GMS Presents:
Summer Training as Research Scholars (STaRS) Program

Research Symposium
Thursday, August 6, 2015

2015 Participants: Steven Soler, Georges Tahhan, Elizabeth Lindsay, M.Bianca Bryant, Brittany Martin, Kingsley Ozongwu, Maria Alavarez, Asia Satchell, Saydee McQuay, Alejandro Sanoja, Annalyn Brown, Christopher Li, Leandro Fernandez, Deandrea King, Alexandra Morquette, Chinaemere Igwebuike (not picture).

BOSTON UNIVERSITY

Division of Graduate Medical Sciences
Summer Training as Research Scholars (STaRS) Program
Research Symposium 2015

Welcome to the 2nd Annual Summer Training as Research Scholars (STaRS) Research symposium, hosted by the Division of Graduate Medical Sciences, for students who are traditionally underrepresented in the biomedical sciences. Our academically talented students were selected for STaRS through a highly competitive application process and have spent the summer conducting research in labs across the BU Medical Campus. We hope you enjoy learning more about their summer research projects.

11:00 – 11:15 am **Welcome**

Dr. Linda Hyman, PhD
Associate Provost, Division of Graduate Medical Sciences

Dr. Maria Ramirez, PhD
Director, Summer Training as Research Scholars

11:15 – 12:15 pm **Oral Presentations**

M. Bianca Bryant
Saydee McQuay
Alexandra Morquette
Georges Tahhan

12:15 pm **Lunch**

12:15 – 12:45 pm **Poster Session: Part A**

Maria Alvarez
Annalyn Brown
Leandro Fernandez
Elizabeth Lindsay
Alejandro Sanoja

12:45 – 1:15 pm **Poster Session: Part B**

Kingsley Ozongwu
Brittany Martin
Asia Satchell

1:15 – 2:00 pm **Presentation of Certificates & Closing Remarks**

Dr. Linda Hyman
Dr. Maria Ramirez

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Maria Alvarez
University of New Mexico

Isolation of a prospective *N. gonorrhoeae* Vaccine Antigen and Evaluation of its Ability to Activate Human Macrophages

Maria Alvarez

Mentor: Ian Francis, Yazdan Shaik-Dasthagirisaheb, Lee Wetzler

Boston University School of Medicine, Department of Microbiology

Additional authors: Ian Francis, Yazdan Shaik-Dasthagirisaheb

Neisseria gonorrhoeae is the causative agent of the sexually transmitted disease gonorrhea. It is one of the most common sexually transmitted diseases in the United States. Gonococcal (GC) infection has been shown to enhance HIV replication and transmission, as well as lead to pelvic inflammatory disease (PID). There have been several attempts to design a successful vaccine against *N. gonorrhoeae,* but many obstacles have prevented an efficacious vaccine from being developed. Research has yet to find an adequate antigen to serve as the basis for a potential vaccine. However, recent studies have provided evidence that anti-P1B antibodies are protective against reinfection with GC of the same serogroup. The outer membrane protein, P1B, is a self- adjuvanting antigen that could be used in a vaccine. Our goal is to advance our understanding of the interaction between host and the GC antigen, P1B. This study focuses on the interaction between P1B and differentiated THP1 cells (derived from an acute monocytic leukemia patient). We expect to see P1B activate macrophages, leading to the release of TNFα and IL6. We will be conducting an experiment to verify our hypothesis by using THP1 cell lines as a source of macrophages, and activate these cells with gonococcal outer membrane protein: P1B. Initial steps of this project were the isolation and purification of P1B from *N. gonorrhoeae*, using column chromatography. THP1 cell lines were grown for 1 week and treated with Phorbol-12-myristate-13-acetate (PMA) for activation of macrophages from these cell lines. Stimulation of macrophages with P1B and quantification assay for cytokines TNFα and IL6 were carried out using ELISA (enzyme-linked immunosorbent assay). These quantifications will serve as evidence that P1B can illicit an response from an innate immune cell (macrophage) and give us more specific knowledge on the role P1B plays as a self-adjuvanting antigen.

