2015 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

2015

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

2015 Summer Undergraduate Research Program

Student Name	Undergraduate School	Mentor Name
Kayla Babbush	Cornell University	**Rubina Heptulla**
Alexandru Barbulescu	CUNY Hunter College	**David Shechter**
Maryia Barnett	University of California Berkeley	**Paraic Kenny**
Abigail Bergman	Stern College for Women	**Florence Marlow**
Matthew Challman	Fordham University	**David Spray**
Anne Chen	Fordham University	**Thomas Leyh**
Kevin Citron Arroyo	University of Puerto Rico	**Bernice Morrow**
Gilad Doron	Case Western Reserve University	**Jayanta Roy-Chowdhury**
Chimere Ezuma	Cornell University	**Dianne Cox**
Lea George	Villanova University	**Antonio Di Cristofano**
Simon Gonzalez Esteva	University of Texas at Austin	**Charles Query**
Michelle Gulfo	SUNY College at Geneseo	**Teresa Bowman**
Mathew Hakimi	New York University	**Ales Cvekl**
Victoria Hamilton	Baylor University	**Konstantin Dobrenis**
Vijay Jani	Rensselaer Polytechnic Institute	**Wei-Li Liu**
Mert Keceli	Hunter College	**Victor Schuster**
Alexander Khamechand	Fordham University	**Pablo Castillo**
Miriam Klahr	Yeshiva University	**Jessica Mar**
Lisa-Qiao MacDonald	Oberlin College	**Myles Akabas**
Steven Maio	College of Mount Saint Vincent	**Naum Shaparin**
Yonatan Mehlman	Yeshiva University	**Mark Suhrland**
Ahava Muskat	Stern College for Women	**David Shechter**
Veronica Natale	The University Of Texas At Austin	**Tom McDonald**
Tierra Ouellette	Tufts University	**Myles Akabas**
Angel Payan	Rochester Institute Of Technology	**Vern Schramm**
Jenna Reich	New York University	**Naum Shaparin**
Zuleirys Santana	Inter American Univ Of Puerto Rico	**David Spray**
Daniel Sapozhnikov	McGill University	**Michael Levi**
Aakash Shah	Bucknell University	**Teresa Bowman**
Michael Shavolian	Yeshiva University	**Ekaterina Dadachova**
Maryam Waris	Arizona State University	**Jon Lai**
Amy Wells	Virginia Polytechnic Institute	**Pablo Castillo**
Julie Williamson	Emory University	**Bridget Shafit-Zagardo**
Kathryn Wilson	Ferris State University	**Thomas Ow**
Mohammad Yaseen	Fordham University	**Saleem Nicola**
Jenny Zheng	Cornell University	**Johanna Daily**
Juin-Wan Zhou	Fordham University	**Elyse Sussman**

Meal Planning Considerations and Nutrition Education in Bronx Soup Kitchens

Kayla M. Babbush[1] and Rubina A. Heptulla, MD[2]

[1] Department of Food Science, Cornell University, Ithaca, New York

[2] Department of Pediatrics, Albert Einstein College of Medicine of Yeshiva University, Bronx, New York

It is estimated that 1.4 million New York City residents rely on food pantries and soup kitchens to feed themselves and their families. With a growing population of homeless individuals in urban America, emergency food programs have struggled to satisfy the needs of this food insecure population. Studies have suggested that emergency food programs often fail to meet recommended dietary guidelines, which may explain the prevalence of obesity among New York's homeless population. The goal of this study was to assess Bronx soup kitchens to gain insight into their meal planning, menu availability and opportunities for nutrition education. Thirty-eight Bronx soup kitchens were identified using The City of New York Website and Internet search engines, and eighteen soup kitchen supervisors agreed to participate in a fourteen question telephone survey. Descriptive statistics and qualitative analysis were used to analyze survey answers. One hundred percent of soup kitchens claim to follow some type of nutrition guidelines when preparing meals, and a majority of these establishments abide by USDA's MyPlate guidelines. However, none of the soup kitchens meet daily recommendations for dairy foods. Fifty percent of soup kitchens keep menus on file, and no soup kitchens have menus available on the internet. Additionally, fifty percent of soup kitchens provide formal nutrition education on-site for clients, and another eleven percent provide some type of informal education. The findings of this study suggest that there are limited resources available for meal planning and education in soup kitchens. Future evaluation needs to be done to determine the effect of limited resources, menu availability and nutrition education on quality of meals served at soup kitchens. We would like to acknowledge Dr. Judith Wylie-Rosett, the staff of The Division of Pediatric Endocrinology at The Children's Hospital at Montefiore and the Summer Undergraduate Research Program at Albert Einstein College of Medicine. We would like to thank soup kitchen staff for participating in this study.

Role of Nucleophosmin (Npm1) as a Histone Chaperone

Alexandru Barbulescu[1,2,3], Chris Warren[2], David Shechter[2]
1 *City University of New York Hunter College, 695 Park Avenue, New York, NY 10065*
2 *Department of Biochemistry, Alberst Einstein College of Medicine, 1300 Morris Pasrk Avenue, Bronx, NY 10461*
3 *Summer Undergraduate Research Program*

Histone chaperone proteins are necessary for the proper association of histones with DNA to form nucleosomes, the basic structural units of chromatin. One such chaperone, Nucleophosmin (Npm1), possesses a wide variety of cellular functions including transcriptional regulation, ribosome biogenesis, nucleocytoplasmic transport, centrosome duplication, and nucleic acid binding. Due to its many properties, little is known about the histone chaperone function of Npm1. Herein, we present a molecular understanding of the *Xenopus Laevis* Npm1 domains important for its histone chaperone ability. Npm1 is 298 amino acids long. Its N-terminus consists of a heat stable pentamerization core while its C-terminus consists of an ordered three alpha helical bundle that is not conserved among family members Nucleoplasmin (Npm2) and Npm3. In between the two extreme termini lies an intrinsically disordered region consisting of two acidic stretches, A2 and A3, thought to be important for histone interactions. Full length Npm1 and three sub-cloned truncations were purified and tested for their ability to 1) prevent non-specific aggregation of histones 2A and 2B with DNA and 2) assemble nucleosomes by depositing hyperacetylated HeLa core histones onto plasmid DNA. Histone aggregation prevention assays indicate the full-length protein exhibits a lower H2A/B aggregation prevention capability than the Core+A3 and Core+A2 mutants while the Core truncation only exhibits modest aggregation prevention. Chromatin assembly assays indicate that increasing molar concentrations of chaperone result in a loss of deposition ability with the full-length protein and the Core+A2 mutant but an enrichment of deposition with the Core+A3 mutant. Interestingly, the Core mutant exhibits modest deposition at all concentrations. Taken together, these preliminary data suggest a structural model of Npm1 in which the basic region near its C-terminus folds back and interacts electrostatically with the third acidic patch, thereby competing with histone binding. This hypothetical model opens up the possibility that post-translational modifications serve to regulate the structure and function of Npm1. A more developed understanding of Npm1 is important to gain further insight into mechanisms of chromatin arrangement and transcriptional regulation. Furthermore, elucidating the mechanism by which Npm1 functions has implications for cancer treatment, as Npm1 is found upregulated in a variety of tumor cells and mutated in approximately two-thirds of acute myeloid leukemia patients with a normal karyotype.

Acknowledgements: I am grateful to all members of the Shechter Lab for contributing to the most organized and well-planned research experience I have ever had. I am also thankful to the SURP for giving me a wonderful opportunity to pursue what I love most.

The Role of Amphiregulin in ERα-positive Breast Cancer Cell Proliferation

Maryia Barnett[2], E. Charles Jenkins Jr.[1], Esther A. Peterson[1], and Paraic A. Kenny[1]

Department of Developmental & Molecular Biology[1] and Summer Undergraduate Research Program[2], Albert Einstein College of Medicine, Bronx, NY

Estrogen is a key hormone required for normal mammary gland development and is a critical driver of proliferation in Estrogen Receptor alpha (ERα) positive breast tumors. The cellular response to this hormone is primarily carried out by its receptor, the transcription factor ERα. Previous work has identified the EGFR ligand Amphiregulin (AREG) as an ERα target gene, among hundreds of target genes, that is upregulated in response to estrogen stimulation. Additionally, knock-out studies have revealed that either ERα or AREG knock-out results in a truncated mammary tree. This suggests that AREG is critical for the proliferative response of the mammary epithelium to systemic estrogen circulation that normally occurs at the onset of puberty. In a cohort of 295 breast cancer patients, Dr. Kenny found that AREG expression was significantly higher in patients with ERα positive breast tumors and, in a panel of 13 human luminal breast cancer cell lines, AREG expression was positively correlated with ERα expression. Given these observations, we hypothesized that the EGFR ligand Amphiregulin is a critical estrogen response gene that is responsible for the proliferative effect of estrogen in human breast cancer.

To test the role of AREG in proliferation of ERα positive breast cancer, AREG knockdown sub-lines were generated from the MCF7 human luminal breast cancer cell line. We have previously demonstrated that AREG knockdown in this cell line significantly decreased growth in 3D cultures and in mouse tumor xenografts in the presence of estrogen. To exclude the possibility that this observation is restricted only to the MCF7 cell line, we created AREG knockdown sub-lines with two different knockdown constructs in two additional ERα positive breast cancer cell lines (T47D and ZR-75-1). Like other EGFR ligands, AREG is present as a full-length protein at the plasma membrane where it undergoes proteolytic shedding to release a portion of the peptide that acts as a soluble receptor ligand. AREG knockdown in luminal breast cancer cell lines was verified using an indirect enzyme linked immunosorbent assay (ELISA) to detect the release of the soluble signaling portion of AREG in conditioned media. Knockdown was additionally verified by qRT PCR. The functional implications of AREG knockdown in these cell lines was assessed using three-dimensional (3D) cell culture in Matrigel (laminin rich extracellular matrix).

We observed that knockdown of AREG significantly reduced the proliferative response of T47D and ZR-75-1 cells to estrogen when cultured in 3D. This result is consistent with what has previously been observed when AREG is knocked-down in MCF7 cells grown in 3D culture or as tumor xenografts. Together, these observations demonstrate the important contribution of AREG to ERα positive tumor growth, and suggest that AREG or AREG producing cells may represent an effective therapeutic target in ERα positive tumors.

Acknowledgments:

This work was supported by Department of Defense Breast Cancer Research Program, The Susan G. Komen foundation, The American Cancer Society, The NIH Institutional Research and Academic Career Development Awards (IRACDA) (K12), and Summer Undergraduate Research Program.

Title: Elucidating the mechanism of Dazap2 mediated germ plasm maintenance

Abigail Bergman[3], Meredyth M. Forbes[1], Florence L. Marlow[1,2]

1. Department of Developmental and Molecular Biology
2. Department of Neuroscience
Albert Einstein College of Medicine, Bronx, NY, USA
3. Stern College for Women, Yeshiva University, NY, NY, USA

Summer Undergraduate Research Program

Abstract: Primordial Germ Cells (PGCs) are the stem cells of the germline. Germline specification can occur through induction by zygotic factors, or through the inheritance of maternal factors called Germ Plasm (GP). GP first localizes to the Balbiani body (Bb) of primary oocytes and is later inherited in the cells that will become the PGCs. When PGCs are formed, the GP components localize in granular structures, or germ granules. While the mechanisms contributing to germ granule development are still poorly understood, our lab has recently identified the scaffold protein Dazap2 as a regulator of germ granule maintenance in embryonic PGCs. Dazap2 mutant ovaries lack overt defects in GP localization and oocyte polarity indicating that there are distinct mechanisms for GP maintenance in the oocyte and the embryo. In order to understand the mechanism by which Dazap2 contributes to germ granule maintenance, we are using biochemical methods to identify and analyze proteins that bind to Dazap2 in oocytes and PGCs. This study will provide further insight into how Dazap2 regulates germ granule maintenance.

