SUMMER TRAINING AS RESEARCH SCHOLARS (STaRS) PROGRAM

Research Symposium

Thursday, August 9, 2018

Boston University Graduate Medical Sciences
Summer Training as Research Scholars

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STaRS Research Symposium Schedule
Thursday, August 9, 2018
2:00 PM – 4:30 PM
Boston University School of Medicine, Hiebert Lounge

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.

2:00 – 2:10 pm Welcome

Dr. Linda Hyman, Associate Provost

Dr. Isabel Dominguez, STaRS Director and Associate Professor

Ms. Farrah Belizaire, STaRS Administrator and Manager of Diversity and Inclusion

2:10 – 3:10 pm Oral Presentations
- Seidu Sumani (2:10)
- William Molina (2:25)
- Jacob Flores (2:40)
- Victor Bacelar (2:55)

3:15 – 4:15 pm Poster Sessions
- Kidest Assefa-McNeil
- Vinson Cobb
- Hasahn Conway
- Zachary Croll-Nesbeth
- Diego De Alba
- Jean Devera
- Anayah Ferris
- Elizabeth Osota
- Sabreea Parnell
- Keyona Pointer
- Shelsea St. Hillien
- Vincent Turnbull

4:15 – 4:30 pm Awarding of Certificates & Closing Remarks

Kidest Assefa-McNeil
Brown University
Class of 2019

Toll-Like Receptor Adjuvants Influence Antigen Deposition on Follicular Dendritic Cells

Kidest Assefa-McNeil, Christina Lisk, Danielle Antos, Rachel Yuen, Dr. Lee Wetzler
Boston University School of Medicine, Section of Infectious Diseases, Boston, MA 02118

Background: Vaccines are a critical medical advancement that have saved the lives of millions of people. There are, however, a multitude of diseases that we lack an efficacious vaccine for protection. Adjuvants are immunostimulatory molecules present in vaccine compositions. Toll-like receptors (TLRs) are immune cell receptors that cause cytokine expression from stimulated cells. TLR agonists have been used in vaccine development as adjuvants. PorB is an adjuvant isolated from the outer membrane of *Neisseria meningitides* bacteria. The Wetzler lab has shown that using the protein PorB increases both hummoral and cellular responses *in vitro* and *in vivo* models. CpG is DNA utilized as a second adjuvant in this study.

In order for antibodies to be created, the antigen ovalbumin (OVA) is deposited on follicular dendritic cells (FDCs) within the lymph node. CD169+ macrophages line the lymph node in the subcapsular sinus. FDCs and CD169+ macrophages have been shown to have cellular connections – B cells move the antigen from CD169+ macrophages and deposit the antigen on to the FDC allowing for the production of antibodies specific to the antigen. The purpose of this project is to investigate how TLR adjuvants influence antigen deposition on FDCs. Utilizing confocal microscopy, a time course study was designed to focus on qualitatively determining the optimum time period after vaccination for antigen deposition with adjuvants.

Methods: Wild type B6 mice were separated into three vaccination groups: OVA, OVA + PorB, and OVA + CpG. OVA was labeled with an Alexa 594 fluorochrome. Each group was further separated into day 1, day 2, and day 3 after injection. Lymph nodes from an animal in each group were isolated and the tissue was cut using a microtome at 5 μm. After placing the tissue on microscope slides, the slides were stained for CD169+ macrophages and FDCs. A Leica SP5 confocal microscope was used to image the tissue.

Results and Conclusion: In correlation with the vaccine regimen previously used in the lab concerning PorB as an adjuvant, we expect to see greater OVA deposition on FDCs in tissue from mice with adjuvant injections. Because PorB causes an up regulation in pro-inflammatory response, we would also like to confirm that pro-inflammatory cytokines are up regulated in the lymph node.

Victor Bacelar
University of Massachusetts, Lowell
Class of 2020

Inhibiting BRCA1-Dependent CPT-Induced Ubiquitination of Topoisomerase I by a small molecule to stabilize topoI, overcome drug resistance and enhance CPT sensitivity.

Victor Bacelar, Dr. Ajit Bharti
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Background: In the presence of camptothecin, CPT-resistant colorectal cancer cells degrade topoisomerase I via a DNA damage response mechanism and successfully replicate their DNA. Irinotecan, a camptothecin analogue, is commonly used to treat colorectal cancer patients. Though it's a first or second line therapy, only a small percentage of patients respond to the drug. CPTs represent highly potent class of drugs however only 12-32% patient respond to the drug and mechanism of resistant is not understood. Bharti lab has demonstrated that CPT induced topoI degradation determines CPT resistance. The cells that degrade topoI rapidly by ubiquitin proteasomal pathway (UPP) are resistant to CPT. They have also demonstrated that BRCA1 ubiquitinates topoI in response to CPT and the lab is working on to discover small molecule that would inhibit topoI ubiquitination by BRCA1. A class of small molecules were discovered to bind to the BRCT domain of the BRCA1 and inhibit ubiquitination.

Hypothesis: With the addition of the small molecule-camptothecin treatment, BRCA1 would be blocked from degrading topoisomerase I which would increase cellular sensitivity to CPT.