Annalyn K. Brown
University of the Virgin Islands

Gb3 Expression on Human Cervical Cancer Cells and Effects of Shiga Toxin-2

Annalyn K. Brown

Mentor: Ji Hye Seo, PhD

Boston University School of Medicine, Pathology & Laboratory Medicine

Recently, there has been an increase in outbreaks of enterohemorrhagic *E.coli (*EHEC*)*. These bacterial infections can lead to the development of hemolytic uremic syndrome (HUS) which may be fatal. In order to treat those suffering from this infectious disease, the effects of these bacteria must be studied *in vitro* before they can be monitored in animal models. Human epithelial cancer cells (HeLa) were challenged with the Shiga toxin type 2 produced by these bacteria to evaluate effects on cell viability. Cells also were incubated with antibodies to determine Gb3 expression (CD77), the receptor on cell surfaces that binds the toxin. RNA from these cells was quantified using qPCR to further confirm results. HeLa cells expressed Gb3 on their cell surfaces and mRNA for alpha galatosidase (GLA), an enzyme important for Gb3 synthesis, was detected. However, HeLa cell sensitivity to Shiga toxin type 2 could not be determined due to inconclusive results. Further tests would need to be performed to obtain reproducible results for HeLa cell sensitivity to Shiga toxins. Understanding the receptor expression and toxin sensitivity relationships in different cell types contribute insight into the pathophysiology of the toxins.

M. Bianca Bryant
Claflin University

ACLP Signaling Enhances Adipose Progenitor Differentiation into Myofibroblasts

M. Bianca Bryant

Mike Jager and Matthew D. Layne

Boston University School of Medicine, Department of Biochemistry

The accumulation of excess extracellular matrix (ECM) or fibrosis in adipose tissue is emerging as a contributor to metabolic dysfunction. Myofibroblasts are critical cells in this process as their deposition and remodeling of the ECM results in a stiff environment surrounding the tissue. Transforming Growth Factor-β (TGF-β) is known to enhance fibrosis; however, there are other mediators in the transition of progenitors cells to myofibroblasts that have yet to be identified. Our lab previously identified Aortic Carboxypeptidase-like Protein (ACLP), a secreted, collagen-binding protein that increases fibrosis and myofibroblast differentiation. The goal of the present project was to test the hypothesis that ACLP enhances the adipose progenitor to myofibroblast differentiation. In order to test this hypothesis, we cultured 10T1/2 cells, murine pre-adipocytes, in adipogenic media, and assessed ACLP expression via western blot analysis as adipogenesis occurred. Then, we isolated the stromal vascular fraction (SVF) from the inguinal adipose tissue of α-SMA-mCherry/collagen I-GFP-topaz transgenic mice, treated the SVF with recombinant ACLP or TGF-β, and analyzed and quantified the expression of α-SMA and collagen I using immunofluorescence imaging. These data show that ACLP expression is lost two days after adipogenic stimulation in the 10T1/2 cells. The immunofluorescence data shows an increase in the expression of the myofibroblast markers in the ACLP-treated SVF; there was a 1.7-fold increase in α-SMA expression and a 1.5-fold increase in collagen I expression. In conclusion, ACLP is rapidly inhibited during adipogenesis, its expression is absent in mature adipocytes, and ACLP has the capability of increasing myofibroblast differentiation in SVF. These findings support the function of ACLP as a stimulator of myofibroblast differentiation.

Leandro Fernandez
Florida State University

Nano Encapsulated Transforming Growth Factor-β (TGF-β) Possibly Attenuates NF-κB Activation Pathway in Pulmonary Hypertension

Leandro Fernandez, Omar Mohtar, Elizabeth Klings, and Jean-Bosco Tagne

Pulmonary Center, Boston University Medical Center

Pulmonary hypertension (PH) is a clinical condition resulting from pulmonary vasculopathy. The epidemiologic associations between PH and systemic hypertension, renal and cerebrovascular disease in sickle cell disease (SCD) suggests a pathologic link amongst vascular complications of SCD. No effective treatment specifically targeting this vasculopathy exists. Candidate gene-based studies done by our group and others have demonstrated that TGF-β, a secreted protein in the superfamily of cytokines that performs many cellular functions is associated with numerous vascular phenotypes in sickle cell disease including stroke, priapism, leg ulcerations and pulmonary hypertension. These functions include the control of cell growth, cell proliferation, as well as cell differentiation and apoptosis. Because of this, TGF-β plays a pivotal role in many diseases such as lung, cancer, diabetes and many others. As a result of TGF-β's wide array of cellular processes, it can be negatively or positively expressed to induce or inhibit these cellular functions.

