and that activation of the receptor via ligand binding results in deacetylation. The exact site of acetylation was discovered using tandem mass spectrometry analysis of analogous peptides from non-acetylated and *in vitro* acetylated PXR; a sole acetylation site was identified at lysine 109 (K109). The goal of this project was to further investigate the molecular, cellular and functional consequences of PXR acetylation at K109.

Site-directed mutagenesis was utilized to create two mutant receptors – an acetylation-deficient lysine mimic (K109R) was generated by replacing lysine 109 with arginine (R), and an acetyl-lysine mimic (K109Q) was produced by replacing lysine 109 with glutamine (Q). Expression vectors containing Wild Type (WT) PXR, K109R and K109Q were transfected into cells, and the effects of these modified receptors were examined through a luciferase luminescence reporter assay and a cell culture assay for phenotypic observation.

The luciferase luminescence reporter assay revealed that K109Q mutants have a significantly diminished capacity to induce gene transcription following activation by Rifampicin and SR12813, two potent PXR agonists. This finding constructs a model of PXR functioning in which ligand binding produces upregulation of gene expression by inducing deacetylation of PXR, thus allowing the receptor to bind cognate DNA promoter response elements.

This model proposes that over- acetylation of PXR diminishes its ability to promote expression of genes required for metabolism and clearance of toxins, making cells more susceptible to noxious chemicals and vulnerable to cell death. In accordance with this hypothesis, a cell plate assay showed that following treatment with Indomethacin, K109Q mutants exhibit a morphological phenotype that is ostensibly more unhealthy than the resultant WT PXR phenotype. The aberrant morphological features displayed by K109Q cells (rounding up, retraction of pseudopods, membrane blebbing and cell shrinkage) are characteristic of apoptotic cells.

Further research will be undertaken to examine K109Q cell viability, and to classify the type of cell death (apoptotic, autophagic or necrotic) displayed in the cell plate assay. A Flow Cytometry Apoptosis Assay (measuring Caspase 3 activation, PS flipping and membrane permeability) will analyze the proportion of K109Q, K109R and WT PXR cells in various stages of apoptosis. Additional molecular assays will probe for defining features of apoptosis (e.g. a TUNEL assay for DNA fragmentation), autophagy (e.g. a Western Blot for LC3-I and LC3-II) and necrosis (e.g. Ethidium homodimer III treatment for membrane integrity). This research will provide a better understanding of the mechanisms by which acetylation and deacetylation of PXR modulates its functioning.

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Effect of *tbx1*/Wnt Signaling on Zebrafish Heart Looping

Nathaniel Tracer, G. Sheela Devakanmalai, Bernice Morrow, Ertugrul Ozbudak
Department of Genetics, Albert Einstein College of Medicine, Bronx, NY

The *tbx1* gene is an important transcription factor and was recently identified to be responsible for cardiovascular abnormalities leading to DiGeorge syndrome in humans. It has also been shown that zebrafish tbx1 mutants show conotruncal defects consistent with *tbx1's* role in Secondary Heart Field development. Thus, zebrafish can be used as a representative model organism for discovery based approaches that may uncover new and potentially clinically relevant genes important for heart development.

Previous studies have suggested a model in which β-catenin-dependent canonical Wnt signaling inhibits cardiac specification. While inhibiting the expression of tbx1 has been found to decrease heart looping in zebrafish, we sought to investigate whether simultaneously blocking β-catenin-dependent canonical Wnt signaling could compensate for the absence of *tbx1* gene expression.

In order to address these issues, we employed a zebrafish line that was transgenic for an inducible inhibitor of Wnt/b-catenin signaling, which inhibited endogenous Wnt/b-catenin signaling at discrete times in development. We assessed whether inhibition of Wnt signaling, which is itself a negative inhibitor, could reestablish the normal looping phenotype in zebrafish. We compared the heart looping angles of batches of embryos that had the inducible inhibitor, TBXMO injections, or both, against controls.

We discovered that TBXMO injections did significantly decrease heart looping when compared with control groups. However, blocking β-catenin-dependent canonical Wnt signaling did not significantly rescue the heart looping lost due to the absence of the *tbx1* gene.