Methods: HCT15, colorectal cancer cells, were grown in medium with fetal bovine serum with essential growth factors and nutrients. An experiment using SDS-PAGE and immunoblot analysis was performed to determine topoisomerase I stabilization in the presence of the small molecule with camptothecin. The cells were pretreated with small molecule and one class of drug that acts as a proteasome inhibitor. After 48 hours with the small molecule pretreatment and 16 hours with proteasome inhibitor, camptothecin was added for 3 hours. Cell lysates were prepared 20 minutes after removal. Lysates of no treatment, camptothecin treatment, and combination treatment were used in the immunoblot analysis to measure their topoI activity. The second experiment was used for capturing topoI stabilization under Leica microscope. This experiment used genome edited colorectal cancer cells with green fluorescent protein in the topoI gene. Before drugs were applied, cover slides were placed in the culture dishes. The cells would adhere to the slides and grow while being treated. Cells were pretreated with small molecule for 48 hours and subsequently treated with camptothecin for 3 hours. The cover slides were washed, stained with DAPI, and fixed onto slides for imaging.

Results: The SDS-PAGE and immunoblot analysis results indicated that topoI is being stabilized by the small molecule in the presence of camptothecin. The control lane had a strong topoI band while the camptothecin alone lane had a dim band for topoI. The lane which had small molecule-camptothecin combination had a strong topoI band. The proteasome –camptothecin combination correlated with past studies and had a strong band as well. The images taken with the Leica microscope displayed topoI stabilization in combination treatment and indication of initial apoptosis. Control images were taken as baseline, and images with cells treated with small molecule alone were imaged and similar to control. Cells treated with camptothecin alone had a darker pigment of topoI. There was much less green fluorescence which meant little topoI in the nucleolus and some cells exhibited irregular shapes indicating the initial stages of apoptosis. The combination treatment had more concentrated topoI in the nucleolus and brighter green in the nucleus of the cells.

Conclusions: These results indicate that topoI degradation is being inhibited through the combination treatment and increasing cell sensitivity to campothecin. The results of both experiments revealed that the addition of the small molecule in combination with campothecin inhibit topoI degradation and increase its stabilization. The dimming of green color in the Leica microscope images were due to topoI leaving the nucleolus and compensating for the degraded topoI. With the combination, topoI remained in the nucleolus because topoI outside the nucleus wasn't being degraded which is shown by the bright green color. SDS-PAGE immunoblot analysis helped to show that there is substantially less degradation of topoI in combination treatment than with camptothecin alone. Future SDS-PAGE and immunoblot analysis experiments with optimized combination concentrations can be used to achieve more similar topoI bands in both the control and the combination lanes. Future experiments can be planned on the bases of these findings to optimize drug concentration to find how much of small molecule and cpt will have the most effective lethargic synergy. These experiments are intended to find an improvement of cancer therapy. Many colorectal cancer patients suffer with the current therapies which use a combination of cytotoxic agents. The answer to camptothecin resistance is this newly discovered drug class. If it can be clearly demonstrated that these small molecules increase cell sensitivity to camptothecin without being toxic to patients, these drugs could be used in clinics to treat millions of patients suffering with colorectal cancer across the world.

Vinson Cobb
Morehouse College, Class of 2017
Boston University School of Medicine, Class of 2021

Capitalizing on Hospitalization to Engage Low Socio-Economic Status Smokers in Low-dose CT (LDCT) Screening

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Background: An estimated 154,050 Americans will die from lung cancer, the leading cause of cancer death, in 2018. Most lung cancers are attributable to smoking, and quitting smoking is the most effective intervention to reduce lung cancer mortality. In addition, lung cancer screening (LCS) is also effective at reducing lung cancer death but is underutilized nationally. At Boston Medical Center, the largest safety net hospital in New England, less than 10% of LCS-eligible patients have been screened. In prior work we identified competing demands and limited time during primary care clinic visits as barriers to shared-decision-making (SDM), a Medicare requirement for LCS reimbursement and treatment for tobacco dependence. Hospitalization may be an opportunity to intervene with two interventions to reduce lung cancer mortality: smoking cessation and referral for LCS. The goal of study is to test the effect of adding an LCS-screening SDM intervention to inpatient smoking cessation counseling on completion of LCS, patient knowledge, and smoking cessation.

Methods: We are currently enrolling hospitalized LCS -eligible patients for a two-arm RCT comparing an inpatient SDM intervention (SDM using a decision aid +smoking cessation counseling) compared to the control condition (decision aid +smoking cessation counseling). We characterized reasons for ineligibility. We performed an interim analysis of early trial enrollment (n=81) of all current smokers who were LCS-eligible. We characterized trial enrollees to date by demographics (sex, age, race, insurance, and education level). We compared knowledge about LCS screening using a 23-item instrument at baseline and 24-hour post enrollment in both groups.

Results: Among our enrollees (n=81), 61.7% were male, 60.5% were African-American, and 70.4% listed Medicaid or MassHealth as their primary or secondary insurance (Table 1). A significant proportion of hospitalized smokers have had a diagnostic CT chest in the past year, limiting LCS eligibility (Figure). Knowledge of LCS screening improved post intervention in some categories, but not all.

Conclusion: Our study found that 15.4% of LCS-eligible smokers appear to be appropriate candidates for LCS screening. Despite, knowledge of LCS risk factors, benefits, and the screening process improve with the introduction of the intervention. Hospitalization at an urban safety net hospital may provide the opportunity to improve screening rates in an underserved population by referring appropriate candidates for outpatient LCS.

Hasahn Conway
Xavier University
Class of 2016

Identification of Novel Transcription Factors that Regulate HIV-2

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Department of Infectious Diseases, Boston Medical Center
Boston University School of Medicine, Boston, MA, 02118

Background: There are 40 million people infected with the Human Immunodeficiency Virus (HIV). HIV is a human lentivirus and contains two subtypes, HIV-1 and HIV-2. Although HIV-1 is responsible for the majority of HIV infection, HIV-2 is prevalent in West Africa having infected 1-2 million people. HIV-1 has been the primary focus of research in this area, whereas HIV-2 has not be studied as extensively. HIV-1 and HIV-2 have differences in their tropism, pathogenesis, course of infection, and latency. HIV-2 is not as virulent as HIV-1 being attenuated in its ability to cause disease. Characterization of the array of transcriptional regulators responsible for HIV-1 and HIV-2 will provide novel insights into their viral pathogenesis.

