GMS Presents:

SUMMER TRAINING AS RESEARCH SCHOLARS (STaRS) PROGRAM

Research Symposium

Thursday, August 10, 2017

STaRS Research Symposium 2017

Welcome to the Annual Summer Training as Research Scholars (STaRS) Research Symposium, hosted by the Division of Graduate Medical Sciences. The students have spent this summer conducting research in labs across the BU Medical Campus and we hope you enjoy learning more about their projects.

10:00 – 10:05 am **Welcome**

Dr. Linda Hyman, Associate Provost
Dr. Maria Ramirez, STaRS Director and Associate Professor
Ms. Lynese Wallace, Manager of Diversity and Inclusion
Mr. Francisco Patino, Program Administrator

10:05 – 11:05 am **Oral Presentations**

- Keyona Pointer (10:05)
- Jenesis Gayden (10:20)
- Luis Marquez (10:35)
- Andres Lojano-Bermeo (10:50)

11:20 – 12:00 pm **Poster Session A**

- Kevin Ruaro
- LeShell Washington
- Selina Gonzalez
- Kharastin Chea-Howard
- Reagan Katulege
- Vangelina Osteguin

12:00 pm **Lunch**

12:15 – 1:00 pm **Poster Session B**

- Diego Trujillo
- Vinson Cobb
- Bria Landry
- Adam Ibrahim
- Sojourna Ferguson
- Kalynn Parks

1:00 – 1:30 pm **Awarding of Certificates & Closing Remarks**

Kharastin Chea-Howard
Norfolk State University
Class of 2019

Characterization of Epithelial Cell Types in Retinoid-Treated Embryonic Lung Slices

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Background: Retinoic acid (RA) is the bioactive metabolite of Vitamin-A. RA signaling has been demonstrated to be essential in embryonic development and tissue homeostasis. The role of RA in lung development during mid to late gestation is essentially unknown.

Hypothesis: RA is crucial for lung epithelial cell differentiation in development during mid to late gestation.
Methods: RARElacZ mice, a reporter of RA activity, were used to model this concept. Embryonic lung slices from wild type and RARElacZ were collected at embryonic day 13.5. They were then embedded in 1% agarose and sectioned in 200 micron slices. Slices were cultured in a transwell for a period of 48 hours in control, BMS (RA receptor antagonist)- containing, DEAB (RA synthesis inhibitor)-containing, and RA-containing medium. X-gal staining was conducted for easy identification of the reporter gene β-galactosidase (LacZ). Immunohistochemistry of markers of different epithelial cell types was performed.

Results: S-gal staining of cultured of RARE LacZ lung slices treated with BMS or DEAB showed abrogation of the LacZ expression, indicating complete blockade of RA signaling. In contrast, LacZ expression was markedly increased in the lung epithelium in lung slices cultured with RA, indicating upregulation of RA activity. When immunohistochemistry (IHC) was conducted for the CC10 secretory marker, there was no signal in any of the lung slices. Most likely, this was due to the fact that CC10 is not expressed at this stage of development. IHC was also done for FOXJ1, a ciliated cell marker. There were much more FOXJ1 positive cells in the epithelium of BMS and DEAB groups than in the control group, and essentially none in the RA group. This leads us to believe that RA deficiency is biased towards ciliated cell types in the epithelium and RA excess is biased toward a downregulation of ciliated cells.

Conclusions: RA signaling affects lung epithelial cell differentiation. Our preliminary data suggests that RA signaling biases lung epithelial differentiation toward a non-ciliated cell type.
Future Work: In the near future, we will do more IHC with KRT5, a basal cell marker, to see the effect that RA has on this cell type. We also hope to further compliment this work in vivo using the Vitamin A deficient, LRAT knockout. This data will be substantial because the LRAT gene will not allow vitamin A to be stored, allowing us to modulate the mouse diet by giving them vitamin A deficient food modeling true vitamin A deficiency. Immunostaining of a secretory marker that is expressed earlier than CC10 (SCGB3A2) will also be conducted to see whether this cell type is up or downregulated by RA signaling.

Vinson Cobbs
Boston University School of Medicine
Class of 2021

Maximizing the Moment: Assessing Tobacco in Hospitalized Patients with Substance Use Disorders

Vinson Cobb, Ryan Seibert MD, Hasmeena Kathuria MD

Background: The rate of smoking in patients with substance use disorders is 2-4 times higher than the general population. Individuals with co-substance use are more likely to die from smoking than from other substances. Studies suggest that quitting cigarettes may help adults in recovery stay sober, highlighting the importance of treating tobacco dependence in patients with substance use disorders. Boston Medical Center (BMC) has established a dedicated inpatient Tobacco Treatment Consult (TTC) service. Hospitalized smokers are highly receptive to the service, but due to limited resources, the TTC team is only able to see 35% of the 550 smokers admitted each month. Smokers with substance use disorder are also often seen by the Addiction Medicine team. If the Addiction Medicine team additionally incorporated tobacco treatment, the number of smokers receiving counseling may be increased. The purpose of this study is to evaluate how the Addiction Medicine team addresses tobacco dependence and to examine provider-level barriers to addressing tobacco dependence in smokers who have concomitant drug use disorders.

