

SUMMER TRAINING AS RESEARCH SCHOLARS (STaRS) PROGRAM

RESEARCH SYMPOSIUM



THURSDAY, AUGUST 7, 2014



2014 Summer Cohort: Jewel Williams, Gregory Patterson, Mayra Cruz, Xavier De Luna, Tyler Pluchino, Niyoyankunze Noel, Briana Toatley, Lynisha Farrell, Brittany Thompson, Kailah Simon, Diego Escobar Garcia, Sydnie Turner, L'Ierret Ailith, Jaileene Hernandez, Sean Munier, Chino Igwebuike, Jennifer Leahy.

Division of Graduate Medical Sciences Summer Training as Research Scholars (STaRS) Program Research Symposium 2014

Welcome to the 5th Annual Summer Training as Research Scholars (STaRS) Research symposium, hosted by the Division of Graduate Medical Sciences, for students who are traditionally underrepresented in the biomedical sciences. Our academically talented students were selected for STaRS through a highly competitive application process and have spent the summer conducting research in labs across the BU Medical Campus. We hope you enjoy learning more about their summer research projects.

11:00 – 11:15 am	Welcome Dr. Karen Antman, MD Dean, Boston University School of Medicine Dr. Linda Hyman, PhD Associate Provost, Division of Graduate Medical Sciences Dr. William Cruikshank, PhD Director, Summer Training as Research Scholars (STaRS) Program	
11:15 – 12:15 pm		
	Jaileene Hernandez	Jewel Williams
	Sean Munier	Gregory Patterson
12:15 pm	Lunch	
12:15 – 1:15 pm	Poster Presentations	
	L'lerret Ailith	Niyoyankunze Noel
	Mayra Cruz	Tyler Pluchino
	Xavier De Luna	Kailiah Simon
	Diego Escobar Garcia	Brittany Thompson
	Lynisha Farrell	Briana Toatley
	Chino Igwebuike	Sydnie Turner
	Jennifer Leahy	
1:15 – 2:00 pm	Presentation of Certificates & Closing Remarks	
	Dr. Linda Hyman, PhD	
	Associate Provost, Division of Graduate Medical Sciences	
	Dr. William Cruikshank, PhD	
	Director, STaRS summer research program	

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L'Ierrét Ailith Xavier University of Louisiana

NOTCH Gene Targeted Airway Cell Differentiation and Proliferation in Mutated Mouse Models

PI: William Cruikshank PhD Mentor: Nathalie Ahyi

Boston University Medical Center, Pulmonary Center

The onset of asthma development begins in the early stages of life when the body is adjusting to its new environment. To study this phenomenon, my lab has specifically focused on airway epithelial cells in mouse models. The notch signaling pathway is key in cell specification and differentiation and closely linked to the growth and development of tracheal and alveolar epithelial cells. In the past, my lab has been able to construct mutated alleles for the notch1 gene and breed mice that have 32 bp tags surrounding the coding nucleic acids. A secondary gene, cre, can be induced to link to these tags and excise the coding DNA leaving the cell incapable of transcribing the full notch1 sequence. Past research has also found that children exposed to cockroach allergens during their toddler stage are predisposed to developing asthma as they grow older. My work involves analyzing in vivo and in vitro data of airway cells and organs to determine whether or not cockroach allergens do in fact interact with the notch gene and alter it in any way making it incapable of coding; and if so, is it dependent on the gene to play a role in asthma development? The in vitro study measures cytokine production variation with tracheal epithelial cells exposed to cockroach or not and involves cell staining to directly view cell differentiation. The in vivo aspect studies variation of airway function with mice exposed to cre and also determines how cockroach allergens having been introduced through the nasal airway over a period of time affect breathing ability in young mice.