Hypothesis: How novel transcription factors are modulated on HIV-2 in specific cell types.

Methods: A yeast-one-hybridization (Y1H) assay was performed, to screen for novel transcription factors that bound the HIV-1 and HIV-2 long-terminal repeat (LTRs). From this screen, fifty transcription factors (TFs) that bind to the long-terminal repeat LTR of the SIV, HIV-1 and HIV-2 were identified. To validate the expression of these TFs in specific cell lines, reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) was performed on macrophages, dendritic cells (DCs), human embryonic kidney cells (293Ts), and CD4+ T cells. The TFs KLF3 and KLF2 both repress HIV-2 transcription when overexpressed in 293T cells

Results: The TFs KLF2 and KLF3 were found to bind all LTRs of three viruses. Other TFs such as PLAGL1 which only bound the HIV-2 LTR, and GABpa only bound HIV-1 and HIV-2. After analyzing mRNA from cells that had been down regulation for KLF2 and other cells with a down regulation in KLF3, the data showed that these two TFs have the ability to repress HIV. We are interested to understand if the repressive characteristics of these two TFs are linked to HIV-2 latency.

Conclusion: Although, KLF2 and 3 have shown repression in 293Ts, the other TFs will inform us of their function as co-repressors in HIV-2 expression through further RT-qPCR and transcription knock down experiments. Future experiments will improve the understanding of these TFs and their impact on expression of HIV-2 and potential role in latency.

Zachary Croll-Nesbeth
Oakwood University
Class of 2019

Aerobic Fitness Negatively Predicts Recollection

Zachary Croll-Nesbeth, Michael Rosario BS, Karin Schon PhD
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Background: Recognition memory is the accurate identification of previously encountered stimuli and can be divided into two components: recollection and familiarity. A dual-process model posits the hippocampus (HC) of the medial temporal lobe as the region responsible for recall, with parahippocampal regions responsible for familiarity. Cardio-respiratory fitness (CRF) has been shown to modulate hippocampal memory in both human participant and animal model research, with rodent model studies showing an increase of neurotrophic factors like brain derived neurotrophic factors (BDNF) and insulin-like growth factor (IGF-1), regulators of neurogenesis and neuroplasticity. Previously, our lab showed a direct relationship between CRF and entorhinal cortex (EC) volume, a primary input structure of the HC, as well as a significant interaction effect of BDNF by aerobic fitness on overall recognition memory.

Hypothesis: 1) CRF predicts recall, but not familiarity. 2) CRF predicts cortical thickness or subcortical volume of the EC and HC.

Methods: Data from a cross-sectional study (n = 62, 64% female) was used to investigate our aims. We used multiple mediation models to examine the relationship between CRF (VO 2 max) and recollection as mediated by BDNF and IGF-1. VO 2 max was measured using a standard graded maximal treadmill test, which was transformed to CRF percentile to normalize data across sex and age. T1-weighted structural MRI volumes were collected in a subsample (n =33) and analyzed using FreeSurfer 6.0, and *a priori* regions of interest were selected (HC and EC) for analysis. Sex and intracranial volume were included as covariates.

Results: Using a mediation model predicting recall from CRF percentile, with IGF-1 as a mediator, we found a significant negative effect of CRF on recollection (p = 0.0383, CI = -0.5036, -0.0144) but no indirect effect of IGF-1 on recall (*effect* = 0.0442, BootCI = -0.0360, 0.1454). The same model did not predict familiarity (p = 0.0823, CI = -.0339, 0.5460), with IGF-1 as a mediator. A subsequent partial correlation analysis yielded similar results for the negative relationship between CRF percentile and recall, while controlling for sex (r = -0.270, p = 0.038). BDNF did not mediate the relationship between CRF percentile and recollection. A multiple regression analysis revealed a negative trend for CRF predicting left entorhinal cortical thickness with intracranial volume and sex as covariates (r = -0.242, p = 0.091).

Conclusion: We found no relationship between CRF and familiarity in our participants; however, we did find a significant negative correlation between CRF and recollection. We also saw a negative trend for fitness predicting left hemisphere entorhinal cortical thickness. This relationship between recollection and fitness percentile is consistent with the dual-process model, but the negative relationship deserves further examination.

Diego De Alba
University of California, Los Angeles
Class of 2019

Neural plasticity following cortical injury: alterations to the dendritic morphology of pyramidal neurons in primate premotor cortex.

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Background: Stroke, as the leading cause of adult disability and the fifth leading cause of death, is an important public health problem in the United States. Using a non-human primate rhesus monkey (*Macaca mulatta*) model of cortical injury, leading to impairment of fine digit movement in the hand, we studied the degree of functional recovery and the effects of treatment with exosomes. Motor tests assessing hand motor function demonstrated that monkeys exhibited varying degrees of recovery of function, with monkeys treated with exosomes exhibiting the highest degree of recovery. In this study, we specifically addressed the fundamental question of whether functional recovery after cortical injury in this model is associated with changes in the structure of individual neurons in perilesional premotor cortices (PMC).

Methods: Once animals reached asymptotic levels of motor function recovery, tissue was harvested from PMC to study assess changes in dendritic morphology of individual pyramidal neurons, which plausibly underlies plasticity to support recovery. Using whole-cell patch clamp, intracellular filling and confocal microscopy, we compared the dendritic architecture of layer 3 pyramidal neurons in non-lesioned control and perilesional PMC. Entire dendritic arbors of these neurons were reconstructed in 3D using image analysis software (VIAS and NeuronStudio) to the quantify and compare dendritic length, branching topology and complexity, between neurons from the two groups.