Aberrant TGF-β signaling has roles in animal models of PH and mutations have been found in some patients with idiopathic PH suggesting that this pathway is an important modulator of gene expression within the pulmonary vasculature. We hypothesize that aberrant TGF-β signaling within the endothelium may play a role in disease modulation in SCD patients with PH. We plan to address the question of whether direct genetic targeting of the endothelium in SCD can alter these interactions and potentially, impact the development of vasculopathy. To do so and using our novel delivery system, we have Nano-formulated TGF-β and started testing its actions on Human pulmonary artery endothelial (HPAEC) cells compared to regular TGF-β. *We plan to explore the functional role of aberrant TGF-β signaling in modulating endothelial dysfunction in SCD.*

HPAEC cells were grown to 70% confluence, treated with both Nano-formulated TGF-β and TGF-β, and compared to non-treated cells. Expression levels of Smad 3, MAP3K7, RelA, and Fibronectin (FN1) were analyzed by qRT-PCR. FN1 expression showed a Nano-formulated TGF-β decrease 1.5 fold compared to TGF-β alone while showing an increase in Smad 3 and a decrease in expression noted in MAP3K7, and RelA which constitute part of the NF-κB pathway. This study showed that in the presence of our Nano-formulated TGF-β, the nuclear factor NF-κB pathway is altered, an indication of the improvement in TGF-β's therapeutic effect in the cure of PH and SCD**.**

Christopher Li
Boston University School of Medicine

Incidence and Prevalence of Elevated Tricuspid Regurgitant Jet Velocity and Pulmonary Hypertension in Children and Young Adults with Sickle Cell Disease

Christopher Li

PI: Patricia Kavanagh, MD

Boston University Medical Center, Department of Pediatrics

Background: Sickle Cell Disease (SCD) affects approximately 100,000 people in the United States (1), and elevated tricuspid regurgitant jet velocities (TRV) and pulmonary hypertension (PHT) in adult SCD patients have been associated with increase mortality (2). But the prevalence of elevated TRV, PHT, associated clinical markers, and clinical progression into adulthood has not been well documented in pediatric patients.

Methods: A retrospective chart review of 210 pediatric patients that presented to an urban academic medical center beginning in 2002 until 2015 was performed to evaluate for the prevalence of elevated TRV and PHT, associated clinical and echocardiography variables, as well as progression at follow-up clinical evaluations within the study timeframe.

Results: TBD

Conclusion: TBD

1: Center for Disease Control and Prevention. Sickle Cell Disease Data & Statistics. Available from: http://www.cdc.gov/ncbddd/sicklecell/data.html

2: J.F. Delgado, M.A. Gómez-Sánchez, C. Sáenz de la Calzada, *et al.* **Impact of mild pulmonary hypertension on mortality and pulmonary artery pressure profile after heart transplantation.** J Heart Lung Transplant, 20 (2001), pp. 942–948

Elizabeth Lindsay
State University of New York, Brockport

The role of the aryl hydrocarbon receptor in oral cancer tumor growth and chemoresistance

Elizabeth L. Lindsay

Mentors: Dr. Elizabeth Stanford, Dr. David Sherr

Boston University, School of Medicine, School of Public Health, Environmental Health