Methods: 1139 Addiction Medicine consults between 7/1/2016 and 6/30/2017 were manually reviewed to assess how often the service addressed tobacco dependence among patients at BMC. Items assessed included how often patients were (1) Asked about current smoking status; (2) Advised to quit smoking; (3) Assessed for readiness to quit; (4) Assisted with a quit attempt; and (5) Arranged for follow-up. We conducted in-depth semi-structured interviews with 9 Addiction Medicine consult attendings at BMC, theme saturation reached. Physicians described barriers and facilitators in addressing tobacco dependence. We audio-recorded, transcribed, and coded interviews. Two coders identified major themes, and reviewed themes with the research team.

Results: 855 of the 1139 chart notes were initial consultations and were included for further review. The addiction medicine team documented smoking history in greater than 70% of visits (643/855). 480 patients with documented smoking history were current smokers (75%). Of the current smokers, 12.5% were advised to quit; 22.9% had their readiness to quit assessed, 30.6% were assisted with quitting, and 14.6% of smokers were given information on smoking cessation resources. Physicians cited a lack of time and tobacco not being an immediate health threat as barriers to counseling patients on tobacco use. Physicians reported counseling when patients had smoking-related symptoms. Addiction consult attendings welcomed a team-based approach to address both substance use and tobacco use disorders.

Conclusion: Hospitalization is a missed opportunity to counsel patients with co-substance use to quit smoking and interventions to promote smoking cessation in these high-risk smokers will need to address provider-level and systems-level barriers.

Sojourna Ferguson
University of the Virgin Islands
Class of 2019

Isolation and Characterization of Activated Stromal Cells in Breast Cancer

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Epithelial derived breast tumors often exhibit alterations in the microenvironment and pathologic stromal activation. These stromal cells are referred to as cancer associated fibroblasts, or CAFs, and are related to activated fibroblasts that expresses both α-smooth muscle actin (α-SMA) and collagen I. When these fibroblasts are activated, they are believed to supports tumorigenesis by stimulating angiogenesis, and enhancing cancer cell proliferation and invasion. The mechanisms of how these cells become activated remains uncertain. By understanding the mechanisms of stromal activation in cancer and the role of their secreted proteins will impact our understanding of cancer. The goal of this project is to identify activated cells in the breast cancer stroma and targeting the stromal derived protein aortic caboxypeptidase-like protein, (ACLP) to examine the effects of this secreted protein have on tumor progression and the remodeling of the extracellular matrix. In this study, we generated a breast cancer model in mice by overexpressing the oncogene Her2 and crossing these animals with transgenic mice harboring fluorescent reporters to monitor both α-SMA and collagen I activation. We analyzed and quantified the expression by both histological analysis, and fluorescent imaging. Using flow cytometry we studied the expression of these reporters, α-SMA and collagen I by adding the EpCam antibody to accurately identify the different cell populations. Immunofluorescence imaging of the tumors showed an increase expression in α-SMA and collagen I. The FACS analysis of the normal tissues, where was 5.74% α-SMA and 3.17% collagen 1 expression in the myoepithelial cells. Additional work is developing a conditional mouse model to understand the role of ACLP in breast cancer progression and epithelial changes. Administration of ACLP to primary epithelial cells induce an epithelial to mesenchymal-like phenotype. The characterization of activated stroma cells and how they contribute to breast tumor progression may, lead to the ability to target secreted proteins such as ACLP. Targeting these proteins that increase in the tumor stroma may also lead to the development of new breast cancer therapies.

Jenesis Gayden
Syracuse University
Class of 2018

Effects of an TIA1 Knockdown on Microglial Activation in the P301S Mouse Model of Taupathy

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Background: Tauopathy is a leading cause of neurodegeneration as seen in conditions such as Alzheimer's Disease, Parkinson's Disease, and Multiple Sclerosis. Studies have suggested that there is a biological link between TIA1 and tau due to the fact that they accumulated together in mouse model and human subject taopuathies (Wolozin et. al. 2012). Studies have also shown that TIA1 knockdown has a rescue effect by preventing tau misfolding and tau-mediated toxicity (Vanderweyde et. al. 2016). As a regulator of the stress response, TIA1 is related to the inflammatory process which has been linked to the exacerbation of tauopathy The objective of this study is to determine the effects of TIA1 knockdown on microglial activation, an indicator of inflammation, in the P301S mouse model of tauopathy, a mouse line containing a gene mutation found in frontotemporal dementia.

Methods: Hippocampal tissue from wild type mice (TIA1 +/+, P301S tau-/-) n=3, tauopathy mice (TIA1 +/+, P301S tau+/-) n=3, TIA1 knockdown rescue mice (TIA1+/-, P301S tau+/-) n=2, and TIA1 knockdown control mice (TIA1+/-, P301S tau-/-) n=2 , was fixed in 4% paraformaldehyde and then processed for immunohistochemistry (IHC). Primary antibodies used during IHC include IBA1 and P2ry12 to visualize all microglia, and MHCII to visualize activated microglia. Confocal microscopy was used to image the dentate gyrus, and Neurolucida software was used to count the microglia. Microglial morphology was categorized as falling into one of the phenotypic categories: "resting", "activated morphology, no MHCII stain", "activated morphology, little MHCII stain", and "activated, highly MHCII positive. Each activation phenotype was numbered 1,2, and 3, respectively. Average total number of microglia was quantified per morphology category, and averages were compared across genotypes. Statistical significance was assessed using a two-tailed Student's t-test and a 2-way ANOVA (with significance defined as $p < 0.05$).