Tissue Clearing and Pain-Mediated Changes in Trigeminal Ganglion Vasculature

Matthew F. Challman[1,2], Zuleirys Santana-Rodríguez[1], Regina Hanstein, PhD[1], and David C. Spray, PhD[1]

[1]Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461, [2]Fordham University, Bronx, NY 10458

Summer Undergraduate Research Program

Background:

Nerve injury is reported to cause an increase in the extent and architecture of blood vessels in the sensory ganglia in rats[1]. However, the methods used in that study to analyze vascularity involved serial section and 3D reconstruction of the ganglia, which is time consuming and risks loss of data. New methods of tissue clearing can achieve transparency of the entire tissue, facilitating 3D imaging without the disruption of tissue architecture. The objectives of this study were **1.) to optimize the tissue clearing and immunohistochemical staining protocols adapted from Yang *et al* (2014), and 2.) to determine whether there are changes in the vasculature of the brain and trigeminal ganglion in an acute orofacial pain model.**

Methods:

Brains and trigeminal ganglia (TG) of wild type, control, and pain model mice underwent clearing and immunohistochemistry. Tissues were imaged using a confocal Leica SP5 microscope and 3D images were constructed using Imaris software. ImageJ was used to determine changes in the vasculature of the sample images. Furthermore, to determine changes in permeability of the blood-brain barrier in pain, Evans Blue Dye was i.p. injected into mice and its penetration into the brain and TG was evaluated.

Conclusion:

The clearing protocol allowed for successful full-tissue imaging of the trigeminal ganglion. Clearing of trigeminal ganglia was achieved in 3 days, whereas clearing of the brain took 4 weeks. We found that antibodies detecting Isolectin B_4 and Von Willebrand Factor both stained the tissue vasculature and higher image quality was achieved when antibody incubation took place at 37°C compared to room temperature. In our mouse pain model, increases in total vasculature volume were statistically significant compared to control mice (Student's t-test, $t(3)=6.82$, $p=0.0064$). Based on the Evans Blue Dye experiment, there is no blood-nervous system barrier protecting the TG. Additionally, CFA-induced pain in mice does not disrupt the blood-CNS barrier and does not increase Evans Blue penetration into the TG.

References:

1. Kubíček, L., Kopáčik, R., Klusáková, I., Dubový, P. (2010). Alterations in the vascular architecture of the dorsal root ganglia in a rat neuropathic pain model. Annals of Anatomy, 192 (2010), 101-106.

2. Yang, B., Treweek, J. B., Kulkarni, R. P., Deverman, B. E., Chen, C., Lubeck, E., Shah, S., Cai, L., Gradinaru, V. (2014). Single-cell phenotyping within transparent intact tissue through whole-body clearing. Cell, 158, 945-958.

Title: Auto-Induction of Human Sulfotransferase 1A1

Authors: Anne Chen, Ting Wang, Thomas Leyh

Department of Microbiology & Immunology

SURP Program

The objective of the current study is to compare the merits of two protein-expression protocols. We hypothesize that it is possible to achieve comparable or better expression of our target protein, human sulfotransferase 1A1 (SULT1A1), using lactose rather than IPTG as an inductant. The expression construct consisted of SULT1A1 positioned downstream of the lac operon in a pGEX-6P vector engineered with a PreScission protease cleavable N-terminal triple-tag. Two lac operon inducers were tested: isopropyl beta-D-1-thiogalactopyranoside (IPTG) and lactose. IPTG, a commonly used inductant, is considerably more expensive than lactose. Because lactose is a relatively poor *E. coli* carbon source, growth media were supplemented with glucose and glycerol in the lactose expression studies. After optimizing culture aeration, we found that lactose induction yielded a two-fold increase in SULT1A1 expression over the IPTG protocol at a two-fold lower cost.

Genetic Modifiers of Cardiac Development in 22q11DS Mouse Models

Kevin Cintron Arroyo, Silvia Racedo, Jonathan Chung and Bernice Morrow
Department of Genetics, Albert Einstein College of Medicine, 1301 Morris Park Avenue, Bronx, NY 10461

Abstract

Over 70% of patients with 22q11.2 Deletion Syndrome (22q11DS) have congenital heart defects. The prevalence and severity of these defects make it critical for us to strive to understand the underlying mechanism of the syndrome by elucidating the gene pathways that are involved in heart development, as their disruption may lead to congenital heart diseases (CHDs). *TBX1* and *CRKL*, the main genes that have implicated in 22q11DS as responsible for patient CHDs, are required for normal development of the heart. It's been suggested that some genes located outside of the 22q11 deleted region can act as genetic modifiers of CHDs in the syndrome and may also be required for normal development of the heart. Three of these genes are *NTRK3*, *WNT5A* and *ROR2*. Mutations in *WNT5A* and *ROR2* were identified in patients with CHDs and 22q11DS, while mutations in *NTRK3* were identified in non-syndromic patients with CHDs. One of the overarching aims of this study is to test whether genetic modifiers of heart defects associated with the 22q11.2 deletion are the same genes as the genes that alter risk for heart defects in non-syndromic CHD patients. First, we must test where these three genes are expressed in normal developing embryonic hearts and we have decided to use the mouse as a model to do this. Digoxigenin-labeled RNA probes for *Ntrk3, Wnt5a and Ror2* were designed and used for *in situ* hybridization in order to observe the expression of these genes in mouse embryos specifically in the heart and regions that contribute to the formation of the heart. *Ntrk3*, *Ror2* and *Wnt5a* were expressed in the pharyngeal arches and heart region**.** The expression of the genes *Ntrk3*, *Ror2* and *Wnt5a* were not only restricted to the heart regions but were also seen in the neural tube area. We also examined the expression of *Ror2* in *Crkl* knock-out embryos, but observed no change. The results may support our hypothesis that *NTRK3*, *WNT5A*, and *ROR2* may be causative of CHDs, since they were found to be expressed in the heart and regions required for heart development, however, more work is needed to prove this. More work is also needed to show whether any of these genes act in the same pathway as genes in the 22q11 deleted region to function in normal heart development.

Development of lentiviral vectors to transduce TERT for enhancing the lifespan of human somatic cells

Gilad Doron[1], Y. Li[1], X. Wang[1], Z. Polgar[1], N. Roy-Chowdhury[1,2], J. Roy-Chowdhury[1,2]
[1] Departments of Medicine and [2]Genetics, and Marion Bessin Liver Center, Albert Einstein College of Medicine, New York

Background: Genetic mutations in somatic cells, such as skin fibroblasts, derived from patients with monogenic liver diseases can be corrected by targeted genome editing, reprogrammed to induced pluripotent stem cells (iPSCs) and then differentiated to hepatocyte-like cells that can be used for regenerative medicine. However, selection of cells that have undergone genome editing requires many rounds of cell division that can lead "crashing" of the cultured cells because of telomere depletion. Therefore, in this project, to improve the lifespan the primary somatic cells, we have developed a lentiviral vector capable of expressing telomerase reverse transcriptase (TERT), the catalytic subunit of the enzyme telomerase. We chose lentiviral vectors because of their high gene transfer efficiency and persistence of transgene expression in dividing cells.
Experimental procedure: Lentiviral transduction plasmids are constructed in which human TERT cDNA is cloned downstream to a ubiquitous cytomegalovirus (CMV) promoter. The plasmid also expresses the marker EGFP from an internal ribosomal entry site and a CMV promoter. The transduction plasmid is cotransfected with the helper plasmids into HEK 293T cells. The released recombinant lentiviruses containing the vesicular stomatitis virus envelope (VSV-G) are concentrated by centrifugal sedimentation.
Results: The lentiviral transduction plasmid expressing TERT and EGFP was engineered successfully. The composition of the cDNA was verified using double digestion and gel electrophoresis. The lentiviral vectors were successfully produced after transfections into HEK 293 cells, with a resulting vector titer of 1.17×10^{10} TU/mL.
Summary: We demonstrated that cotransfection of modified TERT cDNA and helper plasmids initiate the creation of lentiviral vectors that can be used to transduce skin fibroblasts with TERT. Transducing skin fibroblasts with these vectors has the capability to reduce telomere depletion after many rounds of cell division.

Effect of Rab 11 on TNT formation in Macrophage and Tumor Cells

Chimere Ezuma, Kessler McCoy-Simandle, Samer Hanna and Dianne Cox
Department of Anatomy & Structural Biology, Albert Einstein College of Medicine, Bronx

Macrophages, specialized white blood cells, have the ability to communicate with adjacent macrophages and other cell types. Tunneling nanotubes (TNTs) are long thin membranous channels that macrophages use as a form of intercellular communication. This process of intercellular communication allows macrophages to exchange organelles and various cargos and has been suggested to be vital for intercellular processes such as cell development and immunity. Because TNTs are membrane protrusions, we believe that additional membranous material is required. We hypothesize that Rab 11, a member of the Rab GTPase family, is responsible for providing the needed membrane. The purpose of this project was to determine if Rab 11 played a role in the formation of tunneling nanotubes (TNTs). Four Rab11 mutant macrophage cell lines were thawed and fluorescent microscopy was used to visualize TNTs live using a membrane dye, FM1-43. The percent of cells that formed nanotubes in each of the four cell lines were compared. The constituently activated Rab11 GTPase had the highest levels of nanotube formation and the dominant negative Rab 11 GTPase had the lowest levels. Over-expression of wild type Rab11 had no effect on TNTs. From these results, it was concluded that Rab 11 played a role in nanotube formation in macrophages. Since macrophages have the ability to communicate and form nanotubes with other cell types, such as tumor cells, we investigated the role of Rab 11 in nanotube formation between macrophage and tumor cells as well. To visualize this, a similar experiment was performed and nanotubes formed between the mutant macrophages and tumor cells were recorded. As in the macrophage experiment constituently activated Rab 11 GTPase had the highest levels of nanotube formation with tumor cells and the dominant negative Rab 11 GTPase had the lowest levels. These experiments suggest the involvement of Rab 11 GTPases in nanotube formation in macrophages. However, further studies will be needed to validate that Rab 11's role during nanotube formation is providing additional membranous material.

The Effect of Brd4 Gene Knockdown on Tumor Cell Lines

Lea George[1], Arturo Orlacchio[2], and Antonio Di Cristofano[2]

[1]Villanova University, Villanova, PA,
[2]Department of Developmental and Molecular Biology, Albert Einstein College of Medicine, Bronx, NY

Summer Undergraduate Research Program

The BET family is a family of bromodomain proteins, including Brd2, Brd3, and Brd4. JQ1 is a chemical inhibitor that affects the BET family and has other non-specific effects. It influences the expression of multiple genes in a cell, but a significant portion of the results of JQ1 might be due to Brd4 inhibition. Brd4 binds to super-enhancers and is highly active in tumor cells, promoting proliferation. As a coactivator, Brd4 regulates transcription of many genes by bringing transcriptional factors into the proximity of the transcriptional system.

Anaplastic thyroid cancer (ATC) is the most aggressive type of thyroid cancer, resistant to most treatments. JQ1 hinders the growth of ATC cells *in vitro* and *in vivo*. In order to inhibit Brd4, sh-RNA technology was used. This assessed if silencing Brd4 has the same effect as JQ1 on tumor cells. Two cell lines, T3531L and T4888M, derived from mouse ATC tumors, were used for these studies. Both cell lines were transduced with lentivirus sh-RNAs, 778 and 779, specific for Brd4 mRNA. The study of JQ1 treatment will help discover more therapies for ATC.

To elucidate the effect of Brd4 inhibition on tumor cells, plasmids encoding the sh-RNAs specific for Brd4 mRNA were packaged in HEK-293 cells. Two thyroid cancer cell lines were infected with these viral vectors. After the selection, the stable clones carrying the sh-RNA were tested by qPCR for Brd4 expression levels. Growth curves were generated for the silenced clones. Finally, to understand if JQ1 works by inhibiting Brd4, a few genes, with corresponding changes in expression after JQ1 treatment, were randomly selected to be tested. The analysis was performed using qPCR to compare the changes of the selected genes between cells treated with JQ1 and the same cells silenced for Brd4 with respect to the relative controls.

The results displayed how little the T3531L and T4888M cells with Brd4 silencing grew compared to the same cells with no silencing. However, the gene expression changes for Aurka1, Ccna2, Plk1, Ccnb2, Kif20a, Ly6a, and Hk2, observed for both cell lines after Brd4 inhibition are not the same in the case of JQ1 treatment.