The aryl hydrocarbon receptor (AHR) has been shown to play a role in cancer initiation and progression in oral squamous cell carcinomas (OSCC), and other cancers. The AHR is activated by environmental toxins, including polycyclic aromatic hydrocarbons, which are commonly found in cigarette smoke. It is hypothesized that activation of the AHR by these environmental toxins can contribute to the growth and chemoresistance of OSCCs. Nude mice tongues were injected with a human OSCCs cell line, SCC2s, and treated with an AHR antagonist at 25mg/kg daily via oral gavage. Primary tumor growth was measured via calipers and IVIS imaging. RT-qPCR analysis of the harvested tongue tumors and livers was used to examine the activity of the AHR by quantifying the expression levels of *Cyp1b1* and *Cyp1a1*. Based on the results of the *in vivo* experiments, continued testing was conducted to examine the role of AHR inhibition in chemoresistance. Using MTT cell viability assays coupled with dosing of commonly used chemotherapeutics, the effects of the AHR on the chemo-resistance of SCC2s was tested. Three commonly used chemotherapeutics were tested at various dose ranges: Cisplatin (0-10uM), doxorubicin (0-1uM), and 5-Fluorouracil (0-10uM). In addition, cells were co-treated with an AHR antagonist (5uM CH223191) and the chemotherapeutic to determine if decreasing AHR activity increased chemotherapeutic efficiency. ANOVAs were used to evaluate the significance of AHR activity on the effectiveness of the chemotherapeutics. It was determined that AHR antagonism with CB7993113 significantly affected OSCC primary tumor growth *in vivo*. Additionally, it was found that both *Cyp1a1* and *Cyp1b1* expression decreased after treatment with CB7993113 when compared to vehicle alone in the tongue. In the liver, it was found that both *Cyp1a1* and *Cyp1b1* expression also decreased after treatment with CB7993113 when compared to vehicle alone. Interestingly, we also found that decreasing AHR activity with an AHR antagonist CH223191 in addition to treatment with a chemotherapeutic lead to a significant increase in cell death when compared to treatment with the chemotherapeutic alone. This phenomenon was observed in three different frontline OSCC therapeutics. These novel findings implicate the AHR in OSCC initiation and growth, also supporting the development of AHR modulators as potential chemotherapeutics. Overall, these findings support the hypothesis that the activation of the AHR is linked to tumor growth of oral squamous cell carcinomas as well as contributing to the potential chemoresistance of these cells.

Brittany Martin
Jackson State University

The Role of Sphingosine-1-Phosphate Signaling in Triple Negative Breast Cancer

[1,3,4]Brittany Martin, [1]Fabrice Laroche, [1]Hui Feng

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Breast cancer is the second leading cause of death in women in the US. The disease is characterized as Luminal A, Luminal B, Triple negative breast cancer (TNBC), and HER2 subtypes. TNBC is the most aggressive subtype that makes up 10-20% of breast cancer cases. Presently, there is no efficient treatment for TNBC. The subtype is characterized as a basal like tumor that lacks estrogen receptor (ER), progesterone receptors (PR), and human epidermal growth factor receptor 2 (HER2). To understand the role of sphingosine-1 phosphate receptor 1 (S1P1) in TNBC, a TNBC cell line (MDA-MB-231) was genetically modified to overexpress either S1P1 wild type (WT) or a S1P1 mutant protein that is mutated at Threonine 236 to Alanine (S1P1T236A), unable to be phosphorylated for a comparison with cells overexpressing luciferase (Luc) as a non-relevant control. We hypothesize when S1P is overexpressed in MDA-MB-231 breast cancer cells, we expect AKT to be more phosphorylated, which promotes phosphorylation of S1P1. Western blotting analysis was performed to determine the protein levels of Rac1, P-AKT308, P-AKT473, AKT and P-S1P1T236A upon S1P1 WT or T236A overexpression in TNBC cells, using cells overexpressing Luc as controls. Our results demonstrate that MDA-MB-231 TNBC cells are able to overexpress S1P1. Total Rac expresses equal amounts in Luc, WT, and T236A. Overexpression of S1P1 T236A promotes AKT308 phosphorylation. Lastly, overexpression of S1P1 promotes a higher level of AKT473 phosphorylation in WT than T236A. In our future studies, we will elucidate the molecular consequences of these changes in AKT phosphorylation.