Results: Analysis of individual L3 PMC pyramidal neurons showed no significant difference in dendritic morphology between perilesional and control neurons, with both the apical and basal dendrites having similar length and arbor complexity. For both groups, dendritic length and branching were most abundant in the proximal 100-200 um from the soma. These data suggest that after functional recovery from cortical injury, dendritic morphology is either largely unchanged or is reverted back to normative neuron morphology.

Conclusions: The lack of morphological difference in dendritic morphology between perilesional and control PMC neurons suggests that functional recovery after cortical injury is not associated with dendritic hyper proliferation or regression of surviving neurons. Further studies examining neurons at different time points after the onset cortical injury will be important to understand the time course of neuronal changes associated with recovery. Additional inquiry into other aspects of pyramidal neuron morphology, such as spine density, are important to better understand changes in synaptic input that might also play a role in plasticity and recovery. Assessing these changes in dendritic architecture and connectivity will elucidate mechanisms of cortical plasticity and efficacy of exosome treatment in promoting processes of cortical reorganization that are associated with functional recovery.

Jean Devera
University of the Virgin Islands, Class of 2017
Boston University School of Medicine, Class of 2021

Determining the Optimal Medium Condition for AAVDJ/8 Production

Jean Devera, Alessandra Fedoce, Atsushi Sato, Markus Bachschmid
Vascular Biology Unit, Whitaker Cardiovascular Institute, Boston University School of Medicine

Background: Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in the US that is commonly associated with metabolic syndrome, obesity, Type 2 diabetes, hypertension, and cardiovascular diseases. Gene therapy using Adeno-associated viruses (AAVs) have been widely used in the Bachschmid Lab to understand the role of glutaredoxin-1 (Glrx1) via the AAV helper-free system in NAFLD. AAVs provide optimal gene delivery due to their high transduction efficiency, low immunogenicity, and prolonged stable gene expression.

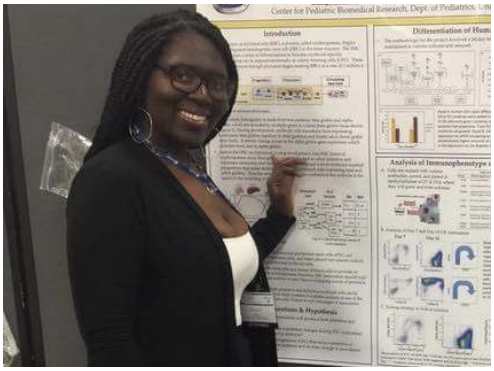
Goal/Hypothesis: Since AAVs are extensively used in the lab, the goal was to determine the ideal conditions for maximal AAV2-DJ/8 production to ultimately improve *in vivo* gene delivery in mouse models.

Methods: To generate AAVs, we used a helper-free system comprised of three vectors: i) AAV expression plasmid ii) AV replication and capsid plasmid (pAAV-DJ/8) and iii) AAV plasmid helper (pHelper). We optimized AAV production by transfection in Human Embryonic Kidney 293 with T-antigen (HEK293T) cells. Cells were transiently transfected using two plasmid, PEI-based transfection. T cells were cultured in Dulbecco's minimum essential medium (DMEM: Gibco) containing deterrents conditions: 0-1% fetal bovine serum (FBS: Promocell), 0-1X GlutaMAX (Gibco), 0-1X HCO3, 0-1X HEPES. On the third and sixth day after transfection, the media were collected for viral titer measurement with real-time PCR.

Results: In this first experiment, the results showed that AAV DJ/8 production was optimized with a daily increase in total AAV production through day 6**.** However, it is not a possible affirmation which conditions are optimal for production yet. Further experiments are necessary.

Conclusion: These results will provide useful information for maximal AAV production for future in vivo gene therapy studies.

Anayah Ferris
University of the Virgin Islands
Class of 2019



Tight Junction Dysfunction: Altered ZO-1 and Occludin in the Diabetic Cornea

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[1]University of the Virgin Islands, St. Thomas, USVI 00802;
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Background: The cornea is the outermost part of the eye and, as a result, can be damaged by debris and other irritants. The cornea can repair itself from minor injuries; however, improper wound repair can further complicate the issue. Type II Diabetes is known to hinder the body's wound healing ability, and in the cornea recurrent erosions are common, with complications in epithelial wound repair being detected in the corneas of diabetic rats. Tight junctions are multiprotein complexes involved in cell proliferation and differentiation, processes required for proper wound healing to occur. Occludin, a transmembrane protein, and ZO-1, a scaffolding protein, are found in the tight junction complex. Prior studies have documented that occludin plays an important role in corneal wound healing and that damage to occludin impairs tight junction integrity. Additionally, our lab has evidence that Crumbs3 (Crb3), a polarity protein associated with ZO-1, is altered in the diabetic cornea. We hypothesized that occludin and ZO-1 localization would be altered in the diabetic tissue.

Methods: Our lab uses the diabetic induced obesity (DIO) mice as a model for Pre-Type II diabetes. Previously we demonstrated a peripheral neuropathy and impaired wound healing in the DIO tissue. Corneal epithelium cells from DIO mice were examined 7.5 and 15 weeks (wk) after onset of a high fat diet and compared to WT C57BL6 mice. Corneas were wounded and allowed to heal for 18 hours after injury. Wounded (W) and unwounded (UW) primary corneal epithelial cells were fixed in 4% paraformaldehyde before staining with antibodies to occludin and ZO-1 and imaged. Fixed corneas were imaged on a Zeiss LSM 700 Confocal Microscope using a 40x- and 63 x-oil objectives and analyzed using FIJI/NIHImageJ.