In conclusion, the two cell lines are similarly sensitive to JQ1 treatment and BET inhibition. Brd4 inhibition does have a significant effect on the proliferation of the mouse ATC cell lines. The preliminary data suggests that JQ1 does not work through Brd4 to regulate Aurka1, Ccna2, Plk1, Ccnb2, Kif20a, Ly6a, and Hk2 genes, signifying that it could work through the other bromodomain proteins to control these genes.

The importance of Brd4 in other ATC cell lines will be investigated including human cell lines. Further studies must include a larger scale of profiling the silenced cell lines to understand Brd4 regulation on other genes. Since JQ1 seems to act through a different mechanism than Brd4, the effect of silencing other BET family members should be studied.

Current funding is provided by the National Cancer Institute, the Irma T. Hirschl Trust, and the Summer Undergraduate Research Program.

The Possible relationship between Macroautophagy and Spliceosomal Regulation

Simon Gonzalez Esteva, Susan Rodriguez- Santiago and Charles Query
Albert Einstein College of Medicine- Department of Cell Biology

ABSTRACT

Structurally, genes contain protein coding and non-coding regions (introns), which need to be removed by a machinery known as the spliceosome. The spliceosome is a multi-mega-dalton ribonucleoprotein (RNP) complex comprised of five snRNPs: U1, U2, U4, U5 and U6 and other proteins. The spliceosome recognizes splice site sequences in gene transcripts to catalyze the removal of introns by two consecutive transesterification reactions. We know a lot about the function, regulation and mechanism of the spliceosome. But, there is a gap in knowledge about how the spliceosome is regulated by different growth conditions, for example nutritional stress.

One of the initial response mechanisms of the cell to nutritional stress is the induction of autophagy. Autophagy is categorized into three different types: Macroautophagy, microautophagy and chaperone-mediated autophagy. In our project we focused on macroautophagy, a mechanism by which the cell uses a double membrane structure known as the autophagosome to degrade cellular components, especially under starvation. Proteins that are targeted by macroautophagy, contain a signaling motif known as LIR(LC3-interacting region). LC3(ATG8 in yeast) is an essential component for autophagosome formation and also interacts with and recruits cargo for degradation in the autophagosome. The LIR motif is characterized by a WXXL cone sequence. Bioinformatic analysis of LIR-containing proteins suggests that spliceosomal components are enriched in LIR motifs, suggesting a relationship between the regulation of the abundance of spliceosomal components by autophagy degradation and its function.

Our project is focused on SF3b1 (Splicing Factor 3b1), which is a subunit of the U2 snRNP spliceosomal component. The U2 snRNP is essential for the splicing reaction and is crucial for its fidelity. Bioinformatic analysis suggests that the splicing factor SF3b1 shares a functional relationship with Atg8, since it possesses two predicted LIR domains at its HEAT repeat containing region in the C-terminal domain. This leads us to hypothesize that SF3b1 levels are changed under nutritional stress through autophagy degradation; possibly having a consequence on splicing function by regulating the levels of the U2 snRNP complex. Here we developed autophagy depleted yeast strains and used sensitive intron reporter assays to test this hypothesis. In this project we were able to successfully develop and validate autophagy knocked out yeast strains by standard procedures in which SF3b1 accumulates in the cell after autophagy induction. The reporter assay showed improvement in recognition of the suboptimal intron U257C, which is an intron that has been shown previously to be sensitive to U2 snRNP mutations. We predict that nitrogen starvation may alter U2 snRNP levels in the cell and thereby affects the splicing reaction.

Characterizing the extent of and response to DNA damage due to *in vivo* depletion of *sf3b1*, a spliceosomal component critical for hematopoiesis

Michelle Gulfo[*], Sara Nik[**], Rosannah C. Cameron[**], Adriana De La Garza-Sauceda[**], Teresa V. Bowman[**]

[**]State University of New York College at Geneseo, Geneseo, NY

[**]Department of Developmental and Molecular Biology, Albert Einstein College of Medicine, Bronx, NY

Summer Undergraduate Research Program

Myelodysplastic syndrome (MDS) is a disorder of hematopoietic stem and progenitor cells (HSPCs) that results in defective hematopoiesis. MDS often progresses into Acute Myeloid Leukemia (AML), a disease that can quickly be fatal. Our goal is to better understand the loss of HSPCs in MDS to provide insight into the disease and potentially help inform regeneration therapies. Recent sequencing studies of MDS patients revealed that one of the most common mutations was in *sf3b1*, an integral component of the spliceosome. We study this mutation to understand how splicing defects might lead to the loss of HSPCs and defective hematopoiesis seen in MDS. It is known that depletion of splicing components in yeast and human cells causes genomic instability. It is also known that DNA damage and apoptosis influence adult HSPC fate decisions and are hallmarks of MDS. We seek to characterize the DNA damage caused by *sf3b1* depletion and the role of DNA damage in HSPC loss in zebrafish embryos, a previously unexplored area. Thus far, the lab has shown that loss of *sf3b1* in zebrafish leads to widespread DNA damage, p53 pathway activation, apoptosis and cell cycle arrest, and elimination of HSPCs. Now we are investigating by Western blotting and immunofluorescence if the canonical ATM/ATR/p53 DNA damage response pathway is activated in mutants and if it is active in mutant endothelial cells, which give rise to HSPCs. We have observed that HSPC precursor endothelial cells are not undergoing apoptosis, yet, canonical DNA damage response factors are elevated in mutants and likely present in mutant endothelial cells. The results suggest that DNA damage might be playing a role in *sf3b1* mutant phenotypes, but further validation of results and characterization is needed to better understand the role of DNA damage in HSPC emergence. These insights will hopefully help inform improved treatment for MDS in the future.

I would like to thank TVB and SN for their vital guidance and insight in the design and completion of my project, and RCC for her generous experimental advice and assistance with imaging. I thank all members of the Bowman lab for an enjoyable summer experience!

Gene Regulation by Pax6 in Forebrain: MicroRNA Connectivity

Mathew Hakimi[1], Elena Martynova[2,3], Yilin Zhao[2,3], and Ales Cvekl[2,3]
[1]New York University New York, NY 10003
[2]Department of Ophthalmology & Visual Sciences and [3]Genetics, Albert Einstein College of Medicine
Bronx, NY 10461
Summer Undergraduate Research Program

Studies on gene regulation to understand brain development at the molecular level require identification of specific gene regulatory networks (GRNs) and their dynamics, and represent a major challenge in the field of genetics. Embryonic development is regulated by a concerted action of DNA-binding transcription factors, transcriptional co-regulators, and extracellular signaling. It has been recognized in the last decade that non-coding RNAs, including lncRNAs and microRNAs, as well as specific RNA-binding proteins, provide another level of gene control at the posttranscriptional level. Among a sparse number of transcription factors, which exert high-level control of cortical development, Pax6 is expressed in a gradient throughout the developing cortex and regulates a wide range of target genes. Pax6 is comprised of a homeodomain and paired domain, and regulates genes involved in radial glial cell differentiation, cell cycle exit control, neuronal differentiation, and dorso-ventral patterning. Recent studies have shown that Pax6 directly controls expression of two microRNAs, miR-135b and miR-204. Establishing a GRN and understanding the connectivity between genes regulated by Pax6 and microRNAs will provide us with a more profound understanding of the genetic underpinnings of cell fate determination and cortical development. Herein we examined 270 transcripts directly regulated by Pax6 using NCBI Gene and UCSC Genome Browser, whether they host miRNAs and/or if miRNAs are found nearby *in vivo* mapped Pax6-binding sites. This analysis predicts 11 down-regulated miRNAs in $Pax6^{-/-}$, including let-7a-2, miR-100, miR-125b-1 (near 2610203c20rik), miR-466d (hosted by Camk1d), miR-124 (100 kb upstream of Kif13b), miR-378b (hosted by Msi2), miR-152 (hosted by Copz2), miR-9-2 (near Pax6 binding region in C130071C03Rik), miR-128-2 (hosted by Arpp21), miR-204 (hosted by Trpm3), and miR-763 (hosted by Hmga2). Computational analysis of predicted targets of these miRNAs (both in human and mouse), revealed a high level of connectivity towards eight genes: Bcl11b, Mafb, Sox2, Stat3, Cdkn1c, Dmrta1, Isl1 and Pax6 (self, via miR-466d, -124, -9-2, and -204). Ongoing experiments are aimed to determine expression changes of these miRNAs in E12.5 and E14.5 $Pax6^{-/-}$ forebrain through real time PCR. Taken together, the present studies generated a testable model of genome-wide gene control of Pax6, through its directly regulated microRNAs.

This study was supported by the R01EY012200 grant. I would also like to thank the SURP at Einstein and the Cvekl Lab for giving me the extraordinary opportunity to contribute to this project.

Mechanisms of Pathogenesis and Treatment of Neurodegenerative Niemann Pick C Disease

Victoria Hamilton, Andrew Smith, Ben Papapietro, Kevin Fisher and Kostantin Dobrenis
Dominick P. Purpura Dept. of Neuroscience, Albert Einstein College of Medicine

Niemann Pick-C1 disease (NP-C) is an autosomal recessive neurodegenerative disorder signified by the lysosomal storage of unesterified cholesterol, as well as accumulation of GM2 and GM3 ganglioside and other lipids, all resulting from a deficiency in a transmembrane protein thought responsible for retroendocytic trafficking of cholesterol. There is no cure for NP-C but two drugs, Cyclodextrin (CD) and Miglustat, have shown efficacy in animal models. The following studies were conducted to both investigate pathogenetic mechanisms and to elucidate the mechanisms by which these drugs, and their combination, correct the NP-C process. This included enzyme assays, immune-assays, and microarray gene expression analyses, using NP-C model mice and brain cell cultures.

The first assay tested whether GM2, in addition to showing intracellular vesicular accumulation, is elevated along the exterior of the cell where it was hypothesized to pathophysiologically impact a variety of neuronal receptors. After incubations to distinguish between surface and internal GM2, followed by immunostaining for MAP2, confocal microscopy indicated GM2 was undetectable on the cell surface, but instead found within intracellular vesicles close to the plasma membrane. The second experiment evaluated the hypothesis that CD stimulates lysosomal exocytosis, in contrast to the idea that CD directly binds cholesterol and facilitates its trafficking from the lysosome to ER. Testing three distinct types of CDs, differing in efficacy in vivo for reducing cholesterol storage and improving morbidity/mortality, the resulting β-hexosaminidase (Hex) (a lysosomal enzyme) levels in treated culture medium supported the opposite: the untreated NP-C brain cultures expressed the most Hex (22±6.8U/well), followed, in descending order, by α-(16±1.6), β-(13±3.4) and γ-(9.4±2.8) CDs. This may be attributed to NP-C cells' heightened Hex expression, and instead suggests that CD reduces Hex expression, rather than stimulating lysosomal exocytosis. Confocal imaging of CD-treated cultures immunostained for the lysosomal membrane marker LAMP2 did not sufficiently support the idea of CD's stimulating lysosomal exocytosis, as none of the cyclodextrin varieties indicated LAMP2 staining sufficiently different from untreated NPC and untreated wild type plasma membrane levels.

Microarray data for 35,916 genes from the cerebral cortex of NP-C and WT mice treated with CD, Miglustat, a combined treatment, or saline revealed approximately 113 genes of interest with statistically significant altered expression as compared to untreated and wild type mice. Multiple pair-wise comparisons (FDR $p<0.05$) of different genotype/treatment groups and logical operations were performed to identify disease and/or treatment effects. Our results indicate that CD treatment is the most effective of the three, with 34 genes with expression returned to wild type levels and 18 genes significantly different from wild type, as compared to the 14 returned to wild type levels and 38 remaining altered with the combined treatment, and, with Miglustat treatment, no genes returned to desired expression and 76 altered from wild type. Cyclodextrin's corrected genes were involved in myelination, lipid metabolism, neural development, and other functions/pathways. Interestingly, the data also revealed unexpected genes that may be utilized to investigate mechanisms unique to CD's success, such as Ttyh2, a member of the Tweety family of genes, encoding a plasma membrane chloride channel which has been implicated in cell aggregation and proliferation. "Side effects" were minimal for all treatments on wild type and NP-C mice, and totaled only three statistically significant genes

Further research must be conducted in order to elucidate the exact mechanisms by which CD is involved in lysosomal exocytosis, if it is indeed so involved, and how it affects cholesterol and ganglioside accumulation, some insights for which might be derived from our microarray data. As these treatments are currently being used in human patients, further research expanding current knowledge of both treatment and disease mechanisms, especially regarding substrate accumulation and removal, are recommended. Supported by NIH grant NS053677.