Saydee McQuay
Syracuse University

Effects of Oleate Fatty Acid on Insulin Secretion and Calcium levels in INS-1 cells

Saydee McQuay

Principal Investigator: Barbara Corkey, PhD
Mentor: Karel Erion
Boston University School of Medicine, Obesity Research Center
Additional Authors: Nathan Burritt

There has proven to be a strong correlation between obesity and type-two diabetes, and as the prevalence of these two diseases rises with the growing waistline of Americans, it is imperative to understand how they are related. In order to determine this, we explored the role of chronic exposure to fatty acids on pancreatic beta cell dysfunction. We set up both temporal and dosage based experiments treating the clonal β-cell line, INS-1 (832/13), with 0.1, 0.15, and 0.2 mM oleate for either 24 or 48 hours. We then assessed glucose-stimulated insulin secretion and total insulin content. Following 24-hours, we observed glucose-stimulated insulin secretion decreased as the dosage of fatty acid incubation increased. Basal insulin secretion was unaffected regardless of the dose. Following 48-hour incubations, increasing fatty acid concentrations dramatically increased insulin secretion at basal glucose, while the cells still failed to respond to elevated glucose. To see if calcium could account for such differences in insulin secretion, we monitored cytosolic Ca^{2+} concentration in response to glucose in control cells and cells exposed to 0.15mM oleate using the fluorescent Ca^{2+} probe, Fura-2. We found that the cells dosed with fatty acid have a longer lag time in response to glucose and have fewer calcium oscillations. In order to determine what accounted for this lag in calcium influx, we further probed into the process of insulin secretion by calculating redox of NAD/NADH in the cells to determine if the cells dosed with fatty acids existed in a more oxidized state. If so, this could account for the lag time in calcium influx in response to glucose. Based on our experiments, there was no difference in the cells dosed with fatty acid basally or in response to glucose. Finally, this led us to investigate if the problem might lie in excocytotic differences in the cells. Preliminary results point to a decreased ability of the fatty acid cells to let in calcium, with potential to recover these cells when they are dosed with high Calcium concentrations. This further supports our thought that fatty acid is affecting the beta cell's ability to influx calcium, causing decreased insulin secretion in response to glucose. These results could help further elucidate the mechanism by which fatty acids cause beta cell dysfunction, hopefully allowing a greater understanding of the relationship between diabetes and obesity.

Alexandra Morquette
Columbia University

Quantification of GABAergic Inhibitory Synapses in Rhesus Monkey Neocortex through Detection of the Vesicular GABA Transporter VGAT

Alexandra Morquette

PIs/Mentors: Dr. Jennifer Luebke; Dr. Maria Medalla

Department of Anatomy and Neurobiology, Boston University School of Medicine

It is important to understand how GABAergic synaptic signaling functions in order to understand the complex inner workings of the cerebral cortex. The two main types of synaptic signaling in the cortex, GABAergic and glutamatergic, have opposite effects on their target neurons; GABAergic neurons inhibit targets through inhibitory postsynaptic currents (IPSCs) and glutamatergic neurons excite targets though excitatory postsynaptic currents (EPSCs). We have previously demonstrated, when comparing two areas of the cerebral cortex with patch clamp recordings, that there is a higher frequency of IPSCs detected in pyramidal neurons of the anterior cingulate cortex (ACC, area 32) in comparison to the lateral prefrontal cortex (LPFC, area 46)(unpublished observation, Luebke and Medalla). The hypothesis of the present experiment was that the levels of vesicular GABA transporter (VGAT) associated specifically with inhibitory synapses are higher in the ACC than in the LPFC. Using the Rhesus monkey as a model, slices of both areas layers 1-3 were extracted from three different animals, fluorescently stained for VGAT using immunohistochemistry, and scanned using confocal microscopy. The computer program FIJI allowed for quantification of the VGAT label. VGAT label was quantified in three different ways: within the entire layer itself, on the surface of individual somata, and on the surface of dendrites emanating from the soma. Results showed that a significantly higher percent area of VGAT label is present in the ACC within each section for layers 1 and a combination of layer 2-3. Results also showed that a significantly higher percent area of VGAT label is found on the surface of the soma and of the dendrites of ACC than of LPFC pyramidal neurons. So far, these results validate the working hypothesis as correct, since higher levels of VGAT label are found in the ACC than in the LPFC. This finding brings us closer to an understanding of how inhibition plays differential roles in different brain areas, and leads to the question of why more inhibition may be required in the ACC to control its main functions.