Results: As expected, there is a significantly higher total number of microglia present in Tauopathy animals in comparison to Wildtype animals (*p=0.01*). Additionally, there are higher numbers of microglia present in animals heterozygous for TIA1 containing tauopathy compared to those without tauopathy (*p=.001*). Interestingly, the knockdown of TIA1 in tauopathy animals does not have a significant effect on total number of microglia *(p=0.87).* Overall, results indicate that the knockdown of TIA1 does not affect total number of microglial cells in wildtype mice (*p= .21*). Furthermore, results yield significant differences based on microglial activation phenotype. As expected, tauopathy animals present with significantly more activated cells compared to wildtype (phenotype 1: *p=.01*, phenotype 2: *p=.01*), while there is a trend towards significantly more resting cells present in the wildtype group (*p=.09*). This pattern is reflected in animals heterozygous for TIA1, with those containing tauopathy yielding higher numbers of activated cells (phenotype 1: *p=.04*, phenotype 2: *p=.03*), and those without tauopathy presenting with significantly more resting cells (*p=0.001*). Interestingly, results indicate that the knockdown of TIA1 in tauopathy animals leads to an increase of MHCII – activated microglia (*p=0.05*).

Conclusions/Future Studies: Preliminary results indicate that knockdown of TIA1 does not affect total number of microglia in the presence of tauopathy. However, the type of microglial activation in this condition may be impacted by a TIA1 knockdown. In the future, a continuance of this study will be conducted with an increased number of subjects. A gene dosage effect study will also be performed to examine the effect of full TIA1 knockout.

Selina Gonzalez
California State University San Marcos
Class of 2018

Pannexin channels regulate cell-cell calcium mobilization and motility during epithelial wound repair

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Departments of Experimental Pharmacology, Biochemistry, Ophthalmology, and Boston University School of Medicine

Background: The cornea is a transparent avascular tissue that transmits light to the retina and covers the lens and iris. According to the World Health Organization, blindness due to injury and disease in the cornea is the fourth leading cause of preventable blindness. Previous research demonstrates that epithelial cells release nucleotides that activate purinergic receptors P2X7 and P2Y2, which induces cell-cell calcium mobilization. This calcium mobilization is reported to potentially play a role in collective cell migration and wound healing. To better understand this specific mobilization, we studied pannexin, a channel forming glycoprotein. ATP is able to move through the Pannexin channel and binds with P2X7.We want to investigate the mechanism of the calcium signal propagation and whether pannexins are involved. We hypothesize that pannexin channels mediate cell-cell calcium mobilization and regulates actin activity in response to injury or agonist stimulation.

Methods: Experiments were performed using 10Panx, an inhibitory peptide for pannexin channels, and scramble Panx, a control peptide. Human Corneal Limbal Epithelial (HCLE) cells were cultured to confluence on glass bottom dishes. Twenty-four hours prior to experimentation, growth supplements were removed. Cells were preincubated with either 100uM 10Panx or 100uM scramble Panx to inhibit pannexin channels. In order to visualize the change in calcium, cells were pre-loaded with 5 μM Fluo-3AM, a fluorescent dye, at a final concentration of 1% (v/v) DMSO and 0.02% (w/v) pluronic acid for 20 minutes at 37°C and 5% CO_2. Images were collected every 3 seconds on a Zeiss Axiovert LSM 880 confocal microscope for a period of 45 minutes after either agonist stimulation or injury. The agonist used for stimulation was BzATP, a synthetic nucleotide that selectively activates P2X7 and UTP, an agonist for P2Y2. Baseline and post-wound frames were captured. Analysis was performed using FIJI/ImageJ and MATLAB programs.

Results: We found that 10PanX correlated with dampened calcium mobilization after injury. In uninjured cultures the decrease was only detected in response to BzATP and not to UTP. Actin activity was also inhibited by 10PanX after injury. MATLAB analysis using heatmap indicates that 10PanX reduces correlation of cells during calcium mobilization.

Conclusion: Our data indicates that P2X7-mediated Pannexin channels play a role in the injury response. This provides greater insight as to the level of involvement of pannexin in calcium signal propagation in response to injury in HCLE cells.

Tarius Hill
Xavier University of Louisiana
Class of 2018

The Effects of Shiga Toxin on Host Cell Immune Response

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Introduction: Shiga-toxins (Stx) are a family of toxins that are produced by certain strains of *Escherichia coli* that can cause patients to experience severe diarrhea, acute renal failure and sometimes death. These Shiga toxins are responsible for more severe consequences of bacterial infection such as hemolytic uremic syndrome which can be lethal. There are two major Shiga toxins from *E. coli*, classified as Stx1, and Stx2. *E.coli* strains expressing Stx2 tend to induce more severe disease in patients.

