GMS Presents:

SUMMER TRAINING AS RESEARCH SCHOLARS (STaRS) PROGRAM

Research Symposium

Thursday, August 8, 2019

Boston University Graduate Medical Sciences

Summer Training as Research Scholars

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Summer Training as Research Scholars

STaRS Research Symposium Schedule
Thursday, August 8, 2019
11:00 AM – 1:00 PM
Boston University School of Medicine, L-206/209

Welcome to the Annual Summer Training as Research Scholars (STaRS) Research Symposium, hosted by the Division of Graduate Medical Sciences.

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.

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.

11:00 – 11:10 AM: Welcome

Dr. Isabel Dominguez, STaRS Director and Associate Professor

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

11:10 – 11:30 AM: Oral Presentations
- Cynthia Flores
- Sabreea Parnell

11:35 AM – 12:05 PM:
Poster Session Group A
- Gina Conroy
- Diego De Alba
- Dylan Dominguez
- Jacob Hernandez
- Shantae Lewis
- Kayley Pate
- Erlin Ravariere
- Gerardo Sequen Rivera
- Paris Taylor
- Carlos Ticas
- Ajah Williams

12:10 pm – 12:40 PM:
Poster Session Group B
- Olumide Ayeni
- Stephanie Camey
- Justin Coleman
- Sojourna Ferguson
- Cynthia Flores
- Braden Garland
- Nina Goodson
- Antonio Lopez
- Bethany Onyirimba
- Sabreea Parnell
- Moises Ramirez
- Chelsey Skeete

12:45 – 1:00 PM: Awarding of Certificates & Closing Remarks

Boston University Graduate Medical Sciences

Summer Training as Research Scholars

Student	Home Institution	Mentor	Department
Olumide Ayeni	University of Illinois at Urbana-Champaign	Vickery Trinkaus-Randall, PhD	Biochemistry
Stephanie Camey	Pitzer College	Jonathan Wisco, PhD	Anatomy & Neurobiology
Justin Coleman	Tougaloo College	Ludovic Trinquart, PhD	Biostatistics
Gina Conroy	Montclair State University	Suryaram Gummuluru, PhD	Microbiology
Diego De Alba	University of California, Los Angeles	Maria Medalla, PhD	Anatomy & Neurobiology
Dylan Dominguez	University of Arkansas	Jeffrey Siracuse, MD	Surgery
Sojourna Ferguson	University of the Virgin Islands	Xaralabos (Bob) Varelas, PhD	Biochemistry
Cynthia Flores	Mount Saint Mary's University	Markus Bachschmid, PhD	Medicine (Vascular Biology)
Braden Garland	Northwest University	Ajit Bharti, PhD	Medicine, Hematology & Medical Oncology
Nina Goodson	American University	David Sherr, PhD	Environmental Health
Jacob Hernandez	Boston University School of Medicine	Haiyan Gong, MD, PhD	Ophthalmology
Shantae Lewis	University of the Virgin Islands	Dennis Jones, PhD	Pathology & Laboratory Medicine
Antonio Lopez	Boston University	Basilis (Vasileios) Zikopoulos, PhD	Health Sciences
Bethany Onyirimba	Columbia University	Jude Deeney, PhD	Medicine (Endocrinology, Diabetes, Nutrition & Weight Management)
Sabreea Parnell	Boston University School of Medicine	Francesca Seta, PhD	Medicine (Vascular Biology)
Kayley Pate	University of Central Oklahoma	Vijaya Kolachalama, PhD	Medicine (Computational Biomedicine)
Moises Ramirez	Salem State University	Thomas Kepler, PhD	Microbiology
Erlin Ravariere	Boston University School of Medicine	Kei Yasuda, PhD	Medicine (Nephrology)
Gerardo Sequen Rivera	El Camino College	Jennifer Luebke, PhD	Anatomy & Neurobiology
Chelsey Skeete	Boston College	Lee Quinton, PhD	Medicine (Pulmonary, Allergy, Sleep & Critical Care Medicine)
Paris Taylor	Louisiana State University	Gareth Morgan, PhD	Medicine (Hematology & Medical Oncology)
Carlos Ticas	University of Massachusetts, Lowell	Neil Ganem, PhD	Pharmacology & Experimental Therapeutics
Ajah Williams	Louisiana State University	Deepa Gopal, MD, MS	Medicine (Cardiovascular Medicine)