Mayra Cruz Queens College, City University of New York

<u>Ultra Structural Effects of Glomerular Capillary Hypertension on Podocytes and Other Glomerular Structures in</u> <u>the Absence of Myh9 Expression</u>

Mentor: Dr. Joel Henderson MD, PhD

Boston University Medical Center, Department of Pathology and Laboratory Medicine Additional Author: Philip Bondzie PhD, Hui Chen MD

Introduction: Glomerular capillary hypertension (GCH) develops in response to any kidney injury associated with loss of functional kidney mass, and causes further progression of kidney damage and ultimately chronic kidney disease (CKD). CKD left untreated can worsen cardiovascular disease, and lead to end stage kidney disease and death. Previous studies have shown that renal susceptibility gene Myh9 encodes an enzyme called non muscle myosin IIA (NMIIA), which is expressed highly in podocytes found within the glomeruli. We have shown that NMIIA is responsible for podocyte contractility, which appears to protect the glomeruli from GCH. When this renal gene is absent, the glomerulus is vulnerable to the effects of GCH, and there is rapid reduction in the function of the kidney, including increases in proteinuria and urinary nephron excretion. Severe glomerular injury is also seen histologically, but the extent of injury to the podocytes specifically and other glomerular structures have not been fully characterized. The intent of this study is to determine the ultrastructural effects of GCH on podocytes and other glomerular structures in the absence of Myh9 expression.

Methods: Podocyte specific Myh9 knockout mice were generated, along with 3 control genotypes, and all were subjected to uninephrectomy and deoxycorticosterone acetate (DOCA) – salt treatment, which is a model of glomerular capillary hypertension. After 6 weeks of uninephrectomy and deoxycorticosterone acetate (DOCA) – salt treatment, kidneys were harvested and processed for electron microscopy. Three glomeruli from each specimen were examined under the electron microscope. A general analysis of morphologic features of glomerular injury was performed, including semi-quantitative scoring of ultrastructural features. A more precise measurement of podocyte foot process effacement, a key feature of podocyte injury, was also performed by quantifying average foot process width in each specimen.

Results: The ultrastructural analysis showed that Myh9 knockout mice exhibited more severe podocyte injury, including and increased extent of foot process effacement, microvillous degeneration, and vacuolization. There was also an increase in features of endothelial injury including cell swelling and loss of fenestrations. Quantification of foot process effacement showed a trend toward increased foot process effacement in Myh9 knockout mice that was not statistically significant.

Conclusion: This study showed that in the absence of Myh9 expression, the uninephrectomy DOCA-salt model of GCH resulted in significant glomerular injury at the ultrastructural level including podocyte foot process effacement and irregularity and endothelial injury, which was more severe than that seen in control mice. This suggests that Myh9 and NMIIA are important for the protecting the glomerulus and for maintaining podocyte structure in the presence of CGH. With this improved understanding of how Myh9 influences renal function, we will better understand the factors that contribute to chronic kidney disease, and may find new ways of treating CKD patients.



Xavier De Luna University of Texas at San Antonio

Detection of Kidney Endoplasmic Reticulum Stress Markers after Shiga Toxin 2 Challenge

PI: Deborah Stearns-Kurosawa PhD Mentor: Caitlin S.L. Parello

Boston University Medical Center, Department of Pathology

Significance: Hemolytic uremic syndrome (HUS) is a potentially lethal complication of infection with Shiga-Toxin (Stx) producing enterohemorrhagic *E. coli* (EHEC). HUS is clinically defined as having thrombocytopenia, microangiopathic hemolytic anemia, and thrombotic microangiopathy, resulting in kidney failure. Production of either Stx1, Stx2 or both defines the bacterium, and production of Stx2 is associated with worse clinical outcome.

Objective: Based on previous *in vitro* data, we hypothesized that the toxins cause apoptosis and endoplasmic reticulum (ER) stress responses in kidney cells which contributes to kidney failure. This hypothesis is supported by mRNA analysis of key ER stress and apoptotic markers CHOP, Bcl2 and DR5, but has not yet been validated with the translated proteins.

Methods: To address this, kidney samples from mice previously challenged with Stx2 were assayed to determine when and how much of the ER stress marker CHOP was present by immunoblotting and microdensitometry. Tunicamycin or media challenged RAW cells were used as positive and negative controls, respectively. Briefly, challenged cells or previously flash frozen kidney was lysed using the Qiagen Tissue Lyser II at 25 Hz for 4 minutes. Cell number or sample mass was compared to previously generated standard curve to determine protein content, and ~100 ug of total protein per sample was subjected to protein gel electrophoresis at 180 V. Following transfer to a PVDF membrane at 30 V and blocking for 1 hour, and washing membrane was incubated with rabbit monoclonal anti-mouse CHOP at 4°C overnight. After washing, membrane was incubated with goat anti-rabbit IgG conjugated to IRdye800 for 1 hour at room temperature, washed again, and analyzed on Odyssey Infrared Western Blot Imaging System.