Biochemical Mapping of the Binding Interactions Between p53 and RNA Polymerase II

Vijay Jani, Sameer Singh, Sharona Levy and Dr. Wei-Li Liu
Albert Einstein College of Medicine of Yeshiva University, Department of Anatomy and Structural Biology, Bronx, NY 10461, USA

Gene expression is the process by which DNA is converted to protein to allow for a cell's many functions and activities to occur. RNA polymerase II (Pol II) plays a key role in gene expression in eukaryotic cells by transcribing DNA into RNA in the process of transcription, where RNA is the intermediary that is translated into protein. Initiation of transcription requires the recruitment of Pol II to the promoter DNA by a pre-initiation complex (PIC).[1] The PIC is a large group of proteins that assembles at the promoter DNA to help Pol II initiate transcription.

p53 is a key tumor suppressor protein that acts as a transcription factor to regulate the recruitment of components of the PIC to the promoter DNA.[2,3] p53 functions in response to various stresses such as DNA damage, hypoxia or resource deficiency.[3-5] p53 activity may result in cell cycle inhibition, apoptosis, DNA repair or senescence. In this way, p53 prevents cells with the potential to become cancerous from dividing and is known to be mutated in over 50% of human cancers.[6]

Although it has been established that p53 can interact with Pol II, the distinct binding locations and structural changes induced by the formation of the Pol II/p53 co-complex are still unknown. In this study, we utilized a photoactivatable protein cross-linking label transfer assay to map the binding interactions between Pol II and p53. In the label transfer assay, we labeled a photo-activatable molecule called Sulfo-SBED (S-SBED) to p53. The Sulfo-SBED is composed of an amine-reactive NHS-ester group that is able to bind p53. After the interaction of p53 and Pol II, exposure to UV light causes the UV light-activatable aryl azide group of S-SBED to conjugate nonspecifically to nearby regions of Pol II. After splitting the S-SBED, the portion of the S-SBED now bound to Pol II contains a biotin group. In order to determine which subunit of Pol II binds p53, SDS-PAGE is used to separate the proteins and western blot analysis is used to detect which subunits of Pol II received the biotin tag. After performing a western blot against biotin, our results indicated that the biotin tag of S-SBED is transferred to two particular subunits of Pol II: RPB1 and RPB2. These results allow use to conclude that p53 interacts with Pol II in the vicinity of the RPB1 and RPB2 subunits. A western blot against RPB1 confirmed the placement of RPB1 on the nitrocellulose membrane.

In the future, we will perform a western blot against the RPB2 subunit to confirm the placement of RPB2 on the nitrocellulose membrane. We plan to perform a label transfer assay in which we solely label the activation domain at the N-terminus of p53 with S-SBED to gain insight into the specific binding location of the activation domain of p53 on Pol II. The knowledge of the binding interactions between p53 and Pol II can lead to research in drug discovery to manipulate the Pol II/p53 co-complex. In this way, drug discovery as well as other following experiments will help elucidate the mechanisms involved in transcription initiation as well as cancer progression.

1. Sainsbury S, Bernecky C, Cramer P. Structural basis of transcription initiation by RNA polymerase II. *Nat Rev Molecular Cell Biology*. **2015** March; 16: 129-143.
2. Laptenko O, Prives C. Transcriptional regulation by p53: One protein, many possibilities. *Cell Death Differ*. **2006**; 13: 951-961.
3. Beckerman R, Prives C. Transcriptional regulation by p53. *Cold Spring Harb Perspect Biol*. **2010**; 2(8)
4. Murray-Zmijewski F, Slee EA, Lu X. A complex barcode underlies the heterogeneous response of p53 to stress. *Nat Rev*. **2008**; 9: 702-712.
5. Vousden KH, Prives C. Blinded by the Light: The Growing Complexity of p53. *Cell*. **2009**; 137: 413-431.
6. Hedau S *et al.* Novel germline mutations in breast cancer susceptibility genes BRCA1, BRCA2 and p53 gene in breast cancer patients from India. *Breast Cancer Res Treat.* **2004**; 88(2):177-86.

Title:
Does prostaglandin transporter PGT mediate uptake of PGs so as to activate PPAR?

Authors:
Mert Kemal Keceli, Run Lu, Victor L. Schuster

Department of Medicine and Physiology & Biophysics, Albert Einstein College of Medicine
SURP Program

Peroxisome Proliferator Activated Receptors or PPARs are nuclear hormone receptors that regulate gene transcription in response to peroxisome proliferators and fatty acids. PPARγ, a subtype of PPARs plays a role in conversion of fibroblasts into adipose cells, suggesting that PPARγ might be a regulator of adipogenesis. PGD2 and 15-deoxy-PGJ2 were determined to be a PPARγ ligands and inducers of adipogenesis. However, these prostaglandins were used as a PPARγ ligand at very high concentrations, as opposed to in vivo concentrations that are several orders of magnitude (picomolar range) below the levels required to induce a biological effect (micromolar range) in the experiments. Prostaglandin Transporter, or PGT, is a protein that imports prostaglandins across the plasma membrane. As shown in figure 1, our hypothesis is that PGT imports PGD2 and/or 15-deoxy-PGJ2 and delivers them to PPARγ. We will be using a nuclear extraction assay to see if there are changes in the levels of prostaglandin-bound PPARγ in the nucleus when comparing PGT null cells and wild type cells. We will also be looking for any differences in the cells' ability to become an adipocyte when PGT is not present on the cell surface.In order to properly test the hypothesis a cell line that can become an adipocyte and which expresses abundant PGT and PPARγ is needed. Though an appropriate assay for liganded PPARγ was set up, of the cells tested, no cell with adipogenic potential has adequate expression of both PGT and PPARγ. We would like to continue to screen additional adipogenic cell lines in order to find one that is appropriate for the assay system.

Contribution of GluN2B containing NMDA Receptors at Medial and Lateral Perforant Path synapses

Alexander Khamechand, Alma Rodenas-Ruano, Pablo E. Castillo
Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine, Bronx, New York, NY 10461, USA.
Diversity Student Summer Research Opportunity Program

The dentate gyrus, located in the hippocampus, is the major terminal point of projections from the entorhinal cortex (EC) and thus, it is considered to be a principal site where information is ultimately converted to memory. Since memory is correlated to synaptic strength, activity-dependent strengthening of synapses or Long Term Potentiation (LTP) is vital for memory formation and learning. Dentate granule cells (DGC) receive and integrate synaptic inputs from the EC via the medial perforant path (MPP) and lateral perforant path (LPP). N-methyl-D-aspartate receptors (NMDARs) are glutamate-gated ion channels capable of influencing long term changes in synaptic structure based on neuronal activity. GluN2A and GluN2B, the primary subunits for NMDARs in MPP-DGC and LPP-DGC synapses, have distinct variations in properties with each affecting the receptor's biophysical, pharmacological and signaling attributes. While an increase of NMDAR mediated synaptic transmission in the form of LTP in DGCs has been established, the mechanisms of induction and expression remain unclear. Preliminary data demonstrates that NMDAR LTP occurs at MPP-DGC, but not at LPP-DGC synapses, however the reason for this input specificity is unclear. One possibility is that NMDAR GluN2 subunits differentially contribute to MPP-DGC and LPP-DGC synaptic transmission, and this discrepancy potentially plays a role in NMDAR-LTP induction. We tested this hypothesis by using electrophysiological and pharmacological techniques to ascertain and quantify the contribution of GluN2B subunit to NMDAR response at MPP-DGC and LPP-DGC synapses in the rat hippocampus. Our results using extracellular field recordings show that, during basal synaptic transmission, in the presence of the GluN2B antagonist Ro25-6981, the percent decline in the amplitude of NMDAR responses is greater in MPP-DGC relative to LPP-DGC synapses. This indicates that the presence of the subunit is greater in the MPP-DGC. Future studies will attempt to determine whether GluN2B is necessary for the induction of NMDAR LTP in the MPP-DGC. Understanding the mechanisms governing NMDAR LTP may shed light on neuropsychiatric disorders that affect memory formation and learning capabilities.

Investigating the Link Between Variability in Gene Expression and Protein Abundance in Ovarian Cancer Patients

By: Miriam Pearl Klahr[1,3]; Samuel Zimmerman[2]; Laurence de torrenté[2]; Jessica Mar[2].

[1]Stern College for Women, Yeshiva University, New York, NY; [2] Department of Computational Biology, Albert Einstein College of Medicine, Bronx, NY; [3] Summer Undergraduate Research Program, Albert Einstein College of Medicine, Bronx, NY.

Abstract

While we understand that genes are up-regulated and down-regulated in a cellular phenotype, we are beginning to recognize that variability in gene expression also has functional consequences. Limited research exists on the relationship between expression variability and average gene expression. There is also a lack of consensus regarding the best statistic to use when studying gene expression variability. Using many transcriptome-wide data sets from different microarray and RNA-seq studies, conducted on both single cell and bulk tissue samples for different cell types, we investigated the nature of how average expression and expression variability are linked across the genome. We also evaluated the performance of three common variability estimators, standard deviation (SD), median absolute deviation and coefficient of variation. The evaluation was based on the degree of correlation between the average expression and expression variability. Our results collectively point to SD being the most stable estimator to use.

Using this information, we conducted an analysis of gene expression variability and protein abundance variability using data on 174 ovarian cancer patients from the Cancer Genome Atlas. We applied an *F*-test to identify genes that had significantly higher levels of variability at the transcriptional level than the protein abundance level, or vice versa. Additionally, we also identified a set of genes that had equal variability in both protein abundance and gene expression. We looked for enrichment of different pathways that was exclusive to each of these three sets of genes in attempt to understand the biological consequences of this variability. We used the NCI Pathway Database for this purpose, as well as other annotation sources, such as MSigDB. We discovered that many of the pathways among the three groups overlapped, meaning that within the same pathway we observed different levels of variability among different genes. This led us to hypothesize that perhaps certain genes are more or less critical to the pathway in that they are expressed at the protein level or transcript level, with greater or less variability in the ovarian cancer patients. This result may even suggest that there are different points of control in pathways that are used with greater consistency. Finding which genes and proteins these points of control correspond to may identify new targets for manipulating tumors.

Title: Quantifying Efficacy of PfENT1 Inhibitory Drugs on TM4 Yeast Mutants

Authors: Lisa-Qiao MacDonald[1]*, Tierra Ouellette[2]*, Avish Arora[3], Myles Akabas[3]
Department, Institution, Location: [1]Oberlin College, Department of Biology, Oberlin, OH; [2]Tufts University, Department of Biology, Medford, MA; [3]Department of Physiology & Biophysics, Albert Einstein College of Medicine, Bronx, NY
**Contributed equally*

Infection of humans with *Plasmodia* parasites through a mosquito vector causes malaria. The growing resistance to artemisinin derivatives validates the urgency for new antimalarial drug development. *P. falciparum* is the most deadly *Plasmodium* species. *Plasmodium* parasites are purine auxotrophs. They import purines from the host cells to generate the purine nucleotides necessary for cellular metabolic processes. Equilibrative nucleoside transporters (ENT) are a family of membrane transporters that transports purine nucleobases and nucleosides. The *Plasmodium falciparum* genome contains four ENT homologues, PfENT1-4. PfENT1 is the primary purine-import pathway for the parasite purine salvage pathway[1]. PfENT1 inhibition leads to parasite death presumably due to purine starvation. A novel yeast-based high-throughput assay was used to identify nine PfENT1 inhibitors[1]. We sought to determine whether residues in the fourth transmembrane segment of PfENT1, TM4, lined the substrate translocation pathway and/or affected inhibitor binding. Fourteen consecutive amino acids, I125 to A138, were mutated, one at a time, to cysteine (Cys) and individually expressed in yeast.