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Investigating the ability of human TRIM5α to bind to the HIV-1 core

Justin Vercellino, Danny Vieira and Felipe Diaz-Griffero

Department of Microbiology and Immunology at Albert Einstein College of Medicine

1301 Morris Park Avenue, Bronx, NY 10461, USA

Abstract

While some progress has been made in recent years, the development of an effective HIV vaccine remains elusive. One alternative towards a vaccine is to understand the intermolecular binding of the restriction factor: human TRIM5α (TRIM5α_{hu}). Restriction factors are key proteins that are able to mount an immune response to infection. TRIM5α_{hu} is unique because it combats retroviruses, specifically. The murine leukemia virus (MLV) is a retrovirus that TRIM5α_{hu} is able to bind to and restrict infection. What we are trying to achieve is in vitro capsid formation of MLV to perform a capsid-binding assay with TRIM5α_{hu}. We have successfully created MLV CA-NC in vitro. To achieve this, we needed to induce cultures of *E. coli* to produce the protein. After we lysed the cells and purified the protein, we tried capsid assembly by leaving the protein in a small amount of buffer at 37°C for one hour, then in 4°C overnight.

TRIM5α_{hu} is a protein of interest because the rhesus monkey TRIM5α (TRIM5α_{rh}) does bind to HIV-1 to inhibit infection despite being a very similar protein. If we can understand the endogenous restriction of TRIM5α_{hu}, we may be able to understand why TRIM5α_{hu} is unable to bind and restrict HIV-1. Understanding the mechanism of restriction can serve as a foundation for future studies of the TRIM5α_{hu} protein, and its interaction with HIV-1.

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Mentor: Dr. Vern Schramm
Department: Biochemistry
Ryan von Kleeck

The Effect of Bond Vibration Dynamics in Enzyme Catalysis of Purine Nucleoside Phosphorylase

Enzymes, like Purine Nucleoside Phosphorylase (PNP), generally catalyze reactions through converting the substrate into a transition state, lowering the activation energy of the reaction, before reacting to form products. It has been well known that large conformational changes (slow changes) in enzymes play an important role in catalysis; however, the effects of femtosecond bond vibrations between atoms still remain unclear. Using ^{13}C, ^{15}N, and ^{2}H labeled PNP constructs to increase the mass of the enzyme, we were able to compare the probability of barrier crossing, or the rate of an enzyme-bound substrate to become an enzyme-bound product for both labeled (heavy) and unlabeled (light) PNP enzymes. Despite an increase in mass, steady state kinetics for both heavy and light PNPs were similar, indicating that substrate binding and product release were not altered by mass. Stopped flow analysis, looking at single turnover events, showed that heavier enzymes were significantly slower at converting substrate to product (chemical step) than their lighter counterparts. Because the chemical step is slower in heavier isotopes, but overall steady state kinetics are the same as 'light' PNP, this suggests that bond vibration dynamics play a role in enzyme catalysis.

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Carbapenem Resistant Gram Negative Bacterial Infections in Solid Organ Transplant Recipients: A Case Control Study

Liat Weinstock[1], Iona Munjal[2], Joel Lindower[3], Esther Benamu[4], Grace Minamoto[4], Victoria Muggia[4], Rebecca Pellett Madan[2]

[1]Stern College for Women of Yeshiva University; [2]Department of Pediatrics, Albert Einstein College of Medicine and The Children's Hospital at Montefiore; [3]Abdominal Transplant Program, Montefiore Medical Center; [4]Department of Medicine, Albert Einstein College of Medicine and Montefiore Medical Center

Background: Carbapenems are a broad class of β-lactam antimicrobials whose structure typically renders them highly resistant to β-lactamases. However, carbapenem-resistant gram-negative bacteria (CR-GN) with direct carbapenem-hydrolyzing capabilities are now highly prevalent, especially among *Enterobacteriacea, Pseudomonas,* and *Acinetobacter*. The heterogeneity of mutations that confer carbapenem resistance complicates the ability to detect and treat these resistant organisms. *Klebsiella pneumoniae* carbapenemases (KPC; Class A) are the most commonly identified carbapenemase in the United States, but metallo-β-lactamases (VIM, IMP, NDM; Class B) and OXA (oxacillinase) β-lactamase (OXA-48,-162,-181; Class D) are also identified with increasing frequency. Resistance can also develop via outer membrane porin mutations; specifically in *Klebsiella* (OmpK-35, 36), *E. coli* (OmpF/C), and *Enterobacter* (OmpF/C). Infection with CR-GN is especially complicated for immunocompromised and chronically ill solid organ transplant (SOT) recipients, and undergoing solid organ transplantation has been identified as an independent and significant risk factor for infection with CR-GN. However, the prevalence of CR-GN among SOT patients is not well defined, and the impact of these organisms on transplant outcomes is unknown.

Objective: We performed a case control study to compare post-transplant outcomes for SOT recipients who were known to be infected with carbapenem resistant gram negative bacteria (CR-GN) vs. outcomes for matched control transplant recipients who were infected with carbapenem susceptible gram negative bacteria (CS-GN).