Results: No evidence of CHOP protein was observed by western blot in kidney samples from animals challenged with either saline or Stx2 at euthanasia endpoint (Fig. 1).

Conclusions: The lack of CHOP protein in Stx2 challenged samples contrasts with mRNA data demonstrating up-regulated CHOP mRNA. It is important to note that, at euthanasia endpoint, kidney CHOP mRNA expression is variable. It is thus possible that, due to this variability, western blotting is not a sensitive enough technique to see CHOP protein in these samples. Even in the positive control, which contains a single, tunicamycin sensitive cell type, the CHOP band is light despite loading 100 ug of total cell protein onto the gel. Therefore, we suspect that the number of cell types and their varying Stx sensitivity present within the kidney samples assayed makes the total protein:CHOP protein ratio too high to be visible by western blotting.





Diego Escobar Garcia St. Mary's University

Mentor: Benjamin Wolozin MD-PhD

Alzheimer's disease (AD) is a devastating neurodegenerative disease that was originally diagnosed over 100 years ago, yet no disease modifying treatment strategies currently exist. Tau is a microtubule binding protein normally expressed in all neurons throughout the brain, but during AD hyperphosphorylated tau forms pathological aggregates to generate the neurofibrillary tangles that are a hallmark of AD. P301L mutant tau has been shown to exacerbate this pathological aggregation in human brain, leading to tauopathies. A hypothesis in the field is that these aggregated species of tau are neurotoxic, and that their removal may reverse or lessen the cognitive deficits associated with disease. The Wolozin lab has identified stress granules (SG) as a major component of AD pathology. SGs accumulate in the brains of subjects with mild cognitive impairment and AD, and in many cases are physically distinct from classic markers of AD pathology. This finding provides insights into putative biological pathways mediating neurodegeneration in AD, as well as novel opportunities for early disease diagnosis. RNA binding proteins (RBPs) are a large group of proteins that regulate RNA translation through formation of RNA/protein complexes. Stress, such as that occurring during neurotoxicity or inflammation, induces alterations in the pattern of protein synthesis, and these stress-induced regulatory events are mediated in part by SGs. SGs are formed via reversible aggregation of RNA and protein, and mediate critical modifications in RNA processing, including sequestration, splicing, degradation and translation. This summer project focuses on understanding the role of Ras GTPase Activating Protein Binding Protein-1 (G3BP1; an RBP that nucleates SG assembly) and its role in neurodegeneration. To determine the effects of pathological tau accumulation on RBP solubility in vivo, the agedependent changes in G3BP1 and other nucleating SGs in two mouse models of AD were analyzed. In addition, preliminary results from our lab indicate that overexpression of G3BP1 decreases the levels of tau RNA in transfected cell lines. Thus, lentiviral shRNA constructs targeting murine G3BP1 were developed to determine whether knockdown of G3BP1 could rescue its effect on tau RNA. With our findings, particularly the knock down experiment of G3BP we hope to further our understanding of the role it plays in neurodegeneration.