Olu Ayeni
University of Illinois at Urbana-Champaign
Class of 2021

Impact of P2X7 Inhibition on corneal epithelial wound healing

Olu Ayeni, University of Illinois at Urbana-Champaign
Dr. Vickery Trinkaus-Randall, Boston University School of Medicine

The cornea plays a vital role in the ocular system to mediate and focus light that comes into the lens of the eyes. When the outermost layer of the cornea is damaged, epithelial wound healing occurs which requires the migration of cells. Our lab has found that during the healing process calcium travels between cells along the wound edge and is linked to changes in cell motility and morphology. Purinoreceptors and pannexins mediate these calcium mobalizations and are essential to the promotion of cell migration during the healing process. Since we demonstrated that when the P2X7 receptors are activated that cell-cell communication increased and we demonstrated that calcium must mobilize from one cell to another for cells to change shape for migration, I examined the role of P2X7 receptors on cell migration and on the activation of a focal adhesion kinase-paxillin. I hypothesized that P2X7 inhibition would decrease the presence of activated paxillin near the wound region. Previously our lab had shown that knockdown of the P2Y2 receptor decreased the phosphorylation of certain tyrosine residues on paxillin. I assessed this by culturing corneal epithelial limbal cells in keratinocyte medium. At the onset of the experiment the cells were washed and maintained in medium lacking nutrients, then inhibiting one group with a competitive inhibitor to P2X7. Fifty percent of the cultures were wounded and incubated for 4 or 20 hours. After incubation, cells were scraped in a Tris buffer containing sodium orthovanadate, and homogenized. Protein determination was performed using the BCA assay. Proteins were loaded as equivalent protein and a 10% SDS-PAGE gel electrophoresis was performed and the proteins transferred and probed for total paxillin, phosphorylated paxillin, rac and activated rac. From the immunoblots I will be able to determine the activation of the proteins compared to total protein after inhibition of specific receptors. The results could provide insight into better understanding the underlying biochemical mechanisms and physiology behind cell-cell communication and cell migration during epithelial wound healing.

Stephanie Camey
Pitzer College
Class of 2021

The Deposition of β -Amyloid Proteins 1-40 & 1-42 in the Myocardium of Alzheimer's and Type 2 Diabetes

Stephanie Camey[1], Alexis Sotelo[2], Shawn Nirody[2], Chloe Amsterdam[3], Jonathan J. Wisco[2]
Pitzer College, Claremont, California[1]
Boston University School of Medicine, Boston, Massachusetts[2]
Cornell University, Ithaca, New York[3]

The onset of Type 2 Diabetes (TD2) is characterized by cellular insulin resistance and excess blood sugar. T2D has an increased risk of heart disease, which could result in damage to the heart musculature and blood vessels. The protein amyloid beta (Aβ) is known to disrupt normal functioning of the mitochondria, which results in a fatal cascade of events that can lead to the development of Alzheimer's disease (AD). Due to the Aβ aggregation, a neuron's communication with other cells is disrupted and immune cells to trigger inflammation resulting in death of brain cells. The 40-residue peptide Aβ (1-40) exerts detrimental effects on the cardiovascular system by promoting vascular inflammation, while the 42-residue Aβ (1-42) plays a major role in the formation of fibrils, leading to significant increase of amyloid deposition extra-neuronally. There is limited research which examines the role of how Aβ (1-40) and Aβ (1-42) affect myocardial function in AD patients with T2D. Our previous studies suggested Aβ co-localizes with iron in the brain, which can be measured from MR images. One of the main challenges for patients with AD and T2D is they are more susceptible to develop extra stiffness in the ventricle, making it more difficult to pump blood. The current study consisted of 20 *ex vivo* AD heart apex biopsies from 20-day old wild-type and two human transgenic (APP/PS1 and TAU) mice. Each heart apex biopsy was harvested and placed in formalin for fixation. We imaged the biopsies with a Bruker 11.7T MRI using the Fast-Low Angle Shot (FLASH), Fast Imaging with Steady-state free Precession (FISP), and Rapid Acquisition with Relaxation Enhancement (RARE) sequences. One image slice of the base of each heart apex was sampled for mean signal intensity within a 2 mm^2 region of interest (ROI) drawn in Horos (https://horosproject.org). A second 2 mm^2 ROI was measured outside the tissue for each image in order to establish a standard signal intensity. The intra-extra tissue mean signal ratio was used for one-way ANOVA statistical analysis. We anticipated a significant decrease in the signal intensity in the T2 based images, FISP and RARE, denoting