We measured each mutant's affinity for three purines: hypoxanthine, inosine, and adenosine. The IC_{50} values (half maximal inhibitory concentration) of each mutant were in the μM range, similar to those of wild type (WT). Thus, the mutations were tolerated and did not impact PfENT1 function.

We also measured the ability of the nine PfENT1 inhibitors to block [^{3}H]adenosine uptake. The IC_{50} values for one or more of the nine compounds were significantly increased for several of the mutants, as determined by a one-way ANOVA ($p<0.05$). Q135C caused a statistically significant increase for several of the compounds. The IC_{50} values of A131C and A134C also differed significantly from WT. Otherwise the effects were rather sporadic except for G132C. None of the nine compounds blocked purine transport by the G132C mutant. This suggests that the mutation interferes with drug binding. However, because G132C showed functional purine transport, G132C alters drug binding but not for purine binding. Of note, V126C grew very slowly and did not import [^{3}H]adenosine. Further studies will aim to characterize other domains of PfENT1 using similar techniques. Once a full understanding of how PfENT1 interacts with each of the drugs is achieved, one of the nine compounds may be selected for further pharmaceutical development.

[1]Frame IJ, Deniskin R, Arora A, Akabas MH. Purine import into malaria parasites as a target for antimalarial drug development. Annals of the New York Academy of Sciences. 1342(2015) 19-28.
[2]Frame IJ, Deniskin R, Rinderspacher A, Katz F, Deng SX, Moir RD, Adjalley SH, Coburn-Flynn O, Fidock DA, Willis IM, Landry DW, Akabas MH. Yeast-based high-throughput screen identifies Plasmodium falciparum equilibrative nucleoside transporter 1 inhibitors that kill malaria parasites. ACS Chem Biol. 2015 Mar 2010(3):775-83.

Clinical Usability Validation of the FORE-SIGHT Elite Tissue Oximeter at Children's Hospital at Montefiore Medical Center

Steven Maio, Naum Shaparin, Singh Nair, Madelyn Kahana

Department of Anesthesiology, Montefiore Medical Center, Bronx

Abstract:

FORE-SIGHT Cerebral Oximetry was approved by the FDA for measuring brain oxygen saturation in adults. This monitor measures oxygen saturation with disposable sensors attached to the forehead which monitors the oxygen saturation of blood flowing to and from the patient's brain. The sensors emit infrared lights and by applying the Beer-Lampert Law of spectrophotometry, an exact value is determined based on the amount of infrared light that the tissue has absorbed. In 2013 CASMED introduced a newer version of this monitor called FORE-SIGHT Elite (FS-E) which has been recently approved for the use in pediatric patients. The purpose of this study is to evaluate the performance, accuracy and user friendliness of these sensors.

This is an ongoing prospective observational study involving ten pediatric (<40 kgs) patients undergoing cardiac surgery under cardiac pulmonary bypass. This study is IRB approved and subjects were consented. All subjects received routine surgical and anesthesia standard of care. Cerebral oximetry sensors were placed appropriately and data was recorded on a research laptop while the patients were undergoing surgery. Venous blood gases (VBG) and arteriole blood gases (ABG) were collected routinely and the results were then compared to the values obtained on the laptop from the FS-E monitor.

The mean age of the study population was 20.8 ± 19.2 months with an average weight of 8.78 ± 5.83 kilograms and 5 (55.5%) subjects were female. The median oxygen saturation (SO_2 (%)) VBG was 79.1 (86.9-96.4) and median cerebral tissue oxygenation ($SctO_2$ (%)) value was 69.8 (63.6-76). When these values were compared, there was an 11.76% difference. Interestingly the SO_2 VBG value when compared to the oxygen saturation in the flank only differed by 3.41%. No skin adverse reactions were reported in any patients.

This intern analysis demonstrated that FS-E cerebral oximetry is an efficient tool to monitor brain and tissue oxygen saturation. However, a larger sample sized study is warranted for a definite conclusion.

A Retrospective Review of "Signet Ring" Features in Cytopathology Specimens with Clinical and Histopathologic Correlation, Montefiore Medical Center (2000-2015)

Yonatan Mehlman[1,3]; Jose G. Mantilla, MD[2]; Sara Maleki, MD[2]; Samer Khader, MD[2]
[1]Yeshiva University, New York, NY; [2]Department of Pathology, Montefiore Medical Center/Albert Einstein College of Medicine, Bronx, NY; [3]Summer Undergraduate Research Program, Albert Einstein College of Medicine, Bronx, NY.

Abstract

Signet ring cells are a well-described histological finding defined by the presence of cells in which the nucleus is pushed by a large cytoplasmic vacuole to the periphery of the cell membrane, hence resembling a "signet ring." Pure signet ring cell carcinoma is a well-defined malignant entity with an associated dismal prognosis. However, the appearance of signet ring cell features in other types of adenocarcinoma, being relatively uncommon, has not been associated with any specific prognostic information. There is only one single prior study correlating the finding of signet ring cells in cytological specimens with their corresponding tissue diagnosis and prognostic implications.

The Clinical Looking Glass (CLG) engine (Emerging Health IT, Yonkers, NY) is a searchable data mining engine that extracts information from hospital information systems. CLG's search features and massive database size establish it as a powerful tool in the construction of retrospective studies. In this study we used Clinical Looking Glass to perform a retrospective review of all cytopathology specimens reported in Montefiore Medical Center from 2000 to 2015 in which the diagnosis contained the term "signet ring," regardless of the site of origin. Based on these results, we extracted and evaluated the demographic information of the patients, the final histological diagnosis, and the survival data.

109 cases were obtained from the initial search for "signet ring" in cytology specimens, showing a marked female predominance: 2.03:1 female:male ratio. The average age at diagnosis was 64 years (standard deviation, ±13.4). Out of all patients, 69 (63%) expired within the first five years from diagnosis (median survival, 92 days; range, 2-1751). Of all cases, 101 (92.7%) were initially interpreted by a cytopathologist as "Positive for malignant cells," while 5 (4.6%) were interpreted as "Suspicious," and 3 (2.8%) as "Atypical."

Cases with initial cytopathology positive for signet ring features were identified (n=101). Within this cohort, the histopathology was analyzed and sorted: 51.5% (n=52) of cases had no available relevant histopathology, 43.6% (n=44) of cases contained signet ring features, and 5.0% (n=5) of cases did not contain signet ring features; cases without signet ring features were manually reviewed to confirm categorization. Thus, ~90% of cases (44/49) with available relevant histopathology confirmed the presence of signet ring cells noted in cytology. The vast majority of the histopathology cases arose in the gastrointestinal tract, followed by mullerian and breast neoplasms, as well as isolated instances of urothelial carcinoma and gastrointestinal stromal tumor (GIST) with signet ring cell features.

Cases with notation of signet ring features in initial cytopathology coupled with signet ring features in histopathology corresponded to a shorter survival time (median survival, 109 days; mean survival, 207 days) as compared to patients with no such signet ring features in histopathology (median survival, 178 days; mean survival 348 days).

The detection of signet ring cell features in cytopathology specimens is generally well correlated with their presence on biopsy or excision specimens (~90%). This indicates a strong correlation between cytological and histological diagnoses of signet ring cell features. The finding of a 2.03:1 female:male ratio is supported by previous literature that notes the prevalence of presentation of signet ring features in females and by a hypothesis suggesting signet ring cell histology may be influenced by sex hormones such as estrogen. Cases positive for signet ring cells in both initial cytopathology and histopathology showed decreased survival time as compared to patients with no histopathological corroboration. However, in our series, a large proportion of cases were observed in body fluids of patients with advanced disease; hence, it is difficult to assess the prognostic significance of these findings without adjusting the survival according to the stage of presentation.

Finally, this methodology of analysis of signet ring features as entities in and of themselves, as opposed to as subcategories of other cancers, is particularly innovative, and opens new angles to understanding this histological diagnosis.

PRMT5-MEP50's Role in Lung Cancer

Ahava Muskat[1]; Hongshan Chen, PhD[2]; David Shechter, PhD2
1. Stern College for Women, 245 Lexington Avenue, New York, NY 10016. 2. Department of Biochemistry, Albert Einstein College of Medicine,1300 Morris Park Ave, Bronx, NY 10461

Our lab studied the effects of PRMT5 and MEP50 on lung cancer cell function, namely metastasis and proliferation. PRMT5 is a type II arginine methyl transferase that catalyzes the symmetrical di-methylation of arginine on many types of proteins. PRMT5 is found in complex with MEP50, a WD repeat protein. MEP50 acts a positive allosteric cofactor for PRMT5 and considerably improves PRMT5's methylation ability. Significantly, PRMT5 is responsible for post-transcriptional methylation of arginine on histone proteins which affects DNA organization, the cell cycle and many other cellular functions.

Most relevant to our research is the PRMT5-MEP50 complex's role in cancer cell proliferation and metastasis. As PRMT5 plays a large role in the cell cycle and proliferation, mutated or misregulated PRMT5 greatly affects cancer cells. Much research has been done showing that over expression of PRMT5-MEP50 is related to cancer and tumorigenesis. Various clinical studies have shown that there is a negative correlation between the expression of PRMT5 in cancer cells and the survival probability of the patient.

There are some studies that indicate that PRMT5 expression affects different types of cancer cells differently. Our objective was to test the role of PRMT5 and MEP50 on highly invasive and proliferative A549 lung cancer cells.

We conducted a series of assays and blots to help us reach this objective. All of the assays and blots that we did were based on the instructions provided by the manufacturer. Firstly, as a positive control, we did a western blot to determine if PRMT5 and MEP50 knockdown cells were actually "knockdown". The results of our western blots showed that the control A549 cells did contain more PRMT5 and MEP50 than the knockdown cells we produced. We also did three western blots that indicated that, compared to the control cells, a lower level of methylated arginine was found on histones 3 and 4 in knockdown PRMT5 cells.

We also conducted a number of functional assays. We localized where PRMT5 and MEP50 are found in cells using immunofluorescence. The immunofluorescence results indicate that PRTM5 is located in the cytoplasm while MEP50 is located right around the peripherary of the nucleus. To test the rate of proliferation of A549, PRMT5 knockdown, and MEP50 knockdown cells, we cultured the cells and counted them over the course of eight days. By the last day we saw that A549 cells had a significantly higher rate of proliferation than the knockdown cells did. We did a colony formation assay (anchorage independent) over the course of 2 weeks and the results indicated that knockdown PRMT5 and MEP50 cells showed less independent colony formation than the control A549 cells. We conducted a series of migration and invasion assays (in matrigel). The results of these assays indicated that migration and invasion of PRMT5 and MEP50 knockdown cancer cells are inhibited when compared to the migration and invasion of A549 cancer cells. This evidence demonstrates the likelihood that PRMT5 and/or MEP50 play a vital role in promoting metastasis of cancer cells. We also prepared a wound heal assay that indicated that PRMT5 and MEP50 knockdown cells migrated significantly less that the A549 cancer cells.

Overall, these results indicated that cancer cells lacking PRMT5 and/or MEP50 are impaired and cannot move or grow as quickly and efficiently as regular cancer cancer cells. In the future, it is important to test regular cancer cells with an inhibitor of PRMT5 and/or MEP50. If the inhibitor produced similar results to the knockdown cells, a possible cancer therapeutic drug could be made specifically targeting PRMT5 and/or MEP50.