Key words: anterior cingulate cortex, lateral prefrontal cortex, immunohistochemistry, pyramidal neuron

Kingsley Ozongwu
University of Wisconsin, La Crosse

Co-infections of HIV-1 and *Porphyromonas gingivalis* predisposed macrophages to persistent HIV-1 infection

Kingsley Ozongwu

Mentor: Andrew Henderson, PhD
Boston University School of Medicine, Department of Infectious Diseases
Additional Author: Luis Agosto, PhD

HIV-1 increases the body's susceptibility to opportunistic infections by targeting cells necessary for immune function, such as T cells and macrophages, which leads to a general immune dysregulation and to a number of secondary diseases in a variety organ systems including the oral cavity. An opportunistic oral pathogen that has been shown to infect many people who suffer from HIV, is *Porphyromonas gingivalis (P. gingivalis)*. This study sought to determine the effects of *P. gingivalis*, on the susceptibility of macrophages to HIV infection. By co-infecting monocyte-derived macrophages with HIV-Luc, an HIV clone that includes the luciferase reporter gene, and heat killed *P. gingivalis*, and measuring luciferase expression, we were able to determine the level of HIV gene expression. We found that macrophages that were co-infected with *P. gingivalis* decreased HIV expression. However, this decrease in HIV gene expression was not due to impaired proviral transcription as the levels of HIV transcripts were not affected. We concluded that co-infection with *P. gingivalis* reduced HIV-1 replication in macrophages at a point after proviral transcription. Our results suggest a novel mechanism for establishing and maintaining HIV persistence within macrophages.

Alejandro Sanoja
University of Florida

Nesting pads primes the immune response in a murine pneumonia model

Alejandro Sanoja, Terry Hsieh, Max Vaickus, Dan Remick, MD

Boston University Medical Center, Department of Pathology

Background: Nesting pads have been found to reduce abnormal behavioral phenomena in animal models by creating a more stimulating environment. While beneficial to animal welfare, recent studies have suggested that the use of these pads may present another variable and interfere with standardization. However, there is little research done on the effects of these pads on inflammatory rodent models, and none on pneumonia models. Our study focuses on the impact of nesting pads on bacterial clearance, cell recruitment, and survival in a murine pneumonia model.

Methods: Enriched female ICR mice groups were exposed to the nesting pad for 1d and 4d prior to pneumonia challenge. These groups were compared to non-enriched controls housed in pad-free cages. Enriched and non-enriched groups were injected with $5x10^7$ CFU *Pseudomonas aeruginosa (Psd.)*, instilled intratracheally. Bronchoalveolar lavage (BAL) was performed 4h post-*Psd*. BAL cell counts quantified by Coulter counter and bacterial load (CFUs) by serial dilutions on 5% blood agar plates. Cells were spun onto slides by Cytospin and stained with Diff-Quick to obtain a differential. Survival was followed for 7d. Cytokines were measured by ELISA. Peripheral blood was collected from the facial vein to obtain a complete blood count with differential via Hemavet.

Results: Enriched mice trend toward having increased WBCs and neutrophils with 4d of housing with the pad. As expected, mice not challenged with pneumonia showed little cell recruitment compared to their challenged counterparts. With 1d of housing, enriched mice trend toward increased cell recruitment and have significantly decreased bacterial burden ($p<0.05$) following *Psd* challenge. However, after 4d exposure, there was no significant difference between groups in both cell counts and bacterial burden. 4d enriched mice trend toward increased IL-6 in BAL fluid while having decreased plasma IL-6 and TNFα.