Lynisha Farrell University of the Virgin Islands

Neurogenesis In Adult Zebrafish Requires Two Nights

Mentor: Irina Zhdanova Md-PhD Alex Stankiewicz, Sharon Fan, Lili Yu

Boston University Medical Center, Department of Anatomy & Neurobiology

Circadian rhythm is an internal, endogenous, biochemical, behavioral and physiological 24-hour timekeeping mechanism that corresponds to the time it takes the earth to orbit on its axis. Circadian rhythms are driven by a biological clock synchronized by external cues called Zeitgebers such as photoperiod or food. Circadian rhythms control the body's sleep rhythms, core temperature, hormone release, and feeding patterns in animals and humans. Although the cell cycle has been shown to oscillate as a function of circadian rhythms, its contribution to adult neurogenesis remains uncertain. The objective of this project is to investigate how circadian rhythms affect the rate of neurogenesis by observing the length of the cell cycle in the zebrafish. We will study this process through the use of the zebrafish, Danio rerio, because similarly to humans, they are diurnal vertebrates. Cell cycle phases are moderated by check points called cyclins that form complexes with Cyclin Dependent Kinase (CDK). We hypothesized that cell cycle occurs over the course of two-nights with a cycle of about 24-hours. Using a CDK4/6 inhibitor, we were able to show a decrease in the number of bromodeoxyuridine (BrdU) labeled cells the following night. Thus, two consecutive nights are needed for cell proliferation in zebrafish brain. Lastly, determining the length of cell cycle phases would help us to understand optimal times and conditions for proliferation. Potentially, this information could be applied to time treatments of patients with neurodegenerative diseases or traumatic brain injuries.

This research was supported by MBRS-RISE 5R25GM061325



Jaileene Hernandez Escalante Temple University

Induction of Cytokine and Chemokine production by Dendritic Cells in Response to N. Gonorrhoeae Major Outer Membrane Protein PIB

Mentor: Lee Wetzler MD

Boston University Medical Center, Department of Medicine, Section of Infectious Diseases Additional Authors: Yazdani Shaik-Dasthagirisaheb, Ian Francis, Michael Reiser, Paola Massari

Gonorrhea is a human sexually transmitted disease caused by the Gram-negative pathogenic bacterium, Neisseria gonorrhoeae. There are approximately over 300,000 cases of gonorrhea reported yearly in the US and it is estimated that there are over 60 million cases per year worldwide. The major characteristic of the disease is inflammation at the site of infection and potentially purulent discharge (especially in men). Increased urgency for control of this infection has occurred due to the recent increased incidence of antibiotic resistance in these organisms. Although past and current studies have focused on the development of a vaccine to prevent gonococcal infection, no vaccine currently exists. Among the gonococcal components that are potential vaccine candidates, a major one is the gonococcal major outer membrane, Protein I (PI or Por). There are two main types PIA and PIB and most of our efforts have focused on PIB. Pors are bacterial porins and play a role in the uptake of ions for the bacterial metabolism and survival. Our group and others have shown that that this protein can be a potential gonococcal vaccine candidate. PIB is known to interact with the host immune system via interaction with the Toll-like receptor 2 (TLR2), a receptor involved in innate immune responses. The purpose of this project is to study the effect of gonococcal PIB on dendritic cells (DC) in vitro via its interaction with TLR2 (and potentially similar to the effect of meningococcal porins). DCs are professional antigen presenting cells and crucially link innate and adaptive immune responses, which are important in the development of effective vaccines. In this study we analyzed the ability of PIB to stimulate DCs obtained from murine bone marrow. Bone marrow was extracted from C57BI/6 wild-type mice and cultured in medium containing GM-CSF (R10 medium) to derive immature dendritic cells. The phenotype of these cells as DCs was confirmed using specific cell surface markers including CD11b, CD11c, MHC II, CD80 and CD86 by flow cytometric (FACS) analysis. To determine the ability of PIB to stimulate these cells, DCs were incubated with purified PIB and with other bacterial products and synthetic stimuli as controls: meningococcal PorB, E. coli LPS (TLR4 ligand) and Pam3CSK4 (another TLR2 ligand). Untreated cells were used as a negative control. After 6 and 24-hours of stimulation, the cell culture supernatants were collected and the levels of TNFa, IL-6, and RANTES were measured by ELISA. Our results indicate that PIB induced significantly higher production of the inflammatory cytokines TNFa and IL-6 and of the chemokine RANTES compared to the un-stimulated control (p<0.05). Further, levels of these molecules were significantly higher with PIB compared with meningococcal PorB. In addition, the other ligands used induced similar significant secretion levels of these inflammatory mediators. In conclusion, our results indicate that purified PIB from N. gonorrhoeae activate bone marrow derived DCs and induce the secretion of inflammatory mediators that may allow for enhanced immune responses when this protein is used as a vaccine candidate.