Thank you to the entire Shechter Lab for all of your support. Funding is provided by the Student Undergraduate Research Program at the Albert Einstein College of Medicine. Funding is also provided by the NIH Grant ROIGM108646

Characterizing HERG in SH-SY5Y Cells

Veronica Natale[1], Marika Osterbur[2], Dr. Tom McDonald[2,3,4]
[1] Department of Kinesiology at The University of Texas at Austin, Austin, Texas, [2]Department of Molecular Pharmacology, [3]Wilf Cardiovascular Research Institute, [4]Department of Medicine, Division of Cardiology Albert Einstein College of Medicine, Bronx, NY

Background:

The hERG gene encodes the alpha subunit of a potassium channel responsible for the I_{Kr} current. This channel is essential for the maintenance of the cardiomyocyte action potential, and allows for repolarization after a heart beat. When mutated, it can cause a cardiac arrhythmia disorder known as Long QT Syndrome, a genetic disease that can cause syncope, heart palpitations and even sudden death. The McDonald Lab is investigating the role of the hERG mRNA in Long QT Syndrome, and was interested in using human cells that endogenously express the hERG channel. Their current method of analyzing the hERG mRNA involves transiently or stably transfecting HEK cells with hERG DNA. However, to validate the findings from the HEK cell data, we would like to compare our data to experiments done on cells endogenously expressing hERG. One cell line known to express endogenous hERG channels is the SH-SY5Y cell line, derived from neuroblastoma cells.

HYPOTHESIS: Our hypothesis is that the SH-SY5Y cells, a cell line with endogenous hERG expression, will serve as a good model of comparison against the hERG-NT transiently transfected HEK cells.

METHODS:

Cell Culture: SH-SY5Y and HEK cell lines were cultured. **Transfection**: HEK cells were transfected with hERG NT plasmid. **Western**: Western was performed to detect hERG protein levels in HEK cells, hERG-NT transiently transfected HEK cells and SH-SY5Y cells. **Patch Clamping:** The SH-SY5Y cells were patched in a 40 mM KCl external solution by Dr. McDonald to identify the hERG Ikr within the cells. **qPCR:** RNA was isolated from hERG-NT transfected HEK cells, HEK cells and SH-SY5Y cells and then reverse transcribed. Finally, the cDNA was diluted 1:10 and qPCR was performed on the cDNA product.

RESULTS:

The results of the western deemed the hERG protein within the SH-SY5Y cells to be undetectable at high levels unlike the hERG protein in the NT cells. After staining the western a third with sodium potassium ATPase, it showed that there is little to no protein within the sample when compared to the controls, HEK and NT. Results from Patch clamping revealed the presence of hERG protein, but the slight detection of the Ikr verified that the amount is too minute to be of any significance. The qPCR reveals a dramatic difference in the amount of hERG mRNA between the SH-SY5Y cells and the hERG-NT HEK cells.

CONCLUSION:

The western data demonstrating a significant band for the hERG-NT HEK cells, and the lack of band for the SH-SY5Y shows that the hERG protein endogenously expressed in the SH-SY5Y cell line is not expressed at a detectable level. The patch clamping data reveals that the hERG protein is present, but at very low levels of protein expression. All in all, the results from this project have showed that hERG in SH-SY5Y cells are far less than that of the hERG-NT HEK cells. These results have lead us to believe that there is not a robust endogenous expression of the hERG protein in the SH-SY5Y cells, and thus these would not be a good cell line to compare the hERG transfected results from the HEK cells.

Transition-State Analogs as Effective Inhibitors Against *Helicobacter pylori* 5'-Methylthioadenosine Nucleosidase

Angel Payan[1], Brett M. Hirsch[2], Vern L. Schramm[2]
[1]School of Chemistry and Materials Science, Rochester Institute of Technology, Rochester, NY 14623
[2]Department of Biochemistry, Albert Einstein College of Medicine, Bronx, NY 10461

Helicobacter pylori is a gram-negative pathogenic bacteria responsible for ulcer progression and contributing to stomach cancer in humans. We targeted 5'-methylthioadenosine nucleosidase (MTAN) in the futalosine pathway, a critical enzyme in menaquinone metabolism. A potent inhibitor, BTDIA, was previously designed from the MTAN transition state. It is a powerful inhibitor of bacterial growth. This study evaluates a small library of compounds stemming from this initial design. Through in vitro and culture inhibition assays, we compare this new series of potential inhibitors against the lead compound BTDIA. Future studies aim to test other analogs as potential therapeutic agents for *H. pylori* as well as to continue pre-clinical trials with the current library of compounds for treatment of *H. pylori* infections.

High and low dose hydromorphone via PCA pump and intravenous push (IVP) in the control of pain in adult patients with a diagnosis of sickle cell disease (SCD) pain crisis.

PI: Arlette Paul NP, Mentors: Singh Nair MD, Naum Shaparin MD, Department of Anesthesia

Sickle Cell Disease (SCD), a condition that affects the oxygen efficiency of red blood cells, is an inherited condition that causes many patients to experience acute painful episodes, also referred to as painful crises. These are characterized as unpredictable, episodic and unpleasant sensory experiences involving one or more areas in the body. These painful episodes can start in infancy and continue throughout the lifetime of the patient. Opioid therapy is the cornerstone to treat sickle cell crisis. Hydromorphone, a derivative of morphine, is a common treatment for patients with SCD. Its higher potency allows for a smaller daily dose, thereby causing fewer risks for the patients. This study will measure pain relief in adult SCD patients using varying daily doses of hydromorphone. The purpose this study is to evaluate the association between varying daily doses of hydromorphone and various outcome measures.

After obtaining IRB approval, a retrospective chart review was performed on patients admitted from 2011-2014 with the diagnosis of sickle cell crisis. The daily doses of opioids (hydromorphone) administered and pain score were collected from the medical records. Additionally, length of stay, adverse outcomes and baseline demographics were collected from the medical records. The association between the amount of opioids used and the pain scores were analyzed using linear mixed effect regression analysis. Date is presented as mean±SD or median (25^{th}- 75^{th}) percentile as appropriate.

Using the medical records of Montefiore Medical Center, 139 patients were identified who were admitted to the hospital due to sickle cell crisis. From these 139 patients, 1133 admissions were recorded. Of these 139 patients, we analyzed the data of 20 patients consisting of 319 admissions. The average dose of hydromorphone that each patient was administered for the first four days of their admission was 5.12 mg, 9.118 mg, 13.19 mg and 12.82 mg with standard deviations (SD) of 16.09 mg, 11.77mg, 23.03mg and 25.82mg respectively. The average pain score that patients provided in the Emergency Department upon being admitted to the hospital was 8.98 with a SD 1.51. Following admission, the average values of pain scores for all patients for the first three days of their admissions were 6.90, 5.68 and 5.26 with SD of 1.76, 1.88 and 2.05 respectively.

While the average total daily dose of hydromorphone increased as the days progressed, the average pain scores remained very similar throughout the patients' first three days in the hospital. Therefore, the data suggests that increasing the dose of hydromorphone does not lead to an average significant decrease in pain scores. Rather, the disparities between average pain scores in the first three days remains minimal. This is significant because it suggests that patients have been given an increased dose of hydromorphone despite the fact that an increase in dose does not enhance the effects of the medication. Risks of prescribed opioids include vomiting, dizziness, drowsiness, nausea and constipation[1]. It is evident that a smaller dose of hydromorphone is preferable if it provides sufficient pain control for patients with chronic pain disorders. The data therefore suggests that further studies are warranted to verify our findings and also to establish the lowest possible dose of opioids that can control pain in SCD patients.

1. Franklin, G. M. "Opioids for Chronic Noncancer Pain: A Position Paper of the American Academy of Neurology." *Neurology* 83.14 (2014): 1277-284. Web.

NEURON AND SATELLITE GLIAL CELLS IMMUNOSTAINING WITHIN TRANSPARENT INTACT TRIGEMINAL GANGLIA

***Zuleirys Santana-Rodríguez*[1,2,3]**, Matthew F. Challman[1,2], Regina Hanstein[1] and David C. Spray[1]
[1]Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461
[2]Summer Undergraduate Research Program, Albert Einstein College of Medicine, Bronx, NY 10461
[3]Inter American University of Puerto Rico, Arecibo, PR 00612

Chronic orofacial pain is a common clinical problem that is poorly managed. Our studies of a mouse model of inflammatory orofacial pain indicate that chronic changes occur in the trigeminal ganglion (TG). These changes include hyperexcitability of sensory neurons and altered expression and function of gap junction channels in neurons and glia. In TG, sensory neurons are surrounded by specialized glial cells known as "Satellite Glial Cells" (SGCs). Both, SGCs and neurons express gap junction proteins (Connexins, Cxs). These are transmembrane proteins that form channels which allow direct intracellular communication between the cells.

The purpose of this study was to optimize a tissue clearing protocol (Yang et al. 2014) for TG whole tissue staining, and to quantify Cx protein expression in mice with inflammatory orofacial pain compared to controls.

Confocal TG images were obtained using a Leica SP5 Acousto-Optical Beam Splitter (AOBS) confocal microscope with 405nm, 488nm and 594nm lasers, and 20X air objective. Images were processed and quantified using Imaris64x, Image J and Photoshop CS5 programs.

We effectively optimized the clearing protocol from Yang, et. al 2014 by adding extra tissue washing steps (For details see Methods section on poster). The analysis of TG anatomy after fixation and clearing showed that neuronal morphology did not change as a result of clearing and fixation. We optimized staining procedures for detection of neurons with anti-NeuN or anti-Cx36 antibody, as well as SGCs with anti-glutamine synthetase or anti-Cx43 antibody. For all primary antibodies used incubation at 37°C for 3 days improved staining quality, either by reducing background or facilitating antibody penetration. Double Staining of TG using anti-Cx43 and anti-Glutamine Synthetase demonstrated overlapping expression of these two proteins at margins of SGCs, neither was detected in neurons. By contrast, Cx36 was detected in neurons, but not SGCs.

Cx43 staining of TG from mice with CFA-induced inflammatory pain showed an upregulation in CFA treated TG in comparison with the controls. We are currently exploring changes in expression of other Cx proteins in pain.

This project was supported by the NIH grant NS092466 and the Summer Undergraduate Research Program. We gratefully acknowledge the advice and assistance of Marcia Urban-Maldonado and Laura de Menezes.

Abstract: Validation of the Focus Simplexa™ Herpes Simplex Virus 1 & 2 Direct PCR Assay for CSF Specimens

Daniel M. Sapozhnikov[1], Michael H. Levi, Sc.D.[2]

[1]McGill University, Department of Biology, [2]Montefiore Medical Center, Department of Pathology

Rapid diagnosis of Herpes Simplex Virus (HSV) encephalitis/meningoencephalitis is important in patient management. The Focus Simplexa™ HSV 1 and 2 assay (FSA) is the first FDA-approved assay for use with cerebrospinal fluid (CSF) specimens. This assay is designed to detect both HSV 1 and 2 in a ~70 minute assay and does not require manual nucleic acid extraction. Before implementing such an assay for clinical use, it is necessary to perform a validation to ensure sensitivity, specificity, accuracy, and precision. To this end, we subjected the FSA to a series of tests. The FSA demonstrated 100% sensitivity with 16 samples that were previously confirmed positive by the Montefiore Medical Center (MMC) molecular laboratory. The FSA also exhibited 100% specificity with 40 previously negative samples from the same laboratory. In addition to the extensive specificity testing provided by the manufacturer, we performed similar analyses with several of the major bacterial, fungal, and viral agents of encephalitis/meningoencephalitis. Again, the FSA detected no false positives for HSV 1 or 2. To investigate the assay's accuracy, ten negative CSF specimens were inoculated with either HSV 1, 2, or nothing by the MMC virology laboratory in a manner such that the tester did not know what each specimen contained. In this case, all results were correct as confirmed by the MMC virology laboratory. A specimen containing each HSV was also tested for three consecutive days and the FSA showed similar results over the three days. To meet the College of American Pathologists (CAP) requirements for PCR assays with internal negative controls, a positive and negative control were run for 20 days, during which no problems were encountered. In addition to the standard validation requirements, we implemented a number of tests to analyze the efficacy of the FSA. We determined the assay's limit of detection (LOD) by performing serial dilutions of inactivated whole HSV 1 and 2 viruses from Zeptometrix. The FSA displayed a 100% detection rate down to 100 copies/mL and was able to detect as low as 10 copies/mL in three of five attempts. However, we determined the LOD to be ~30 copies/mL as the FSA detected the viruses 15 out of 16 times. The LOD proved to be below the viral load that is typical for HSV central nervous system disease. We also tested possible contaminants of CSF specimens – chlorhexidine, isopropyl alcohol, and blood – and found no significant inhibition of the FSA's ability to detect HSV 1 or 2. Additionally, the FSA also demonstrated similar results whether CSF specimens were stored at -20 ^{o}C, 2-8 ^{o}C, 25-27 ^{o}C, and 37 ^{o}C for a period of 10 days. Finally, several specimens containing HSV 1 and 2 were tested, boiled to inactivate the virus, and re-tested with similar results prior to and after boiling. Ultimately, the FSA has proven to be a simple, sensitive, and accurate assay with an LOD which is below the viral load expected in encephalitis/meningoencephalitis. The assay was specific for HSV 1 and 2 and robust in the sense that it was not inhibited by a number of interfering agents or temperature conditions.