Results: We demonstrated that the localization patterns of occludin and ZO-1 were altered in the UW and W DIO tissue at both the apical and basal cell layers of the corneal epithelium. ZO-1 is apically located in the UW control tissue, while occludin is present throughout the tissue. Upon wounding, staining patterns of occludin are maintained, while ZO-1 staining is observed at the apical and basal cells. In the UW 7.5 wk DIO tissue, staining of occludin and ZO-1 appear fragmented at the apical cell layers, while staining at the basal cell layers is similar to the W control tissue except for a loss of occludin staining at the cell membrane. Upon wounding, staining at the apical cells is similar to the UW control tissue and staining at the basal cells is similar to the W control tissue, however occludin and ZO-1 staining is also localized along the wound edge. In the

UW 15 wk DIO tissue, staining is similar to that of the UW control tissue. Upon wounding, staining is similar to the W control tissue; however there is a loss of ZO-1 staining at the apical cell membranes. The change in continuous ZO-1 occurs prior to changes in occludin.

Conclusion: These results support the results demonstrating that Crb3 is altered. Since Crb3 mediates trafficking of ZO-1, our data suggest that there is an irregularity in tight junction stability and membrane polarity in the diabetic tissue.

Jacob Flores
Houston Baptist University
Class of 2020

HIF-1α regulation by Glrx under High Glucose condition and Hypoxia in Skeletal Muscle cells

Jacob Flores, Beatriz Ferran Perez PhD, Brian Chong MS, Yuko Tsukahara PhD, Deyar Dashti BA, Reiko Matsui MD

Background: Diabetes is one of the most important risk factors for the progression of peripheral artery disease and is the leading cause of non-traumatic lower limb amputations. In order to develop an effective therapy for the attenuated vascularization seen in diabetes, further understanding of the relating mechanisms is needed.

Hypothesis: Studies have shown that the suppression of gluterodoxin-1 (Glrx), a cytosolic enzyme that regulates protein-GSH adducts, activates HIF-1α and promotes angiogenesis after ischemia. It has also been reported that Glrx levels are increased in high glucose levels. Therefore, we hypothesize that high glucose down regulates HIF-1α stability in hypoxia through the increase of Glrx levels.

Methods: C2C12 skeletal muscle cells were used *in vitro* to evaluate the combining effects of hypoxia, high glucose, and Glrx levels on HIF-1α. An adeno-associated virus (AAV) containing the sequence for an RNA short hairpin loop complementary to the mRNA of Glrx (shGlrx) was used to lower Glrx protein levels. C2C12 cells were infected with an AAV containing shGlrx and an AAV with a short hairpin that has no targeted mRNA as a control(shCT). C2C12 cells were also cultured in low (5 mM) and high (25 mM) glucose DMEM for 4 days, and normoxic (20% O_2) and hypoxic (5% O_2) conditions for 6-16 hours. The protein from the cells were then extracted and analyzed by a western blot to quantify Glrx and HIF-1α protein levels.

Results: AAV-shGlrx inhibited Glrx expression to 10% of control. High glucose shows an increased Glrx expression. In low glucose condition, hypoxia-induced HIF-1α levels were further increased by shGlrx after 6 hours of hypoxia. However, in high glucose condition, the suppression of Glrx by shGlrx did not have much effect on stability of Hif-1α levels. After 16 hours of hypoxia, Glrx protein expression was increased, and HIF-1a induction was rather inhibited by shGlrx.

Conclusion: We have confirmed that inhibiting Glrx can enhance HIF-1a stability in mild hypoxia, but these preliminary data indicate that it may not happen in high glucose. **Future direction**: RNA is also extracted from the cells and we will to analyze mRNA levels of Glrx and angiogenic factors by RT-qPCR. Effects of high glucose on HIF-1α stability may not be regulated by GSH adducts. Further studies will elucidate mechanism of impaired angiogenesis in diabetes.

William Molina
University of Puerto Rico, Mayaguez Campus
Class of 2019

Determination of the Role of LOX-1 during *Escherichia coli* Pneumonia

William Molina, Filiz Korkmaz, Ph.D., Elise Symer, B.A., Matthew Jones, Ph.D., Joseph Mizgerd, Sc.D., and Lee Quinton, Ph.D.
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Background: Pneumonia is an acute lower respiratory tract inflammation that is primarily caused by infection from bacteria, virus, and/or fungi. It is responsible for severe morbidity and mortality worldwide. Pneumonia is the foremost reason for children's hospitalizations in the United States and accounts for more than 935,000 deaths globally. In mice with severe bacterial pneumonia it is known that the pathophysiology of pneumonia is due to the overt activation and recruitment of host innate immune cells. Data generated in our lab has shown that there is an induction of the *OLR1* gene that expresses lectin-like oxidized lipoprotein receptor 1 (LOX-1) in the lungs of mice that are more susceptible to tissue injury during pneumonia. LOX-1 is a receptor that is expressed in many cells, including host innate immune cells and it is commonly associated with atherosclerosis and inflammation. Herein, we aim to understand the role LOX-1 plays during Gram-negative bacterial pneumonia. We have previously shown that local neutralization of LOX-1 within the lungs during pneumonia caused a significant increase in total tissue injury and inflammatory cytokines, indicating that LOX-1 may have a protective effect.