Objective: Previous *in vitro* studies have shown that human monocytes exposed to Stxs have increased pro-inflammatory cytokine expression (TNFα and IL-8). Interleukin-23 (IL-23) is a cytokine that is essential during intestinal inflammation that has not been investigated in the presence of Shiga-toxins. Our goal is to evaluate the effects of Stx1 and Stx2 on IL-23 expression in human macrophage-like THP-1 cells.

Methods: THP-1 cells were differentiated into macrophage-like cells via incubation in media supplemented with 100 nM phorbol 12-myristate 13-acetate (PMA) for 72 hours, then serum-starved for 24 hours. Following serum starvation, cells were incubated with: Media alone, lipopolysaccharide (LPS, 100 ng/ml), LPS (100 ng/ml) + Stx1 (0.1 ng/ml), or LPS (100ng/ml) + Stx2 (10 ng/ml) for 24 hrs. After 24 hrs, the RNA for each sample was isolated and reverse transcribed into cDNA. From this a quantitative polymerase chain reaction (qPCR) was performed to determine *TNFa* and *IL23p19* transcript content.

Results: THP-1 cells incubated with Stx2 showed a larger decrease in the expression of both IL-23 and TNFα mRNA during LPS stimulation. Unlike Stx2, the samples incubated with LPS+Stx1 showed no change in expression of IL-23 and TNFα mRNA compared to cells stimulated with LPS alone. Cells incubated with media alone had a baseline cytokine transcript content for comparison.

Conclusion: Based on this experiment, Stx2 suppressed IL-23 and TNFα mRNA levels during LPS stimulation in differentiated THP-1 cells. Stx1 had no apparent effect on mRNA levels of these cytokines in LPS-stimulated cells. Reduced cytokine mRNA may mean reduced production of these important cytokines and reduced ability of the host to mount a good immune responses against the toxin-producing *E.coli*.

Adam Ibrahim
SUNY, University at Albany
Class of 2018

Characterization of the aorta of vascular smooth muscle cell-specific BCL11B knockout mice.

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Background: Aortic stiffness (AS), or loss of compliance of large arteries, is an independent predictor of cardiovascular events, in addition to other risk factors such as diabetes and obesity. Our laboratory discovered that the transcription factor BCL11B protects against AS. However, the molecular mechanisms used by BCL11B to regulate AS are unknown. The goal of this study was to characterize the vascular phenotype of vascular smooth muscle (VSM) cell-specific BCL11B knockout mice (BSMKO) and to understand how this deletion affects the aorta on a cellular and molecular level.

Methods: 24-hour continuous blood pressure (BP) recordings were acquired by radio telemetry in BSMKO (n=8) and wild type (WT; n=7) mice before and after angiotensin II infusion (1 µg/kg/min) to determine the effects of VSM BCL11B deletion on BP regulation. Aortic diameters were measured on echocardiographic images of aortic arches of BSMKO (n=8) and WT (n=6) mice to determine whether VSM BCL11B deletion would affect the structure of the aorta. To characterize cellular and molecular mechanisms, we used 10 µm aortic sections from WT (n=1), BSMKO (n=1), WT/angII (n=3) and BSMKO/angII (n=5) mice for immunohistochemical staining of the following targets: tissue morphology (hematoxylin and eosin), elastin and collagen (with a modified Van Gieson staining), apoptosis (with TUNEL staining), calponin-1 (a VSM cell marker), galectin-3 and CD68 (two markers of inflammation).

Results: VSM-specific deletion did not significantly affect BP or aortic diameters in BSMKO mice compared to WT. However, angII infusion caused aortic aneurysms in BSMKO mice. TUNEL staining of aortic aneurysms revealed increased number of apoptotic cells, lack of calponin-1 and inflammatory markers in BSMKO/angII aortas compared to WT/angII mice, suggesting increased VSM cells death but lack of inflammation in BSMKO aneurysmal aortas.

Conclusion: At baseline, BCL11B deletion in VSM cells does not affect blood pressure or gross morphology of blood vessels in mice. However, VSM BCL11B is crucial to maintain the structural and functional integrity of the aorta.

Reagan Katulege
University of Massachusetts Amherst
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The Association between Depressive Symptoms and Cortical Thickness as Modulated by Aerobic Fitness

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Background: Prior studies have demonstrated that adult hippocampal neurogenesis (AHN) occurs in rodents, non-human primates, and humans. Previous research has shown that AHN in rodent models is upregulated by aerobic exercise. In comparison, depression functions in an opposite capacity, being directly related to the down-regulation of AHN. While AHN cannot yet be measured directly in humans in vivo, research with human participants has shown that higher aerobic fitness is directly related to structural changes in the adult brain: Previous research in our lab identified greater aerobic capacity (VO2 Max) being positively correlated to greater right entorhinal cortical volume using voxel-based morphometry. Here, we use a surface-based analysis to assess the association between depression scores and entorhinal cortical thickness as modulated by aerobic fitness.