Identification of novel HSPC and hemogenic endothelial markers in zebrafish

Aakash Shah[*], Rosannah C. Cameron[**], and Teresa V. Bowman**
[*]Bucknell University, Lewisburg, PA
[**]Department of Developmental and Molecular Biology, Albert Einstein College of Medicine, Bronx, NY
Program: SURP

Hematopoiesis, the production of blood cells, has long been studied in the model organism *Danio rerio* (zebrafish), providing critical information on the emergence of multi-lineage hematopoietic stem and progenitor cells (HSPC's) early in development. The existence of hemogenic endothelial cells, a subset of endothelial cells with the potential to give rise to HSPC, was previously demonstrated in zebrafish using time-lapse imaging and fate mapping studies. Despite the knowledge that hemogenic endothelial cells exist during development, we currently lack robust markers to uniquely identify and label these cells in zebrafish. To Identify candidate genes that might distinguish hemogenic endothelial cells from HSPCs, we examined gene expression data from purified murine endothelial and hemogenic endothelial populations as well as HSPCs. Based on differential expression between hemogenic endothelial cells and HSPCs, we selected five genes to test by *in situ* hybridization in zebrafish including *nfix, hlf, mpl, zfhx3,* and *sox17.* The *nfix* and *hlf* genes are duplicated in the zebrafish genome, so we will be testing both genes (*nfixa* and *nfixb)* and *(hlfa* and *hlfb).*In order to carry out this study, we first needed to clone the open reading frame for these genes into plasmids that can be used for *in vitro* transcription. We first extracted mRNA from embryos to create cDNA and then used this cDNA to amplify genes of interest using reverse transcription PCR (RT-PCR). PCR products were then cloned into the pCRII vector, which contains SP6 and T7 primer sequences that can be used for *in vitro* transcription. We have successfully cloned *hlfb* and will continue to work on the other genes. The ultimate goal is to generate digoxygenin (DIG) RNA probes for each of the genes and determine their pattern of expression in 24 hours post fertilization zebrafish embryos. If successful, hematopoietic researchers can utilize these new markers to study the endothelial- to hematopoietic transition (EHT) through which an HSPC is born. Insight into that process could have important implications on the generation of HSPC from pluripotent embryonic stem cells.

I would like to thank everyone in Dr. Bowman's lab for providing guidance and an exceptional learning opportunity. Although I was fairly new to laboratory research, at the end of the two months, I have gained an extensive tool kit comprised of new skills especially that of a critical mind. I would like to give a special thanks to Dr. Rosannah Cameron; you were my first lab mentor and in the midst of your research you gave me knowledge and your patience. Lastly, I would like to express my gratitude to Dr. Teresa Bowman. Despite my missteps and errors (especially in the beginning), Dr. Bowman remained cool and positive. While she did teach me an incredible amount, she also helped me develop an analytical mind pushing me to understand mechanism and the "WHY" question. However unrefined, I will continue to utilize these skills as I continue on my journey. Thank you.

Radioimmunotherapy with ^{225}Actinium Shows Promise

Michael Shavolian[1], Abdullah Norain[2], Ruth Bryan[2], Zewei Jiang[2], Ekaterina Revskaya[2] and Ekaterina Dadachova[2]

[1]Summer Undergraduate Research Program; [2]Albert Einstein College of Medicine, Bronx, N.Y.

Abstract

Recent biomedical research has demonstrated the newly discovered efficacy of α-emitting RIT (radioimmunotherapy) in targeting leukimas, which exhibit more radiosensitivity than their solid counterparts. In particular, RIT using ^{225}Ac has shown potential as an alternative to full-body myeloblative radiation as a conditioning regimen prior to hematopoietic stem cell transplantation. CD45 is a pan-leukocytic antigen widely expressed on white blood cells to the measure of more than 10^5 sites per cell to which BC8, an immunoglobulin G monoclonal antibody, binds effectively. BC8 monoclonal antibody can be radiolabeled with ^{225}actinium using chelating agent DOTA in order to deliver theranostic doses of radiation. In this experiment we aimed to evaluate the efficacy of conjugation, labeling and immunoreactivity as well as biodistribution of the promising BC8-DOTA-^{225}Ac complex.

Both BC8 and 18B7, a control which binds to a polysaccharide in C. neoforman, were conjugated and labeled. A series of assays was carried out to identify the efficacy of the conjugation and labeling as well as the immunoreactivity and subsequent biodistribution of the radiolabeled antibody in healthy mice. We were able to achieve nearly 50% labeling efficacy, as demonstrated by High Performance Liquid Chromatography, and showed by flow cytometry that conjugated BC8 results in a significantly lesser degree of immunoreactivity than BC8 alone. While 18B7 exhibits negligible median fluorescence intensity, an immunoreactivity assay for the same MAb yielded a percent bound value of 19.4% of the labeled MAb. These, apparently confounding, results may be due interactions between the secondary antibody and the DOTA-MAb complex. Instant Thin Layer Chromatography yielded final purity of 78% for the sample utilized in the biodistribution.

We conclude that the absence of cancer in the healthy murine models may have led to a higher radioactivity biodistribution in the tested organs. Additionally, the immunoreactivity of the radiolabeled MAb tested before hand was below the ideal 90%. Lastly, this experiment allows the future calculation of effective dose per organ to assess the maximum theranostic dose of this promising complex.

Engineering Cross-Neutralizing Antibodies that Target Multiple Filoviruses

Maryam S. Waris, Elisabeth K. Nyakatura, Daniel Hofmann, and Jonathan R. Lai*
Department of Chemistry and Biochemistry, Arizona State University, Tempe, AZ 85287 and *Department of Biochemistry*, Albert Einstein College of Medicine, Bronx, NY 10461

Filoviruses Marburg (MARV), Ravn (RAVV), and five species of Ebola: Zaire (EBOV), Sudan (SUDV), Bundibugyo (BUDV), Tai Forest (TAFV), and Reston (RESTV) are all causative agents of severe and fatal hemorrhagic fever. Recently, the filovirus outbreak has reached an unprecedented scale in both magnitude and geographic spread, and with no commercial vaccines or antiviruses approved to treat the disease, the need for broadly effective treatment options is greater than ever. Thus far, mainly monospecific antibody cocktails have been shown to protect against EBOV in non-human primate studies. We intend to design bispecific (bsAbs) and trispecific (tsAbs) antibodies that can cross-react with and cross-neutralize multiple different filovirus strains, aiming to improve post-exposure therapeutics in cases where the infecting species of virus is unknown. Our results show the bsAbs and tsAbs generated exhibit promising cross-binding activity in ELISA binding assays using EBOV, SUDV, and MARV glycoprotein (GP) targets. Furthermore, colocalization of the antibodies and viral particles was confirmed via fluorescence microscopy. These results favor performing imminent neutralization assays to determine if the antibodies can successfully inhibit infection, ultimately leading us down the road to creating broad-spectrum antibodies that have the potential to revolutionize filovirus treatment and the field of immunotherapy.

EXCITATORY/INHIBITORY BALANCE IN AN ENDOCANNABINOID SENSITIVE CIRCUIT

Amy E. Wells[1], Kyle R. Jensen[2], and Pablo E. Castillo[2]

[1]Department of Neuroscience, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061 [2]Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461

Memory and learning are thought to occur by activity-dependent changes in synaptic strength, known as plasticity. In the hippocampus, synaptic plasticity has been extensively characterized at glutamatergic synapses that relay information to and from each of the three major hubs of the trisynaptic circuit. Much less is known about synaptic plasticity within the hetero-associative circuit of the dentate gyrus (DG), a circuit that is proposed to be important for the generation of associative memories, pattern separation, and conjunctive encoding. In the DG, hilar mossy cells (MCs) and CCK^+ GABAergic interneurons (INs) form synapses with dentate granule cells (DGCs) in the inner molecular layer (IML). Notably, the IML has one of the highest levels of the type 1 cannabinoid receptor (CB1Rs) in the entire brain. CB1Rs are metabotropic receptors that can powerfully modulate synaptic transmission in both a long- and short-term fashion. Intriguingly, CB1Rs are expressed at presynaptic terminals of both MCs and CCK^+ INs. Our lab has evidence that endocannabinoid (eCB)-mediated long-term plasticity does not occur at MC-DGC synapses, but it is still unknown whether inhibitory synapses onto DGCs can be modified by eCBs in a long-term fashion. To test the possibility that inhibitory transmission onto DGCs can be modulated by eCBs, we performed extracellular field recordings of evoked postsynaptic inhibitory field potentials (eIPSPs), at IN-DGC synapses of rat hippocampus. We found that application of the CB1R agonist WIN (5uM) and the group I mGluR agonist DHPG (50uM) each caused robust long-term depression of inhibitory transmission (iLTD) at the IN-DGC synapse. This is the first evidence of eCB-mediated iLTD at this synapse. It is an important find because this form of iLTD could dynamically shift the excitatory/inhibitory (E/I) balance onto DGCs in favor of excitation, which could therefore alter DGC output and profoundly affect dentate-dependent computations. Current experiments are being performed to determine: 1. The mechanisms of this iLTD 2. If this form of iLTD can be synaptically induced, and 3. If this form of plasticity can alter DGC output by shifting the E/I balance onto DGCs. The preliminary experiments shown here depict a form of plasticity that could powerfully regulate DGC output and could play a role differentially affecting dentate-dependent processes such as pattern separation.

$Gas6^{-/-}Axl^{-/-}$ mice demonstrate fewer oligodendrocytes in response to cuprizone toxicity and a reduced immune response during MOG-induced EAE.

Julie Williamson[1,2,3], Ross Gruber[1], Alex Ray[1], and Bridget Shafit-Zagardo[1]
[1] Department of Pathology, Albert Einstein College of Medicine, Bronx, NY 10461.
[2] Emory University, Atlanta, GA, 30322.
[3] Summer Undergraduate Research Program, Albert Einstein College of Medicine, Bronx, NY 10461.

MS is a disease of unknown etiology in which the body's immune cells target myelin, the protective and insulating coating of nerves in the CNS. The resulting CNS damage causes wide-ranging symptoms, including fatigue, muscle weakness, numbness and paralysis in the estimated 2.3 million people affected by the disease worldwide. Several animal models of demyelinating diseases aid in the study of MS, including myelin oligodendrocyte glycoprotein (MOG)- induced experimental autoimmune encephalomyelitis (EAE) and cuprizone induced demyelination. EAE is the most widely used model of MS, as it is characterized by pathological similarities including infiltrating CNS inflammation, axonal damage and demyelination. The cuprizone model is useful for understanding demyelination and the remyelination process in the absence of infiltrating T cells observed in the EAE model. Cuprizone introduced to the diet for 4-6 weeks kills mature myelin-producing oligodendrocytes, causing demyelination and activated glia, microglia, and astrocytes in the corpus callosum. By week 6, remyelination begins even in the presence of cuprizone.

Gas6 is the sole ligand for the Axl receptor, a member of the TAM tyrosine kinase receptors shown to have anti-inflammatory and pro-myelinating effects. Previous research demonstrates that $Gas6^{-/-}$ and/or $Axl^{-/-}$ single knockout mice undergo more severe demyelination and more inflammation than WT mice in response to EAE. Additionally, delivery of Gas6 to the CNS dampens the immune response and improves the clinical outcome of this disease. Furthermore, cuprizone fed $Axl^{-/-}$ and $Gas6^{-/-}$ single knockout mice exhibit a lag in debris clearance, contributing to their delay in recovery upon cuprizone withdrawal.