Methods: To determine the effect of LOX-1 inhibition systemically, an intraperitoneal injection with 10 µg of □LOX-1 antibody was administered to neutralize LOX-1 in C57BL/6J mice that were subsequently infected with *E. coli*. Mice were euthanized 24 hours after infection and bronchoalveolar lavage fluid (BALF) samples were collected. In an *ex vivo* system, mouse lung epithelial (MLE-12) cells were similarly treated with □LOX-1 antibody to observe the effect of neutralizing LOX-1 on epithelial cell death. BALF from infected and uninfected mice was also added in the *ex vivo* model to more accurately recreate the conditions of the alveolar space. Total protein from MLE-12 cells was then collected 24 hours after stimulation for analysis and characterization.

Results: Overall, the systemic neutralization of LOX-1 in C57BL/6J mice during bacterial pneumonia did not significantly affect leukocyte recruitment or lung injury. In addition, *ex vivo* LOX-1 neutralization in MLE-12 cells did not affect apoptosis as measured by cleaved caspase-3 on a western blot.

Conclusion: Inhibition of LOX-1 in both *ex vivo* and *in vivo* models did not significantly limit tissue injury in mice with E. coli pneumonia. Further studies involving measurement of resistance and resilience of lung tissue are underway and will more completely characterize the role of LOX-1 during pneumonia.

Elizabeth Osota
University of Georgia
Class of 2018

Utilizing RT-qPCR to Measure Relative HIV titer

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Background: Human Immunodeficiency Virus (HIV) is a retrovirus that has infected about 37 million people worldwide. A distinct step in the retroviral replication cycle involves the reverse-transcription of viral RNA into DNA by the reverse transcriptase (RT) enzyme. In the United States alone, around 85.8 million adults (age 18-64) reported getting tested for HIV. Similar to other viruses, finding efficient methods to quantify the viral titer or load of a HIV sample is important in both clinical and research settings. Common titering methods include enzyme-linked immunosorbent assay (ELISA) and TZM-bl assay. While ELISA measures both infectious and noninfectious viral by detecting HIV antibodies and antigens, TZM-bl measures only infectious particles by using reporter cells, TZM—bl, that expresses reporter genes, beta-galactosidase and luciferase, only in the presence of HIV trans activator protein, tat. The problem is these standard assays are expensive and time consuming taking 1-2 days for the ELISA and 2-3 days for the TZM-bl assay. We hypothesized a more efficient method would use a reverse transcriptase quantitative PCR (RT-qPCR) based assay that utilizes the novel reverse transcription step of retroviruses to detect HIV and quantify relative amounts of virus.
The RT-qPCR assay would be done in less than four hours, inexpensive, and more sensitive compared to other titering methods.

Methods: In the first step of the two-step RT-qPCR assay, reverse transcriptase from a patient sample, lab generated virus stock, or standards with known amounts of commercially available purified reverse transcriptase enzyme (SuperScript II) was used to reverse-transcribe bacteriophage MS2 RNA into MS2 cDNA. In the second step, DNA Taq Polymerase was used to amplify the first strand MS2 cDNA product from the first step. Amplification was assessed using SYBR Green cDNA-specific fluorogenic labeled probe in a QuantStudio 3 Real-Time PCR System.

Results: A preliminary standard curve was created using varied concentrations of commercially available Superscript II RT enzyme. The RT-qPCR assay is currently being further optimized to be applied to laboratory and clinical virus samples.

Conclusion: The MS-RNA RT-PCR based assay to detect virus and estimate quantity will require further optimization prior to implementation with both laboratory and clinical samples.

Sabreea Parnell
Morgan State University, Class of 2018
Boston University School of Medicine, Class of 2022

Analysis of gene expression in aortas of vascular smooth muscle Bcl11b knock out mice

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Background: BCL11B is a transcriptional repressor known to be crucial in the formation of neurons and T-cells, however there is little research regarding its role in the smooth muscle of the vasculature. When BCL11B is knocked out of the vascular smooth muscle of mice treated with the potent hypertensive agent angiotensin II, development of aortic aneurysms is observed. With aortic aneurysms accounting for a common health risk to those with increased blood pressure and current lack of therapeutic options, it is imperative to understand the role of BCL11B in the formation of aortic aneurysms as a prevention therapy may arise.

Methods: RNA sequencing performed on the aortas of wild type (WT) and vascular smooth muscle BCL11B knockout (BSMKO) mice treated with angII for 3 or 7 days identified 74 genes with a statistically significant increased expression of at least 2 fold in BSMKO/angII than WT/angII. Using Ingenuity Pathway Analysis (IPA®), a software that allows to organize genes in functional clusters and networks based on a curated literature database, 15 genes were selected to validate the results of RNA sequencing. qRT-PCR with specific Taqman probes measured gene expression of the selected genes and ANOVA statistical analysis was performed.

Results: qRT-PCR confirmed that of the 15 genes selected for further analysis, 9 (SOX9, CASP8, PPBP, MCM4, MYO5A, CALR, MMP3, ORAI2, and E2F3) were expressed in the subject mice, 6 were undetected (CADH11, TERT, P2RY12, TLR5, MYO1B, and ARRB2), and 1 (CASP8) showed a significant increase in BSMKO/angII (13.1 ± 6.0; n=9) vs WT/angII (1.4 ± 0.4 n=9). One gene, caspase-8, known as crucial mediator of apoptosis, was chosen for further validation studies including Western Blot and TUNEL analysis, to measure apoptosis of aortic smooth muscle cells.

Conclusion: The results suggest that the identified genes with an increased expression in BCL11B knockout mice treated with angII may have a role in the development of aortic aneurysms and could be potential targets for gene therapy in prevention of aortic aneurysms.