Jennifer Leahy Boston University School of Medicine

Use of a Multiwall Chemotaxis Chamber to Study Neutrophil Chemotaxis

PI: Daniel Remick MD Mentor: Terry Hsieh

Boston University School of Medicine, Department of Pathology and Lab Medicine

A multiwall chemotaxis chamber allows for the rapid study of the chemotactic activities of different cells. In order to use such a chamber, different factors must be considered, including membrane pore size, incubation time, method of staining, and chemoattractant concentration. Preliminary results indicate that conditions to study the chemotactic characteristics of neutrophils from murine blood include use of a membrane with 5µm pore size, incubation time of 45 minutes and the use of DRAQ5, a far-red DNA stain. Initial work has been completed examining chemoattractant concentration, however, much work is still needed before this instrument is fully functional. The ultimate goal of the chemotaxis chamber is to study the neutrophil chemotactic properties of an established murine traumatic brain injury model.



Sean Munier Loyola University, Maryland

Real-Time In vitro Quantification of the Unfolded Protein Response through ATF6-Driven Luciferase Expression

Mentor: Andrew Wilson, MD

Boston University Medical Center, Center for Regenerative Medicine

The endoplasmic reticulum (ER) is responsible for folding and processing newly translated proteins. ER stress occurs when the volume of proteins entering the ER lumen for folding exceeds the ER's capacity to process them. This stress causes what is known as the unfolded protein response (UPR), a reaction meant to restore balance to the ER by inhibiting synthesis of ER client proteins while simultaneously upregulating expression of genes responsible for enhancing ER function. While the UPR is meant to compensate for ER stress and restore normal cell function, evidence suggests that prolonged activation of the UPR can induce cell death. Further research has since linked the UPR to certain types of inflammatory lung and liver disease; however, these findings remain poorly understood. In order to track the UPR in real-time *in vitro*, we created a lentiviral reporter vector (designated "lenti-ATF6-Luciferase-W") designed to activate transcription of firefly luciferase in the setting of nuclear translocation and binding of the transcription factor Activating Transcription Factor 6 (ATF6), a molecule important in initiating the UPR. Following transduction of human kidney 293 cells with our ATF6 reporter vector, we treated cells with a reagent known to induce the UPR and quantified ATF6-driven luciferase expression. Our results showed a viral dose-dependent increase in ATF6-driven luciferase expression, suggesting its applicability for real-time quantification of the UPR in inflammatory disease models.



Niyoyankunze Noel Fayetteville State University

Vascular Smooth Muscle Sirtuin-1 is Beneficial Against Obesity-Induced Aortic Stiffness

PI: Richard Cohen MD Mentor: Francesca Seta PhD

Boston University Medical Center, Division of Cardiovascular Medicine Additional Author: Jessica L. Fry

Background: Aortic stiffness (AS) is a gradual deterioration of large artery elasticity and an independent predictor of cardiovascular events. AS is an indicator of adverse, structural, and functional changes within the vessel wall and possibly a precursor of hypertension, a major cardiovascular risk factor. We previously showed that in C57Bl/6J mice fed a diet rich in fat and sucrose (HFHS), mimicking the human metabolic syndrome, AS develops within 2 months and precedes hypertension by 5 months. Resveratrol, a polyphenol known to activate sirtuin-1 (SirT1), a NAD⁺-dependent deacetylase with anti-inflammatory and anti-oxidant effects, prevented HFHS-induced AS.

Aim: The goal of this study was to determine whether vascular smooth muscle (VSM) SirT1 could be beneficial against HFHS-induced AS.

Methods & Results: We generated novel mouse strains with SirT1 deletion (VSM-SirT1-KO) or over-expression (VSM-SirT1-Tg) in VSM, the major component of large arteries, or whole body (SIRBACO), and we confirmed the correct genotypes by PCR on tail DNA. We assessed arterial wall thickness on H&E and Picrosirius red-stained aortic sections after feeding mice HFHS for 8 months. Pulse wave velocity (PWV), an *in vivo* index of AS, was measured by Doppler ultrasound. We did not find any significant differences in arterial medial thickness between experimental groups, however PWV was significantly reduced in HFHS-fed VSM-SirT1-Tg and SIRBACO mice compared to WT.

Conclusion: VSM SirT1 is beneficial against HFHS-induced AS despite having no significant effect on aortic hypertrophy. Further studies are in progress to determine the cellular and molecular mechanisms by which SirT1 is beneficial against HFHS-induced AS.