Methods: A cross-sectional study was conducted in order to investigate two aims. The first aim sought to assess the relationship between depression scores and entorhinal cortical thickness. The second aim sought to assess the relationship between fitness and entorhinal cortical thickness. Baseline data were collected from a cohort of 58 young adults (18-35 years, 65% female) who enrolled in an exercise study at Boston University School of Medicine. Additional regions of interest (ROIs) included the hippocampus, amygdala and insular cortex because these regions have previously been cited in studies involving major depression disorder (MDD). Participants underwent magnetic resonance imaging (MRI) to obtain T-1 weighted images. These images were then processed by FreeSurfer 6.0 for cortical thickness and subcortical volumes, which were used as outcome variables. VO_2 Max was estimated using a treadmill with a modified Balke protocol. Predictor variables included fitness percentile, which was calculated based on the participant's estimated VO_2 Max, age, and sex, and Becke Depression Inventory II (BDI) scores, used as a measure of depressive symptoms.

Results: In a multiple regression analysis, left entorhinal cortical thickness was found to have a significant positive relation with fitness percentile while controlling for the effects of intracranial volume, age and sex (r = .520, R^2 = .213, p = .001). In a partial correlations analysis, individuals of lower fitness displayed a significant positive relationship between depression score and left insular cortex (r = .516, R^2 = .266, p = .010) and right insular cortex (r = .516, R^2 = .266, p = .010).

Conclusion: The relationship between depression and cortical thickness while present is not well-represented in our young adult sample. Participants with more variability in their depression scores may be needed in order to elucidate this relationship. However, consistent with our hypothesis and previous research, fitness is positively associated with structural changes in the entorhinal cortex, a brain region of the medial temporal lobe memory system that has direct anatomical connections with the site of AHN, the dentate gyrus subfield of the hippocampus. Future work in the Brain Plasticity and Neuroimaging Laboratory will examine this relationship in older adults and in participants with and without depressive symptoms.

Bria Landry
Xavier University of Louisiana
Class of 2018

Correlation between Protein Kinase CK2 and Myc in T-cell Acute Lymphoblastic Leukemogenesis

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Background: T-Cell Acute Lymphoblastic Leukemia (T-ALL) is an aggressive hematologic malignancy that results from the malignant transformation of T-cell progenitors. T-ALL accounts for about 10-15% of all pediatric cases, and about 25% of adult cases. A substantial amount of T-ALL cases are also fatal. Because the frequent use of multi-agent cytotoxic drugs lead to disease relapse and high toxicities, there is a great need for targeted therapies. The pro-oncogene, Myc, is over-expressed in many T-ALL cells. It also acts as an enhancer that increases gene expression. Myc and CK2 subunits are both over-expressed in T-ALL cells, but their inhibitor can kill T-ALL cells. The combination of the two inhibitors can increase the ability of killing T-ALL cells.

Methods: In order to determine the correlation between Protein Kinase CK2 and Myc, a variety of scientific techniques were used. Specifically, the subcloning of shRNA into PLKO1, cell culturing, quantitative polymerase chain reaction (qPCR), and western blot.

Results: From the research, it was found that CK2 expression is elevated in human T-ALL cell lines together with NOTCH1 and MYC. It was also found that Myc protein levels were down regulated in shluc and shMyc Jarkat cells. Also, CK2a1 protein levels was down regulated at day 4, but up regulated on day 6, in the shluc and shMyc Jurkat-BCL2 over-expressed cells, which opened many questions that will be later evaluated.

Conclusion: MYC pro-oncogene can regulate CK2a1, CK2a2, CK2b in T-ALL cells. That is the reason that when MYC levels were high, CK2a1, CKa2, Ck2b were over-expressed.

Andres Lojano-Bermeo
CUNY, Hunter College
Class of 2018

Using Red Fluorescent Protein to find potential targets to treat HIV

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Background: Although potent combination antiretroviral therapy can effectively block viral replication in the host, human immunodeficiency virus (HIV) persists due to the existence of transcriptionally silent proviruses residing primarily in a very small population of resting CD4+ T cells. These silent infections do not generate viral particles and are thus resistant to antiretroviral therapy. In addition, these silently infected cells have long lives and can be stimulated to produce live HIV at any moment, making live long therapy of infected individuals necessary. Therefore, understanding how this population of latently infected cells is generated and regulated is essential if HIV infection is to be cured. We hypothesize that a process called HIV cell-to-cell transmission can generate latently infected cells by direct infection of resting CD4+ T cells.

Methods: We confirmed that silently infected T cells are generated after HIV cell-to-cell transmission and these cells can be induced to produce HIV upon stimulation. To further investigate which subpopulations carry latent HIV after cell-to-cell transmission, we needed to develop a reporter virus that can help us track infected cells. In order to investigate this, we developed an HIV-based reporter virus carrying a constitutively active red fluorescent protein. Results: Our preliminary work suggests that this virus can be used to track by flow cytometry silently infected cells generated *in-vitro.* . Finally, we hypothesize that cellular transcription factors recognize proviral sequences outside of the canonical proviral promoter and regulate proviral expression. To test this, we generated PCR amplicons that expanse the full HIV genome and will be applied to a high throughput screen for human transcription factors.

Conclusion: Altogether, our work aims at further understanding the mechanisms for the generation and regulation of HIV silent infection in T cells with the goal of elucidating new potential targets to treat HIV infection.