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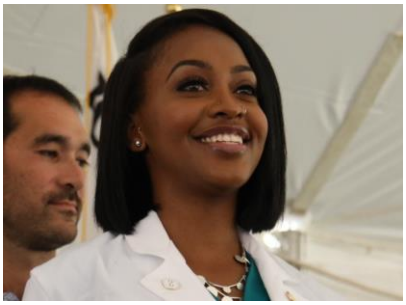

Management of Asymptomatic Patients with Significant Carotid Artery Stenosis

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Background: Carotid endarterectomy (CEA) and carotid artery stenting (CAS) are common vascular procedures performed for treating patients with asymptomatic carotid stenosis. In the CREST trial, patients were randomized to undergo CEA or CAS and the trial demonstrated a 30 day risk of stroke or death among asymptomatic patients was 1.4% for CEA and 2.5% for CAS. In the asymptomatic carotid artery study (ACAS), patients with at least 60% carotid artery stenosis were randomized to receive medical therapy only or CEA and medical therapy. The study demonstrated a reduction in risk of stroke by 5.9% over 5 years for those who underwent CEA. One criticism of this study is that it was completed prior to the induction of current intensive medical therapies such as statins which have significantly lowered the risk of ipsilateral stroke or transient ischemic attack (TIA) since the mid-1980s with the annual risk of ipsilateral stroke in patients with asymptomatic carotid stenosis being as low as ~0.5%. However, there are patients who present with 70% to 80% asymptomatic carotid stenosis and management of these patients are often unclear, particularly when there is progression of disease, even with the implementation of modern intensive medical therapy alone such as plavix, warfarin, aspirin, and statins.

Objective: Our study characterizes current treatment plans for patients with asymptomatic carotid stenosis between 70% and 80%, and analyzes outcomes such as stroke/TIA, myocardial infarction (MI), intervention (CEA or CAS), and death.

Methods: In this retrospective case review study, 350 patients with asymptomatic carotid artery stenosis from 70% to 80% were identified from 2010 to 2018. Patients with asymptomatic carotid artery stenosis were defined as having atherosclerotic extracranial internal carotid artery narrowing who presented without a history of TIA or stroke within the last 6 months. A carotid duplex ultrasound was utilized to evaluate the percentage of stenosis, systolic and diastolic velocities, and internal carotid artery/common carotid artery ratio. The impact of medical management was measured by outcomes which included stroke/TIA, MI, significant disease progression requiring intervention, and death.

Results: Our preliminary results indicate that asymptomatic patients with an initial carotid artery stenosis from 70% to 80% who undergo intensive medical therapies have very low risks of stroke or death.

Conclusion: If the risks of stroke or TIA can be lowered by medical therapies alone, CEA and CAS procedures should be withheld from patients with a 70% to 80% stenosis until patients become symptomatic or significant carotid artery stenosis progression is observed.

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A General Methodology for Understanding Decorin

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Background: Infirmities such as schizophrenia, bipolar disorder, major depression, substance addiction, and Alzheimer's disease can be a burden to people diagnosis with said disorder; one thing that tends to be common across the board for each is the the alteration of brain extracellular matrix formation during development. Decorin, a component of the extracellular matrix, is also altered in those with neurological diseases. Despite this useful information, how decorin is altered in normal physiology and disease is still yet unknown. The purpose of this study is to better understand the structure of decorin in a healthy individual in hopes to understand in the future how it alters in one suffering from a neurological disease.

Methods: We use two methods to better comprehend decorin and its decorations: method one which looks at the glycoforms and method two that looks at the composition of disaccharides. For method one, some of the decorin samples is reduced and alkylated and then undergo a Trypsin/Lys-C and Glu-C digestion. After the protein digestions, we perform an enrichment of GAG-linked peptides via a 10 kDa filter which separates the N- linked glycopeptides from the peptides with chondroitin sulfate (CS) chains.The peptides with CS chains go through a chondroitinase ABC (ChABC) digestion which catalyzes the removal of CS side chains of proteoglycans. Once the digestion is done, the peptides with CS chain Linker attached undergo C18 tip cleaning, which leaves us with clean CS peptide linkers. The second method consist of the decorin samples going through a ChABC digestion, which is followed by separation of the chondroitin sulfate from the proteoglycan via a 10 kDa filter. Afterwards, we use size exclusion chromatography to attain 4S/6S disaccharides to be analyze with liquid chromatography mass spectrometry.

Results: We used two software programs to analyze decorin and its post translational modifications (PTM): Peaks and GlycReSoft. Peaks showed our decorin sample had a 53% coverage and the sequence of proteins in the sample; the software made us aware of specific PTM that occurred such as HexNAc, phosphorylation, and carbamidomethylation. GlycReSoft gave us more information about the *N* -glycosylation sites: out of the three *N* -glycosylation sites decorin has, two of the three site where found within the decorin sample we analyzed. The first *N* -glycan is composed of five Hex and two HexNAc whereas the second *N* -glycan consist of two fucose, four Hex, and four HexNAc. This is important because understanding how the structure of a healthy decorin should look will help us better distinguish it from the decorin structure in an individual suffering from a neurological disease.

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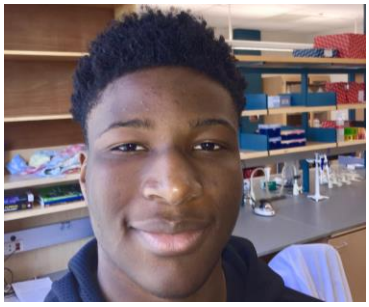

Determining the impact of α1-adrenoceptor antagonism on NCC activity

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Background: According to new AHA guidelines 1 in 2 American adults are hypertensive. Hypertension (HTN) significantly increases the risk for myocardial infarction, stroke, chronic kidney disease, and contributes to 10% of deaths globally. Understanding the mechanisms involved in the development of HTN is essential for creating effective treatments. The sodium chloride cotransporter or NCC which is located in the distal convoluted tube of the kidney influences blood pressure by modulating sodium reabsorption. Prior research has shown that norepinephrine (NE) infusion increases NCC activity and contributes to the development of salt sensitive HTN in Sprague Dawley (SD) rats. NE may exert its effects on NCC via an alpha adrenoceptor pathway.