Gregory Patterson Cheyney University

Maximum Eosinophil Recruitment to the Peritoneal Cavity

Principal Investigator: Daniel Remick MD Mentor: Evan Chiswick

Boston University Medical Center, Department of Pathology

Introduction: Eosinophils are a specific white blood cell that is part of the innate immune system. Eosinophils have been known to induce inflammation in peripheral tissues and could be related to asthma and other allergic diseases as well. Eosinophils were discovered by Wharton Jones in 1846 while its cytoplasmic granules were revealed by Paul Ehrlich, which ultimately gave a characteristic of eosinophils. Eosinophils aren't as numerous as the other white blood cells and normally they are studied from the bone marrow. Current methods for this include sacrificing mouse, harvesting their bone marrow and culturing the cells ex-vivo for 1-2 weeks. This procedure takes a lot of time and is extremely expensive. Previous experiments in the lab have found eosinophil recruitment to the peritoneal cavity after an initial lavage. Using this information, along with knowledge of surface markers of other immune cells, we were able to identify max eosinophil recruitment in the peritoneal cavity after an initial lavage was performed.

Methods: Peritoneal lavage. A peritoneal lavage was performed on ICRCD1 type mice which was anestitzed with isofluorane. Warm HBSS w/EDTA 3.38mM HBSS/EDTA was used to lavage mice. 10mL total volume was used for lavage, with no more than 5mL injected at a time in order to prevent tearing the abdomen. Cells were collected and placed on ice. Cell Counting. They cells were collected and then separated from the plasma by centrifugation (conditions were 4 degrees Celsius, 450 X g and 10min . Once the cells were separated they were suspended in 1ml of PBS/.05%BSA. The cells were then counted manually using a hemocytometer (5 fold dilution was used on the hemacytometer). Cytospin apparatus. Cells were placed into cytospin apparatus along with cytospin fluid so they could place on a slide. The slide was then dried for at least an hour and no more than 24 hours. Once dried they were stained with blue fixative solution, red eosin solution, hematoxylin solution and deionized water. Cell differential was then performed on the slides.

Results: (hemacytometer) This data was taken during each lavage procedure performed on the mice. Eosinophils were the most abundant cell type post lavage (72 hour) was performed.

Conclusions: Normally to identify and analyze eosinophils you would have to use the bone marrow and this procedure takes time, money and kills the mice. Knowing that there is a high amount of eosinophils after an initial lavage offer not only another procedure in studying eosinophils, but also keeping the mouse alive as well so other procedure can be performed on the same mouse.



Tyler Pluchino State University of New York at Binghamton

Purification and Characterization of Haemophilus Influenzae Type B Pili

Mentor: Esther Bullitt PhD

Boston University Medical Center, Department of Physiology & Biophysics Additional Author: Chelsea R. Epler PhD

Haemophilus influenzae is a pathogenic bacterium that invades the nasopharynx to target host cells and cause pneumonia, epiglotitis, and meningitis. The ability for *H. influenzae* to infect via the nasopharynx is dependent on hair-like structures present on the cell membrane known as fimbriae. Fimbriae, or pili, are used to attach to surfaces as an adhesion factor, making *H. influenzae* virulent. Although *H. influenzae* is preventable through immunization, there are many developing nations and unvaccinated infants that suffer from this pathogen. By determining the structure of pili and how they adhere to surfaces, novel treatments against pili may be created and used to treat disease. Pili from *Haemophilus influenzae* were imaged using a transmission electron microscope using samples that were negatively stained or frozen hydrated. Images were then analyzed and models of *H. influenzae* pili were developed using UCSF Chimera.