Earlier studies focused on single knockout mice (e.g. $Axl^{-/-}$ or $Gas6^{-/-}$). However, in order to completely silence this pathway, we created a $Gas6^{-/-}Axl^{-/-}$ double knockout mouse (DKO). Here we show preliminary results using DKO mice in an animal model of MS as well as in the cuprizone model of demyelination/remyelination. When sensitized with MOG peptide, DKO mice display an atypical EAE disease course, noted by the significant lag in both disease onset and recovery compared to WT mice. In one experiment, the DKO mice show similar peak disease relative to the WT, unlike both single knockout mice. Levels of pro-inflammatory (IL-2, IFNγ, and IL-17) and anti-inflammatory (IL-4) cytokine mRNA are noticeably lower in lymph nodes isolated from DKO mice during the initiation of the disease, indicating a reduced immune response following MOG sensitization. However, following cuprizone toxicity, DKO mice show significantly fewer oligodendrocytes in the corpus callosum at 5 weeks cuprizone treatment, with little to no remyelination apparent at 6 weeks, indicating a lag in remyelination. Ongoing studies will examine the time points beginning 1-, 2- and 3-weeks post-cuprizone withdrawal to determine whether DKO mice remyelinate in a timely manner. This demonstrates that Gas6-Axl signaling may play a crucial role in the disease onset and recovery from pro-inflammatory injury and demyelinating disease, and suggests that this signaling pathway may play different roles in regulating the peripheral immune response, and in myelinating cells of the CNS.

TARGETING BCL-2 FAMILY MEMBERS TO TREAT HEAD AND NECK SQUAMOUS CELL CARCINOMA

Kathryn Wilson[1,7,8], Carlos Thomas[1], Alan Alfieri, MS [2], Michael B. Prystowsky, MD, PhD[1], Thomas J. Belbin, PhD[1], Nicole Kiwachi[1], Evripidis Gavathiotis, PhD[4,5],
Chandan Guha, MB,BS, PhD[1,2,5], Thomas J. Ow MD[1,6]
[1]Department of Pathology*, [2]Department of Radiation Oncology*,
[3]Department of Biochemistry*, [4]Department of Medicine (Cardiology)*, [5]Department of Urology*,
[6]Department of Otorhinolaryngology, Head and Neck Surgery, Montefiore Medical Center/Albert Einstein College of Medicine
[7]Ferris State University, Big Rapids, MI 49307, [8]Summer Undergraduate Research Program*
*Albert Einstein College of Medicine, Bronx, NY 10461.

Apoptosis involves two pathways, the extrinsic and intrinsic pathway. For this project, the intrinsic pathway of apoptosis was investigated. The BCL-2 family of proteins is one of the key factors of the intrinsic pathway. Two family members were specifically targeted for this study: BAX, a BH1-4 domain protein, and BCL-2, a BH1-3 domain protein. For this project it was hypothesized that targeting BCL family proteins will lead to significant cell death in HNSCC cells.

A Cell Viability Assay was performed on four cell lines of the Head and Neck Squamous Cell Carcinoma: HN5, HN30, MDA686 TU, and UMSCC6. These cell lines were treated with two different drugs. The drugs included, Navitoclax, a high sensitive inhibitor of anti-apoptotic BCL-2 family members including BCL-2, and BAM38, an activator of a pro-apoptotic BCL-2 family member, BAX. The cells were exposed with either Navitoclax or BAM38 at concentrations that were prior determined to induce response in a MTT Assay. These cells were harvested after 24 hour and 48 hours of exposure and stained with Trypan Blue, and counted for viability. Some resistance to BAX activation was noted at 24 hours but appeared to be overcome at 48 hours. Relative resistance to BAM38 was recorded, correlating to low baseline BCL-2 expression.

An Annexin A5 Assay was completed, using these two drugs and HN5 and HN30. The cells were exposed to Navitoclax and BAM38 at the appropriate concentrations. The cells were then harvested after 24 hours, and quantified on a flow cytometer. There was a general increase of late and early apoptosis measured for each cell line, treated with Navitoclax or BAM38.

Finally, the four cell lines were treated with the appropriate concentrations of Navitoclax or BAM38 and harvested after 24 hours of exposure. A Caspase Cleavage Western Blot was performed using Caspase 3 and Caspase 9 antibodies. These two caspases are vital to the occurrence of apoptosis. Cleavage of Caspase 3 and 9 was present in all cell lines. UMSCC6 showed resistance to Navitoclax despite evidence of caspase cleavage when the drug was present.

Based on the results from the Cell Viability Assay, Caspase Cascade and Annexin V Assay there is not a definitive advantage between Navitoclax and BAM38. However, each drug does have a positive effect on activation of apoptosis for each of the investigated HNSCC lines.

Further studies will further characterize apoptosis signaling molecules as biomarkers of response or mediators of resistance.

Effects of Optogenetic Activation of Ventral Tegmental Area Dopamine Neurons During a Cued Reward-Seeking Task

By: Mohammed Yaseen, Cindy Reyes and Saleem Nicola
Summer Undergraduate Research Program and Neuroscience Department of Albert Einstein College of Medicine, Bronx, NY

The nucleus accumbens (NAc) and its dopaminergic (DA) innervation from the ventral tegmental area (VTA) are involved in promoting reward-seeking behavior. When an animal initially receives a reward, there is firing in the NAc as well as DA release. Over time, when a cue is presented preceding a reward, DA release and firings occur in conjunction with the cue. This suggests that NAc DA can play a role in reinforcement by strengthening the association between cue and reward as well as promote reward seeking behavior. Investigation in our lab has provided evidence to support this reward seeking hypothesis. Many NAc neurons are excited by cues that predict reward and these excitations occur before movement onset. Use of DA antagonists injected into the NAc has shown a decrease in response to the cue as well as decreasing NAc cue evoked firing. While these studies strongly support DA activating reward seeking by increasing cue-evoked firing it is not known if activation is sufficient to increase cued approach behavior.

This study aims to investigate the sufficiency of DA neuronal activity for cued approach behaviors and reinforcement. We hypothesize that DA neuronal activity is sufficient to increase cued approach to reward, and that DA neuronal activity during reward presentation is sufficient for reinforcement during cued tasks.

We targeted dopaminergic projections by injecting *TH:Cre* rats with a double floxed AAV vector containing channelrhodopsin-2. Then we implanted optic fibers aimed at the VTA, which allowed us access to selectively activate dopaminergic neurons. This will allow us to investigate the sufficiency of dopamine release in the NAC for cued approach and reinforcement in an operant task. We then trained the *TH:Cre* rats to respond to certain condition stimulus (*CS)* task, where one cue (CS+), indicates the availability of a liquid sucrose reward, and the rat makes a nose poke to obtain the reward. The other cue (CS-), is a neutral, non-rewarded cue. Once trained, animals will be photo stimulated during the task at different time points. This will allow us to investigate the role of DA in cued reward seeking and reinforcement.

Our preliminary data shows that during extinction (i.e. when sucrose is no longer delivered), substituting photo stimulation for sucrose is sufficient to prevent the decline in behavioral responding normally caused by extinction. This result suggests that dopamine neuronal firing plays a role in reinforcement of behavioral responding.

Acknowledgements:

We would like to thank the Nicola lab for all their help and support. Additionally we would like to thank the SURP Program and CSTEP. Funding for this project was provided by NIDA, NIMH, NARSAD and the Klarman Foundation.

Title: Nrf-2 and TNF-α modulate *P. falciparum* sequestration to brain endothelial cells

Authors: Jenny Zheng[1,2], Neida K. Mita-Mendoza[2], Catherine Feintuch[2], Karl B. Seydel[3], Terrie E. Taylor[3], Johanna P. Daily[2]

Department:

[1]Cornell University, College of Agriculture and Life Sciences, Ithaca, NY, 14850, United States

[2]Albert Einstein College of Medicine, Bronx, NY, United States

[3]Blantyre Malaria Project, Blantyre, Malawi

Program: Summer Undergraduate Research Program

Abstract:

Malaria remains a significant global issue, with 584,000 deaths estimated in 2013. Cerebral malaria (CM), a severe complication of *Plasmodium falciparum* malaria is associated with high rates of mortality and neurological complications. Infected Red Blood Cells (iRBCs) sequester to brain endothelial cells and this phenomenon underlies the neuropathogenesis of CM. Testing *in vivo* identified putative modulators of iRBC sequestration to brain endothelial cells is the goal of this project. In a recent study, we identified an association of activated neutrophils with iRBC sequestration in pediatric CM. In contrast, the Nuclear Factor Erythroid 2-Like 2 (Nrf-2) pathway was associated with protection from iRBC sequestration. Prior studies have reported that TNF-α, a product of activated neutrophils, increases ICAM-1 expression and iRBC cytoadherence to human brain microvascular endothelial cells (HBMVEC). The Nrf-2 pathway provides cellular resistance to oxidative stress and toxins and has an anti-inflammatory effect. Furthermore, upregulation of the Nrf2 pathway can directly modulate endothelial cell biology by increasing the surface receptor expression of MCP-1 and VCAM-1. In this study, we evaluated the effects of activated neutrophils, TNF-α and dimethyl fumurate (DMF) which upregulates the Nrf-2 pathway, on iRBCs sequestration to HBMVEC. We pretreated HBMVECs with Phorbol Myristate Acetate-activated neutrophils, 20ng/ml TNF-α, 50uM DMF, or a combination of both prior to iRBCs cytoadherence in a semi-static binding assay in 8-well chambered slides. We determined that activated neutrophils increase parasite sequestration in a subset of strains and TNF-α consistently increase parasite sequestration. In addition, in one laboratory strain (ITgICAM-1), DMF negated the effects of TNF-α and reduced parasite sequestration down to basal levels. This suggests that the effects of DMF through the Nrf-2 pathway may have potentially protective effects against high levels of parasite sequestration for parasites that bind to endothelial cells surface ICAM-1. Further studies will be performed to characterize the effects of DMF on endothelial cell surface receptor expression over a time course as well as to identify the optimal exposure time to upregulate the Nrf-2 pathway in HBMVECs prior to the cytoadherence of iRBCs. Additional studies can characterize and determine if other activated neutrophil products have similar effects on parasite sequestration and if there are potential therapeutics to provide protection against iRBC sequestration to the endothelium.

Acknowledgements

We thank the Blantyre Malaria Program for the Malawi isolates (3173 and 3005) and Joseph Smith for the lab lines (ITv19 and ITgICAM-1). This work was supported by the Einstein Summer Undergraduate Research Program (SURP). Lastly, I thank the Daily lab for all your help this summer and opening your arms to me so readily. It has been great getting to know all of you!

Preliminary evidence for neural processing of task-irrelevant streams in complex auditory environments

Juin-Wan Zhou[1], Sally Cole[2], Renee Symonds[3], Elyse Sussman[3]

[1]Department of Physics and Engineering Physics, Fordham University — Bronx, NY
[2]Department of Science, Math, and Computing, Bard College — Annandale-on-Hudson, NY
[3]Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine — Bronx, NY

The auditory system is constantly bombarded with sound, and yet is able to differentiate one sound stream from the mixture of streams that enters the ears. This process is called auditory scene analysis (ASA). To what extent the background sound is processed is not fully known. It is unclear whether the auditory system segregates the unattended background sounds into distinct streams or if it is undifferentiated noise. Recent studies using only frequency as the differentiating cue have shown stream segregation, but with limited processing. We hypothesized that strengthening streaming by characterizing sounds through multiple cues: location, sound envelope, sound type, and frequency would reduce the attentional load and allow more resources to be available to process unattended streams. Subjects were presented with auditory stimuli while EEG was recorded. Processing of unattended streams was indexed using the mismatch negativity (MMN) component of event-related potentials (ERP). The presence of MMN in unattended streams provides preliminary evidence that streaming occurs, and demonstrates further processing of task-irrelevant streams. The results of this study can be applied to future research methods regarding ASA, and provide a better understanding of the process. The findings of ASA studies can be applied to clinical settings in improving the quality of life in patients with auditory processing disorders, autism spectrum disorders, and age-related hearing loss.