Hypothesis: Antagonism of α1-adrenoceptors will prevent the development of salt sensitive hypertension by downregulating NCC activity.

Methods: Groups of male SD rats were given a subcutaneous (s.c.) infusion (600 ng/min) of either NE or saline and were put on a normal salt (0.6% NaCl) or high salt (4% NaCl) diet for 14 days. Additional groups of NE infused SD rats were given terazosin, an α1 antagonist, and placed on a normal or high salt diet for 14 days. On day 14, MAP and NCC activity was measured. NCC activity was measured by recording changes in urinary sodium excretion in response to hydrochlorothiazide. Expression of NCC and pNCCT58 were measured by immunoblotting protein samples from the homogenized kidneys of the rats (N=4).

Results: Control rats infused with s.c. saline maintain normal MAP and downregulate NCC activity and expression on a high salt diet. In NE infused rats on a high salt diet, MAP significantly increased indicating the development of salt sensitive hypertension. NCC activity and expression in these rats was not downregulated despite their high salt intake. Alpha 1 antagonism reduced MAP, NCC activity, and expression in NE infused rats on a high salt diet.

Conclusion: The data shows that α1 antagonism prevents the development of salt sensitive HTN by restoring the ability of the NE infused rats to downregulate NCC activity in response to high sodium intake. The mechanism by which NE influences NCC activity is likely mediated by α1 adrenoceptors. The results of this study suggest that α1 antagonism represents a therapeutic target for treating salt sensitive HTN.

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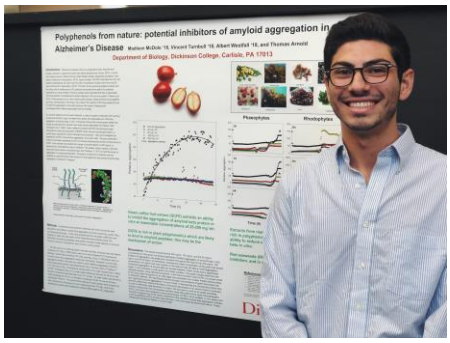

The effects of aging on the morphology of L3 pyramidal neurons in the lPFC of the Rhesus monkey and amelioration by curcumin

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Background: By 2060 the total number of Americans aged 65 or older is predicted to double. Finding a way to inhibit the effects of aging on cognition is necessary and is the driving force for this study. It has been known that aging causes neuronal atrophy resulting in a widespread loss of neurons, dendrites, and spines. These changes in the brain are correlated with cognitive decline. An important brain area in which to study the effects of aging is the Lateral Prefrontal Cortex (LPFC) which is associated with regulating complex cognitive tasks, including “working memory.” This area of the brain encodes and transiently stores sensory-motor information. Turmeric, a natural spice, contains curcumin which is known to have antioxidant and anti-inflammatory effects within the brain.

Methods: Old female (n=3) and young male (n=2) rhesus monkeys were the subjects used in this study. The old monkeys were 18.5, 15.3, and 13.8 years old while the young monkeys were 5.2 and 6.9 years of age. Layer 3 of the LPFC in each primate was removed and sectioned to be imaged under the confocal microscope and gather morphology and electrophysiology data.

Results: By comparing the neuronal morphology changes and electrophysiology data of young monkeys to old monkeys, the effects of aging will be revealed and the possible ability of curcumin to prevent age-related changes can be evaluated. Evidence showing curcumin diminishing the effects of aging on neurons could lead to preventing cognitive decline with age and could potentially be extended to those with neurological diseases where cognitive decline is a major factor.

Boston University Graduate Medical Sciences

Summer Training as Research Scholars

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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Kidest Assefa-McNeil	Brown University	Lee Wetzler, MD	Infectious Diseases
Victor Bacelar	University of Massachusetts, Lowell	Ajit Bharti, PhD	Hematology & Medical Oncology
Vinson Cobb	Boston University School of Medicine	Hasmeena Kathuria, MD	Pulmonary Section
Hasahn Conway	Xavier University	Andrew Henderson, PhD	Infectious Diseases
Zachary Croll-Nesbeth	Oakwood University	Karin Schon, PhD	Anatomy & Neurobiology
Diego De Alba	University of California, Los Angeles	Jennifer Luebke, PhD	Anatomy & Neurobiology
Jean Devera	Boston University School of Medicine	Markus Bachschmid, PhD	Vascular Biology
Anayah Ferris	University of the Virgin Islands	Vickery Trinkaus-Randall, PhD	Biochemistry
Jacob Flores	Houston Baptist University	Reiko Matsui, MD	Vascular Biology
William Molina	University of Puerto Rico, Mayaguez Campus	Lee Quinton, PhD	Pulmonary Section
Elizabeth Osota	University of Georgia	Manish Sagar, MD	Infectious Diseases
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Keyona Pointer	Boston University School of Medicine	Jeffrey Siracuse, MD	Surgery - Vascular
Shelsea St. Hillien	Carleton College	Joseph Zaia, PhD	Biochemistry
Seidu Sumani	University of Massachusetts, Amherst	Richard Wainford, PhD	Pharmacology & Experimental Therapeutics
Vincent Turnbull	Dickinson College	Jennifer Luebke, PhD	Anatomy & Neurobiology