Kailah Simon Central Connecticut State University

Relationships Between Infection Site and the Abilities of Pneumococcus to Activate Macrophage NF-KB

Principal Investigator: Joseph P. Mizgerd ScD Mentor: Fadie T. Coleman

Boston University Medical Center, Pulmonary Center

Background: Every year, the United States spends roughly \$20 billion dollars on bacterial pneumonia. *Streptococcus pneumoniae* (pneumococcus) is a major cause of disease worldwide and the most common community acquired pneumonia in the U.S. Clinical outcomes of pneumococcus range from acute otitis media, sinusitis, meningitis and pneumonia. Clearance of pneumococcus requires expression of cytokines from alveolar macrophages. The transcription factor nuclear factor kappaB (NF-κB) is essential in regulating cytokine production, a crucial trigger in eliminating microbes in the lung. Activation of the transcription factor has been found to help clearance of bacteria, including pneumococcus. However, pneumococcal pneumonia is understood to avoid or subvert effector mechanisms of innate immunity. It has been observed that even within a given serotype, different isolates vary in their ability to cause disease. We hypothesize that pneumococcal isolates amongst different serotypes and within a serotype vary in their ability to activate macrophage NF-κB dependent upon their site of origin.

Methods: To evaluate NF-κB activation by different pneumococcal patient isolates, a high throughput macrophage bioassay that reports NF-κB-mediated gene transcription developed in-house was used. In this model, pneumococcus is used to stimulate a macrophage-like cell line that expresses luciferase upon NF-κB activation. RESULTS: Isolates from 100 patients with pneumococcal disease and those without pneumococcal disease have been screened in our bioassay and show a wide range of NF-κB induction. There were significant differences in the capability to activate NF-κB between serotypes as well as within a given serotype. Previous studies with serotype 19A isolates from the pleural fluid of patients with Empyema activated less macrophage NF-κB than serotype 19A from the nasopharynx or blood. This finding suggested that inflamed environments might allow low NF-κB activators to survive better. To further our understanding of how infection site associates with NF-κB activation, I compared serotype 33F isolates from cerebral spinal fluids (CSF) of meningitis patients or from the healthy nasopharynx. Within serotype 33F, isolates from the CSF had consistently lower activation of macrophage NF-κB than isolates from the nasopharynx. Ongoing studies are examining other serotypes and sites of infection to test if this is a universal phenomenon.

Conclusion: The level of NF- κ B activation within serotype 33F associates with the site of the infection. Whether this holds to be true across infections and serotypes is still yet unknown. Future studies will further test the hypothesis that low NF- κ B activators predominate in infections of otherwise sterile sites.



Brittany S. Thompson Kennesaw State University

Long non-coding RNA, LL18/NANCI as an in vitro tool for Nkx2-1 induction in ESC-derived anterior foregut endoderm

Mentor: Laertis Oikonomou PhD

Boston University Medical Campus, Center of Regenerative Medicine Additional Author: Steven Cincotta

Long non-coding RNAs (IncRNAs) are indicated to have instrumental roles in regulating gene transcription; however, the specific function of IncRNAs is unclear. Recently, the IncRNA LL18/NANCI (Nkx2.1-associated noncoding intergenic RNA) which is 2.5kb downstream from thyroid transcription factor-1 (Nkx2-1, a homeodomain transcription factor) was shown to act upstream of Nkx2-1 and regulate lung endodermal differentiation in vivo. Nkx2-1 is the earliest known marker of lung development and has important roles in lung function postnatally. In order to investigate the function of NANCI in the development of lung/thyroid progenitors in vitro, we decided to overexpress and knock-down NANCI during directed differentiation of mouse embryonic stem cells (mESCs). The ultimate goal of this research was to complete a successful differentiation of NAX2-1^{GFP} mESCs to lung/thyroid progenitor cells. Prior to the differentiation, amplified plasmids for NANCI shRNAs (NANCI shRNA 7, NANCI shRNA 1, pIKO-GFP Scrambled (control)), NANCI overexpression (pLenti7.3/v5 NANCI) and Empty Vector (control) were packaged in 293T cells and the resulting lentiviruses were titered using Fluorescence Activated Cell Sorting (FACS). In a preliminary directed differentiation experiment, Nkx2-1^{GFP} mESCs were transduced with the NANCI lentivirus and then were cultured for direct differentiation over a period of two weeks. Cells were then FACS-sorted and analyzed via qPCR to determine whether or not the use of NANCI would induce overexpression of Nkx2-1⁺ lung/thyroid progenitors.