Methods: All microbiology labs were reviewed for patients who underwent SOT at Montefiore Medical Center between January 1, 2007 and July 1, 2013. Patients with a culture positive for CR-GN (*Enterobacteriaceae, Pseudomonas aeruginosa,* and *Acinetobacter baumannii)* were identified as cases. Patients with a culture positive for CS-GN were identified as controls. Cases were matched to controls (1:2) based on organ transplanted, pre vs. post transplant infection, age, sex, race, and infecting bacterial species.

Results: 572 transplantations were reviewed (78 pediatric, 494 adult). 8/78 (10.3%) pediatric patients were found to be infected with CR-GN compared to 42/494 (8.5%) of adults (Odds ratio=0.81 [95% CI 0.37, 1.8] [p=0.67]). Infection pre transplant with CR-GN vs. CS-GN was not associated with a significant difference in post transplant length of stay, readmission, one-year survival, or one-year graft failure ($p>0.05$ for all). However post transplant infection with CR-GN compared to CS-GN was associated with a significant increase in average post transplant length of stay (95% CI -45.75, -11.44 [p=0.002]) and risk of readmission (95% CI -46.17, -8.661 [p=0.005]). We did not identify an association between post transplant CR-GN and one-year survival or graft failure.

Conclusions: We identified a significant risk of longer post transplant length of stay and readmission in patients who were infected with CR-GN post transplantation. Further studies are needed to determine if this association remains significant in a multivariable analysis and if this association is related to bacteria-specific virulence factors or patient co-morbidities.

HPV Infection and CDKN2A CpG Methylation are Associated with the Overexpression of CDKN2A Splice Variants and ANRIL in Head and Neck Squamous Cell Carcinoma

Joshua A. Wetzler[1], Miriam M. Ben-Dayan[1], Richard Smith[2], Michael B. Prystowsky[1], Nicolas F. Schlecht[3] & Thomas J. Belbin[1]

Departments of [1]Pathology, [3]Epidemiology & Population Health: Albert Einstein College of Medicine, [2]Department of Otolaryngology: Montefiore Medical Center

Background: Head and Neck Squamous Cell Carcinoma (HNSCC) is associated with Human Papillomavirus (HPV-16) infection. HPV+ tumors are most prevalent in the oropharynx and patients with these tumors exhibit improved survival when treated. This would suggest that HPV+ and HPV- HNSCC are molecularly different diseases, with different genome-wide methylation, gene expression patterns, and patient outcomes. Previous research has distinguished HPV+ and HPV- tumors using a CpG methylation profile. HPV may induce tumorigenesis through the production of E6 and E7 oncoproteins, which promote the degradation of host p53 and Rb tumor suppressors, respectively, which are regulated by the CDKN2A splice variants, ARF and INK4a. Thus, any disturbance of the CDKN2A locus leads to the transcriptional down-regulation of the splice variants, ARF and INK4a, as well as lncRNA ANRIL. We hypothesized that since ARF and ANRIL share a promoter, their expression may be correlated if transcription initiates at the ARF-ANRIL promoter and that in HPV+ tumors, they will be overexpressed.

Methods: In the present study, we examined ARF, INK4a, and ANRIL expression levels in tumor and normal oropharyngeal tissue using real-time reverse transcriptase polymerase chain reaction (qRT-PCR). HPV- and HPV+ paired tumor-normal samples, and individual tumor samples were analyzed. As a validation of our patient samples, we utilized genome-wide expression and DNA methylation data from the Cancer Genome Atlas (TCGA) database based on RNA-Seq data for HPV- and HPV+ oropharyngeal samples.

Results: We observed a significant increase in expression of CDKN2A and ANRIL in HPV+ tumors ($p<0.0001$ and $p<0.0005$, respectively), but only ARF expression significantly increased in HPV+ tumors when compared to HPV+ normal tissue ($p<0.05$). These changes were confirmed in tumor sample data derived from the TCGA. Furthermore, ANRIL expression mirrored ARF expression patterns.

Conclusions: This study confirmed the hypotheses that CDKN2A CpG hyper-methylation correlates with over-expression of ARF, INK4a, and ANRIL in HPV+ oropharyngeal tumors. Additionally, ARF, INK4a, and ANRIL are overexpressed in HPV-positive tumors, suggesting that the transcription occurred at the ARF-ANRIL promoter. We validated our findings using TCGA data, which also suggest that ARF expression is associated with HPV+ oropharyngeal SCC. While this study focused on the CDKN2A/B locus, it supports the argument that HPV+ and HPV- tumors are essentially different diseases. Future studies will determine the mechanism by which HPV induces CDKN2A and ANRIL expression.