Asia Satchell
Xavier University of Louisiana

Mir-200c, a microRNA, and its role in RAS Pathways

Asia Satchell

Mentor: Dr. Anurag Singh

Boston University School of Medicine, Department of Pharmacology, Laboratory of Cancer Pharmacogenomics

The RAS proteins play a key role in controlling signaling pathways in cancer. They also regulate aspects of normal cell growth as well as fatal cell transformation. In many different types of cancer, mutations turn KRAS into a malignant oncogene, which abruptly disrupts the signaling pathways. Each protein involved in the RAS pathways affects the cells in different ways. It has been shown that the microRNA, mir-200c, directly targets the KRAS oncogene and contributes to tumor suppression by inhibiting epithelial-mesenchymal transition in cells. After overexpressing mir-200c in the lung cancer cell lines, we observed the effects of TAK1 inhibitor and AZD6244 inhibitor, which inhibit the MEK kinase, on the epithelial and mesenchymal cells by drug treatment assays. We observed that the mir-200c cell lines were more resistant and therefore less sensitive to the TAK1 and AZD6244 inhibitors in comparison to the control cell line, which was H460-RFP. The RFP cells were more sensitive to the drug treatments. It was also observed that the TAK1 inhibitor caused morphological changes to the cells in both the RFP and mir-200c cell lines. Western blots were carried out to analyze epithelial markers and apoptosis of the cells. The western blot showed a substantial amount of E. cadherin epithelial marker in the mir-200c cells, while not being as abundant in the RFP cells. These results verify the mir-200c cells as epithelial cells due to their amount of E. cadherin, and also verifies the RFP cells as the mesenchymal cells due to lack of E. cadherin. When measuring the amounts of Cleaved PARP by western blot, the results showed the C. PARP fragments to be more prevalent in the RFP cells. This shows that apoptosis, or programmed cell death is more present in the RFP cells than in the mir-200c cells. It has been shown that oncogenic changes disrupt apoptosis. Furthermore, we assessed levels of total ERK and phosphorylated ERK following MEK inhibitor treatment to validate the downstream effects of the inhibitor. Lastly, we carried out immunofluorescence microscopy to observe the levels of E. Cadherin, vimentin, and mitotic cells. Overall, our findings suggest that the RFP cells were more sensitive to the TAK1 and ADZ6244 inhibitors, in comparison to the mir-200c cells, which were more resistant to the drug inhibitors. In addition, due to the change in cell morphology observed in the cells treated with TAK1 inhibitor, we can conclude that the TAK1 protein plays an important role in cell morphology of both cell lines. In addition, we verified higher levels of E. cadherin in the epithelial mir-200c cells, and higher levels of vimentin in the mesenchymal RFP cells.

Steven Soler
BUSM II

The Role of the Deacetylase Sirtuin-1 in Diet-Induced Arterial Stiffness

Steven Soler

Mentor: Francesca Seta

Boston University Medical Center, Vascular Biology Unit

Background: The aorta is an elastic and functionally compliant vessel. However, risk factors, such as age and obesity, can cause stiffening of the aortic wall, leading to loss of compliance, with deleterious consequences for the cardiovascular system, including hypertension. Our lab showed that mice fed a high fat, high sucrose (HFHS) develop arterial stiffness, as measured in vivo by pulse wave velocity, within two months compared to normal diet (ND)-fed mice. The drugs resveratrol and SRT1720 are activators of sirtuin1 (SirT1), an NAD^+ dependent deacetylase, known to have antioxidant and anti-inflammatory effects, and activated by calorie restriction, exercise, and polyphenols. Our lab showed that resveratrol and SRT1720 protect mice from HFHS-induced arterial stiffness.

Aim of Study: To determine the cellular and molecular mechanisms by which SirT1 can prevent HFHS-induced arterial stiffness.

Methods: Mice were fed HFHS or ND for up to 10 months and a subset of WT mice on HFHS received the SirT1 activator SRT1720, for a further two weeks. Aortas from the different experimental animals were harvested and sectioned and stained for collagen, using Picrosirius Red. ImageJ was then used to measure collagen deposition and aortic media thickness, from digital images of aortic sections acquired under polarized or bright light, respectively. Because of the well-established metabolic effects of SirT1, several markers of metabolism were also assessed in the various experimental groups. Livers were sectioned to 10um and stained for lipid deposition using Oil Red O stain. The intensity of staining and percent lipids in digital images of each liver section, acquired with an inverted microscope at 10x magnification, was quantified using the program ImageJ. Plasma, collected from each mouse, was measured fro triglyceride and cholesterol levels. We used a colorimetric assay, based on the comparison with a standard curve, which was prepared by serial dilutions of a triglyceride or cholesterol standard (200mg/dL), from which the unknown concentrations of plasma samples were inferred.