Briana Toatley Albany State University

The Effects of Expression and Activation of the A2bAR Adenosine Receptor on Stress Fiber Formation

Principal Investigator: Katya Ravid PhD Mentor: Shenia Patterson

Boston University Medical Center, Department of Medicine

Vascular smooth muscle cells compose the medial layer of the blood vessel wall. These cells are necessary for the regulation of blood pressure and are able to do so by contracting and relaxing to alter the luminal diameter of the arteries. This contraction and relaxation provides constant maintenance to blood pressure. Vascular smooth muscle cells contain stress fibers composed of actomyosin bundles. These stress fibers are the framework of the major contractile structures present in vascular smooth muscle cells. They are also involved in the adhesion, migration and mechanotransduction of the cells. Stress fibers are composed of actin molecules that have been cross-linked by the protein alpha-actinin-1. It has been found through colocalization and Co-ip experiments in HEK cells that the A2b adenosine receptor is a novel binding partner of alpha-actinin-1. Therefore, we hypothesized that stress fiber formation is regulated through the A2b adenosine receptor (A2bAR). This receptor is one of four gprotein coupled receptors of the vasodilator adenosine. The four receptors are A1 and A3, which are inhibitory receptors for cyclic adenosine monophosphate (cAMP), and A2a and A2b, which serve as stimulatory receptors for cAMP. To test this theory, stress fibers were visualized in vascular smooth muscle cells using immunofluorescence after either knock-down of the A2bAR or cytochalasin D wash out with BAY stimulation. We found that changes in stress fiber formation after knock-down of A2b or wash out of cytochalasin D with BAY stimulation were not noticeably visible. Future research may include cytochalasin D washout of knock down A2bAR VSMCs as well as VSMCs overexpressing A2bAR to determine the role of the A2bAR in the formation of stress fibers.



Sydnie Turner Xavier University of Louisiana

Phospholipid Biosynthesis as an Anticancer Target

PI: Raphael A. Zoeller PhD

Boston University School of Medicine, Department of Physiology and Biophysics

The receptor tyrosine kinase inhibitor tyrphostin AG879 has been determined as a potential anti-cancer drug, however the exact mechanism of how it is effective against cancer cells is unknown. We found that tyrphostin AG879's severely inhibited phospholipid biosynthesis in the human cervical cancer cell line, HeLa, as measured by fluorescent fatty acid uptake into phospholipid pools as well as labeling with ³²P-inorganic phosphate. Tyrphostin AG879 was tested in toxicity curves using HeLa cancer cells in order to determine the concentration or the amount of time necessary to significantly reduce cell viability. The EC₅₀ for growth inhibition was approximately 3 μ M, similar to the EC₅₀ for phospholipid biosynthesis inhibition. While growth inhibition was observed in two separate cell lines, cytotoxicity was observed in the HeLa cell line within 24 - 48 hours. These data show that the mechanism behind tyrphostin AG879 may be associated with its ability to inhibit phospholipid biosynthesis and that phospholipid biosynthesis may be a valuable target is the search for novel anticancer regiments.



Jewel Williams St. Mary's College of Maryland

Effects of Chronic Exposure of Simvastatin and Pravastatin on Insulin Secretion from a β -Cell Line, INS-1 Cells

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Statins are a class of drugs used primarily to lower cholesterol and prevent the onset and progression of atherosclerosis. Despite epidemiological evidence indicating that the risk of diabetes is higher for individuals receiving statins, little is known about the effect that statins on insulin secretion from pancreatic β -cells. To determine this we treated clonal β -cells with simvastatin and pravastatin for 48hrs at 100nM and 5uM, respectively. Basal insulin secretion at low glucose (2 mM) was unaffected. Glucose-stimulated insulin secretion (8 mM) was inhibited in cells that were treated with simvastatin. Interestingly, pravastatin had no effect on glucose-stimulated insulin secretion. To further examine the effects of simvastatin and pravastatin on glucose-stimulated insulin secretion, the possibility was considered that the inhibition of insulin secretion could be due to a reduction of Ca²⁺ influx. Contrary to expectations, no differences were observed in Ca²⁺ raising the possibility that other metabolic pathways that are involved in the inhibition of insulin secretion by simvastatin. With the ever-growing diabetes epidemic and the frequent administration of statins it is imperative we clarify the pathway involved in the inhibition of insulin secretion by simvastatin.