Luis Marquez
Xavier University of Louisiana
Class of 2018

Return to Classroom sports related TBI-A Pilot Study

Boston University School of Medicine

Marquez L, Ritchie M, Raskins A, Patel R, Stucky J, Lerner E.

Introduction: In 2013, the Center for Disease Control and Prevention found that approximately 2.8 million people in the United States suffered from traumatic brain injury (TBI) related illnesses that resulted in emergency department visits, hospitalization, and death. Boston University's Ryan Center diagnoses and rehabilitates student athletes as well as other individuals from the greater Boston area who suffer from acute TBIs. Prior research suggests that non-invasive technology that measure biomarkers may be especially useful in finding related symptoms that may go unnoticed during a clinical visit. Understanding the relative symptoms may recovery rates help to quicken the recovery process. Noninvasive biomarkers that have been suggested to measure changes in brain function include cognition, balance, motor, sleep, quality of life. Technologies that measure these biomarkers for acute TBI's have yet to be validated.

Methods: A case-control study will be conducted. Non-concussed patients will be randomly selected. Patients enter the clinic seeking care for acute-TBI or Non-TBI injuries. They will receive the current Ryan Center standard of care, which assess their health history and cause of injury. In addition to this protocol, patients will also be assessed using new technology during their visit(s) and while they on mobile and home devices. We will assess patients for an average of 4-6 weeks until symptoms finally subside and they return to their normal daily activities.

Results: A review of past literature suggests that traumatic brain injuries will be the third leading cause of death by the year 2020 (Hyder, 2007; Meaney, 2014). Major causes of TBI include road traffic injuries (60%), falls (20-30%), violence via domestic or military (10%), and injuries sustained while working, including sports (10%). In regards to technological incorporation, studies have found goal setting feature of mobile applications to especially be helpful for patients to adhere to medical goals (Evald, 2014). The primary objective of the prospective study is to implement technology to reduce time to recovery. The secondary objective is to incorporate technology that is used to monitoring acute TBI symptoms. We hypothesize that implementing new technology will reduce time to recovery and improve the healing process.

Conclusion: TBIs may eventually lead to excessive medical visits and neurological disabilities/diseases. Based on the current review, the study will implement several outcome measures that may effectively help in determining biomarkers and quicken recovery. In relation to this pilot study, we will be focusing on several outcome measures to monitor patient recovery: cognition, balance, motor, quality of life, sleep quality, etc. Implementation of these devices will lead to lower health care cost, validation of technological use for recovery after a TBI, and broaden the scope of a shareable e-platform for future research.

Vangelina Osteguin
University of Texas-San Antonio
Class of 2019

Examination of ER Visits After an Infrainguinal Bypass

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Background: Infrainguinal bypasses are notorious for ER visits and readmissions 30 days after the procedure. We wanted to analyze the reasons behind the ER visits to see where falls in patient care can be avoided during the index encounter and to also see how they differ from ER visits within 90 days of the surgery.

Methods: A retrospective review and analysis of all of Boston Medical Center's non-emergent infrainguinal bypass patients between 2007 and 2017. The patient demographics, past medical history, procedure details and reasons for visits to the hospital after bypass surgery were extracted from medical records.

Results: A total of 421 patients that underwent an infrainguinal bypass within the last 10 years were identified. Patients were on average 65 years old (SD 10.5), most of the time either Caucasian (47.3%) or African American (39.2%), and mainly males (62.2%). As for comorbidities, hypertension (86.7%), current/former smoker (73.3%), and diabetes (59.9%) were of highest occurrence. Indications for the bypass were due to either rest pain or tissue loss (73.6%) and most of the tissue loss were due to ulcers on the foot (75.7%). Most bypasses consisted of a common femoral origin (65.8%) to an above knee popliteal target (26.8%) using an autologous vein (69.4%) and had an average post-operative length of stay of 7 days (SD 7). About a quarter of the patients have their first visit to the ER within 30 days while for 90 days there is an increase to 36.8%. For the 30-day visits, surgical site infections were the main reason for visiting at 24.3%. The main reasons for visits between 31-90 days were other infections not related to the surgical site (14.9%), foot wounds, (10.6%), and falls or other traumas (8.5%). Of the 155 that visited the ER at all, 29 of those patients continued to visit the ER up to at least 3 times and these visits took place within 31-90 days with the main reasons being diabetes related or contralateral leg issues.

Conclusion: The short term (30-days) and long term (90-days) visits vary in reasons from focusing on the bypass whether it be surgical site infections or graft complications to preexisting comorbidities and the contralateral leg needing more attention. Since the majority of the visits were due to infections related to the index procedure, interventions can come about from pointing out good techniques for caring for the extremity.

Kalynn Parks
Tennessee State University
Class of 2018

Sympathoexcitation and Increased Sodium Chloride Cotransporter Activity in Hypertensive Aged Sprague Dawley Rats

Kalynn Parks, Alissa Frame, Jonique George, Franco Puleo, Richard Wainford

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Boston University School of Medicine Division of Graduate Medical Sciences

Background: As of now hypertension affects about one in three American Adults, and the prevalence increases to two in three adults above the age of 65. Hypertension has a wide variety of causes, including increased sympathetic tone and impaired renal sodium handling.