Results: Aortic media thickness was significantly increased in HFHS-mice treated with SRT1720 compared to ND-fed and HFHS-fed mice (118um vs 77um and 81um, respectively). Collagen content in the aortic media was significantly changed by the HFHS feeding when compared to ND or HFHS-fed plus SRT1720 (1.01 vs 0.63 and 0.66, respectively). HFHS-fed mice had significantly higher cholesterol levels than ND-fed mice (122.71mg/dL in WT vs 172.89mg/dL in HFHS). HFHS feeding did not affect plasma triglycerides, compared to ND. After lipid quantification of livers, it was noted that the Oil Red O method did not adequately stain all mouse groups consistently, so a new and improved method will be employed in future studies.

Conclusions: Our preliminary findings suggest that the beneficial effect of resveratrol and SRT1720 in preventing HFHS-arterial stiffness, is not due to an improvement of HFHS-induced aortic medial thickness or collagen deposition. Our results also show that HFHS slightly, but significantly, increased cholesterol, but not triglycerides, in our animal model.

Works Cited:

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3. Gano, et al. Treatment with the SIRT1 activator SRT1720 reduces large elastic artery stiffness, superoxide production and inflammation in old mice. The FASEB Journal 2011.

Characterization of 30-day Vascular Surgery Readmissions

Georges Tahhan, Jeffrey J. Siracuse

Division of Vascular and Endovascular Surgery

Boston University School of Medicine, Boston Medical Center

Background: Thirty-day readmission is increasingly being used as a quality of care indicator and is a target for financial penalties to institutions. Vascular surgery patients have historically been at high risk for readmission. We wanted to analyze our institution's readmissions to gain a better idea of how to potentially lower postoperative complications in this high-risk population.

Methods: A retrospective review and analysis of Boston Medical Center's admission and discharge data was conducted from October 2012 to March 2015. All in-patients who were discharged as inpatient status from the vascular surgery service and subsequently readmitted as an inpatient within thirty days were included.

Results: There were 135 readmissions out of 649 (20.8%) discharges. The patients were Caucasian (59.52%) males (64.44%) with an average age of 66 (SD 11 years), and average BMI of 27.81 (SD 6.62). The most common comorbidities were, diabetes (56.30%), coronary artery disease (40%), chronic heart failure (CHF) (23.70%), and chronic obstructive pulmonary disorder (19.26%). Index operations were open lower extremity procedures (38.52%), diagnostic angiograms (34.81%), endovascular lower extremity procedures (16.30%), dialysis access (7.41%), carotid/cerebrovascular procedures (7.41%), above and below knee amputations (5.93%), and aorta/abdominal procedures (5.19%). In this same patient population, 7.41% of patients did not have any surgical procedures, 4.44% had other procedures, and 10.37% had a concurrent podiatry procedure. Index LOS was on average 7.48 days (SD 6.73 days). Readmissions within 30 days were for planned procedures (21.48%), surgical complications (37.78%), and medical causes (40.74%). Planned readmissions included procedures at the same site (79.31%), different site (13.79%), and planned podiatry procedures (6.90%). Surgical causes for readmission were surgical site infections (64%), graft failure (20%), bleeding complications (8%), and unplanned podiatry procedures (10%). Causes for medical readmissions most commonly include general malaise/failure to thrive 27.27%, CHF complications 16.36%, other infectious causes 10.91%, and acute renal failure 9.09%. Index readmission LOS was on average 7.43 days (SD 7.22 days). Sources of readmission included the vascular surgery clinic 38%, emergency room 56%, and direct readmission 6%. There was no difference in comorbidities, index operation, or reason for readmission for days 0-7 and days 8-30.

Conclusions: This analysis of 30 day readmission rate shows that the causes are multifactorial including many planned readmissions and unrelated medical readmissions in high risk vascular surgery patients. Characterizing the readmissions could help lower readmission rates and help adjust benchmarks for targeted readmission rates.