Methods: Mean arterial pressure (MAP) was monitored in 3-month, 8-month, or 16-month conscious male Sprague Dawley rats maintained on a lifetime normal salt diet. Measures of sympathetic tone (global and renal norepinephrine [NE] content and peak depressor response to hexamethonium), sodium chloride cotransporter (NCC) activity (peak natriuresis to hydrochlorothiazide [HCTZ]), and renal protein expression were also evaluated. Separate groups of 3 and 16 month rats underwent bilateral renal denervation or chronic subcutaneous HCTZ treatment and MAP was monitored on day 14.

Results: Basal MAP and NCC activity increased with age in rats (MAP [mmHg]; 3-month 124±2 vs 8-month 140±1 vs 16-month 149±3, $P<0.05$: peak ΔUNaV to HCTZ [μeq/min]; 3-month NS 9±1 vs 8-month NS 18±2 vs 16-month NS 15±5, $P<0.05$). Measures of sympathetic activity, including global and renal NE levels and peak depressor response to hexamethonium, also increased with age in rats on a NS diet (plasma NE [nmol/L]; 3-month 44±4 vs 8-month 55±3, $P<0.05$: renal NE [pg/mg]; 3-month 612±36 vs 8-month 835±48 vs 16-month 974±39, $P<0.05$: peak depressor response to hexamethonium [mmHg]; 3-month -33±4 vs 8-month -64±5 vs 16-month -60±3, $P<0.05$). Renal protein expression of total WNK1 and phosphorylated SPAK/OxSR1also increased with age. Bilateral renal denervation and chronic subcutaneous HCTZ treatment improved MAP in 16-month old rats.

Conclusion: Hypertensive aged rats displayed increased sympathetic tone, NCC activity, and renal protein expression and activity of kinases involved in NCC activation. Importantly, removal of renal sympathetic outflow and chronic NCC antagonism reduced blood pressure in aged rats. We hypothesize that increased renal sympathetic outflow drives NCC activation through a WNK1-SPAK/OxSR1 pathway to promote age-related hypertension.

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Characterization of Neisseria gonorrhoeae derived Outer Membrane Vesicles (OMV)

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Background: The human bacterial pathogen Neisseria gonorrhoeae is the causative agent for the sexually transmitted disease, gonorrhea. Since this bacterium has become increasingly more antibiotic resistant, new treatments or preventative therapeutics are needed. Currently, there are no vaccines available for gonorrhea. Outer membrane vesicles are spherical buds released from gram negative bacteria containing lipooligosaccharides (LOS), proteins, lipids, and other molecules. These are produced by many gram-negative bacteria, including Neisseria gonorrhoeae. We wanted to characterize the immunostimulatory ability of natural outer membrane vesicles (OMV). The poly antigenic ability for nOMVs could help increase the likelihood of immune resistance in the case that the adaptive immune system is equipped with antibodies that can recognize more than a single antigen upon infection.

Methods: A Lowry protein Assay and gel electrophoresis were used in the characterization of the nOMVs. We performed an ELISA (Enzyme linked immunosorbent assay) on 2 aliquots of OMVs taken in February 2017 and September 2017 to access and indications of immunostimulatory capability. The Elisa utilized TLR 1, TLR 2, and TLR 4 to monitor Pathogen Associated Molecular Pattern specific immune stimulation.

Results: The characterization techniques indicated the presence of both LOS and P1B as OMV components. The results of the ELISA indicated a positive immune stimulation from LOS and P1B.

Kevin Ruaro
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Nano formulation effects of miR-200c and miR-1195 on lung cancer cell lines

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Background: microRNAs have been shown to possess post-transcriptional editing properties in the regulation of certain gene products. The following microRNAs: miR-200c and miR-1195 have been shown to play a role in regulating transcription factor gene products essential to cellular growth and division; however, the exact molecular mechanisms by how this occurs is still unclear. By using a liposomic nano-vehicle encasing the microRNAs as treatment, we hope to understand the molecular machinery behind their regulatory roles.

Method: RNA-Seq and Quantitative reverse transcription polymerase chain reaction (RT-qPCR) was used to target specific gene products under investigation when three lung cancer cell lines (KW634, 821T4, 821LN) were treated with the microRNAs (miR-200c and miR-1195), with and without the liposomic nano-vehicle. A migration assay was also carried out to observe the inhibitory effects of our miR-200c Nano formulated in two of the three lung cancer cell lines.

Results: When the cancer cell lines were treated with miR-200c, both encased within the Nano-vehicle compared to the non-encapsulated, we found an altered expression pattern in NKX2-1, Six4, Six1, Sox2, Myb, Nfib, BCL2, and Gtf2ird1 transcription factors with slight variance between cell lines. In the migration assay, we found a decrease level of migration in the 821LN metastatic cell line.

Conclusion: These studies suggest that the treatment of lung cancer cell lines with Nano-encapsulated miR-200c compare to non-encapsulated, better affect downstream gene products that play a role in regulating transcription factors essential to cellular growth and division with a decrease in cellular migration.

Diego Trujillo
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Understanding the Molecular Mechanism of *Hnrnph1* Underlying Methamphetamine Sensitivity

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Background: Methamphetamine (MA) is a psychostimulant that is highly addictive and causes thousands of premature deaths and hospitalizations every year. The LAG at BU is dedicated to understanding the MA addiction as a whole, in order to combat the widespread abuse of MA. Currently, addiction is largely misunderstood due to the fact that genetic research concerned with properties of addiction is fairly new. Understanding the mechanisms of addiction is a necessary step towards engineering an efficient treatment for MA sensitivity, which could save countless lives. *Hnrnph1* was identified as the gene responsible for methamphetamine sensitivity using quantitative trait locus mapping. This was confirmed using TALENS mediated knock-down of the *Hnrnph1* gene. Hnrnph1 knockout mice showed a reduced sensitivity to the stimulant and rewarding effects of MA. After successfully finding that the *Hnrnph1* gene is responsible for MA sensitivity, the Laboratory of Addiction Genetics at BU is now investigating how this gene regulates MA sensitivity.

Methods: *Hnrnph1* is expressed ubiquitously in the brain. In order to make the jump from what genes cause addiction to how do genes cause addiction, we must understand mechanism of action of *Hnrnph1* in the reward circuit. Understanding the brain-region and cell-type specific function of this gene would help elucidate its mechanism of action in the regulation of MA. For this analysis, a knock-in mouse model was engineered with loxP sites flanking the first coding exon of *Hnrnph1* via TALENS-mediated gene editing. The Cre-loxP system can then be used to finely manipulate the expression of Hnrnph1 in specific brain regions and cell type within the reward circuitry. The goal of this project is to determine that that loxP sites inserted in the right place in the genome using long-range PCR. The presence of the loxP sites will be confirmed using PCR genotyping & gene sequencing. To do so, we needed to optimize our PCR assay. Optimization of the PCR assay included designing primers, as well as adjusting the annealing temperatures, DNA content, and specific Taq polymerase used.

Results: I have successfully optimized a long-range PCR assay for confirmation of loxP sites in the heterozygous knock-in mice. Currently, we are working on cloning the amplified DNA bands to send in to Genewiz for sequencing. Once loxP insertion sites are confirmed, the lab will go forward with viral mediated Cre recombinase delivery into specific brain regions to investigate which brain region is responsible for the function of *Hnrnph1* in the regulation of MA.

Conclusions: Understanding the mechanism of how *Hnrnph1* regulated MA and other psychostimulant would give insight into novel therapeutic targets for treatment of MA dependence.

LeShell Washington
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The Function and Regulation of Lectin-like Oxidized Low-Density Lipoprotein Receptor -1 (LOX-1) During Pneumonia

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Background: Pneumonia is a debilitating condition commonly caused by various bacterial, viral, and/or fungal pathogens in the lower respiratory tract. Infection triggers an inflammatory response in the alveoli, which results in the accumulation of leukocytes and proteinaceous edema fluid. Despite substantive advances in many areas of biomedical research, pneumonia mortality is virtually unchanged since the discovery of antibiotics, and pneumonia persists as the leading cause of death for children under the age of 5. Our group is focused on understanding the balance between bacterial clearance and host tissue resiliency during pneumonia. Our long-term goal is to identify specific processes controlling lung injury during pneumonia. To this end, transcriptional profiling of lung epithelial cells revealed expression of the lectin-like oxidized low-density lipoprotein receptor-1 (LOX–1) in mice with pneumonia. Although previous studies have linked LOX–1 to the pathogenesis of atherosclerosis, another important inflammatory condition, neither its role in epithelial cells nor its influence on pneumonia have been considered. We hypothesized that the inhibition of LOX-1 would reduce lung injury in mice with pneumonia.

Methods: Both *in vitro* and *in vivo* studies were performed to assess the roles of LOX -1 during pneumonia. MLE-12 cells, which represent an alveolar epithelial cell line, were stimulated with live *E. coli* or bronchoalveolar lavage fluid (BALF) collected from mice (n=6) 0 or 24h after an intratracheal (IT) *E. coli* challenge. LOX-1 mRNA expression was measured by qRT-PCR in lysates from MLE-12 cells and purified lung epithelial cells. To determine the influence of LOX-1 on pneumonia, mice (n=6) were co-instilled IT with *E. coli* and either a neutralizing anti-LOX-1 or control antibody. BALF was collected to assess cellularity (using a hemacytometer) and proteinaceous edema (using a BCA total protein assay).

Results: Both *E. coli* and pneumonic BALF significantly increased epithelial LOX-1 mRNA expression. No significant differences in BALF neutrophil or macrophage numbers were observed following LOX-1 neutralization. Surprisingly, a significant increase in the total amount of protein was noted in BALF from *E. coli*-challenged mice treated with anti-LOX-1 (vs. control antibody), indicating enhanced edema.

Conclusion: Contrary to our original hypothesis, our data suggest that LOX-1 is induced in lung epithelium to limit tissue injury during pneumonia. However, future studies are necessary to better understand if LOX -1 influences pneumonia outcome.

The STaRS program would like to acknowledge the achievements of our students and the faculty and researchers across BU Medical Campus whose mentorship has enhanced both the quality of our students' work and their overall summer experience. Thank you!

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