the iron-associated pathogenesis of AD and T2D. The statistical model, comparing abeta, tau, and wild type heart FISP signal was not significant (p=0.833), however. Our results may be due to several factors: an insufficient amount of pathological proteins is deposited in the heart wall by 20 days, and therefore no additional iron deposits in the myocardium; or, any additional iron associating with AD pathology is overshadowed by the endogenous iron present in the myocardium. To confirm either of these hypotheses, we will follow-up with histological analysis. In addition, we will perform MRI and histology studies on heart apices from mice at 3, 6, 9, and 12 months when we expect the AD pathology to progress.

Justin Coleman
Tougaloo College
Class of 2020

Systematic Review of Temporal Trends of the Incidence and Prevalence of Atrial Fibrillation

Justin Coleman; Tinuola Ajayi; Emelia Benjamin, M.D., Ludovic Trinquart, Ph.D.
Department of Biostatistics, School of Public Health, Boston University School of Medicine, Boston, MA

Background: Atrial Fibrillation (AF) is a cardiac arrhythmia marked by random contractions of the atrial myocardium, causing a totally irregular rapid atrial rate. AF is associated with increased mortality risk, increased risk of stroke and heart failure and considerable health-care costs. Several studies have suggested that the incidence and prevalence of AF are increasing but results vary widely. Our objective was to perform a systematic review of the epidemiological trends of AF in the United States (US) and other parts of the world.

Methods: We selected studies that examined temporal trends in the incidence and/or prevalence of AF. Eligible studies were cohort studies and studies using electronic health record data, regardless of location. We discarded studies that examined trends in hospitalization for AF and studies where participants were selected on the basis of special health conditions. We searched PubMed and developed the search equation with the assistance of an information specialist. The selection was performed by two independent reviewers in duplicate. One reviewer collected all data while a second reviewer independently compared all data against the full-text papers. We extracted incidence and prevalence by using an online digitization tool, when needed.

Results: We selected 13 studies. Electronic health records represented 85 % of the studies. Of the three different regions, the average prevalence rate in the US at 6% nearly tripled Asia's average prevalence rate at 2% and Europe's average prevalence rate at 2.33%. Europe's highest incidence rate was 2.6 per 1000 person-years. Asia's highest incidence rate was 1.75 per 1000 person-years. The United States' highest incidence rate was 24 per 1000 person-years. These rates were proven consistent with the US showing the highest incidence and prevalence rates. However, even though the findings are limited to the US, UK, Iceland, Taiwan, Japan, and China, our findings show that AF incidence is definitely on the increase in

these regions and while having the least amount of reported AF cases, there is a higher AF incidence and prevalence in the US than in Europe and Asia.

Conclusions: The United States expressed the highest incidence and prevalence rates, showing that there is a serious AF problem in America. More funding and research should be prioritized in areas where AF incidence and prevalence rates have risen and continue to rise and find more ways to combat this problem.

Gina Conroy
Montclair State University
Class of 2021

Evaluating Expression of Gene SP110 On Cell Susceptibility to HIV-1 Infection

Gina Conroy, Jacob Berrigan, Dr. Rahm Gummuluru
Department of Microbiology
Boston University School of Medicine, Boston, MA 02118

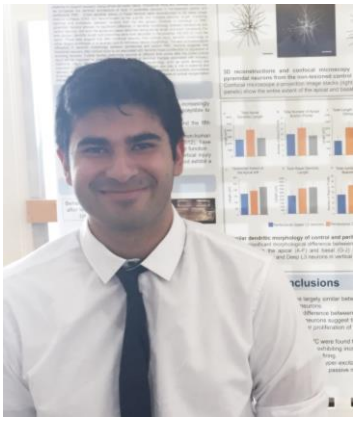
Background: The protein, Sp110, expressed in monocytes and macrophages, has shown to play a part in immune response to diverse pathogens. Nuclear body protein Sp110 is induced by activity of type I interferons. Type I interferons assist in a cell's innate defenses against bacterial and viral pathogens. Because of SP110's role in the immune system, the gene is being studied to evaluate the extent to which its expression influences cell susceptibility to HIV-1 infection. Furthermore, recent studies show that in a mouse model of tuberculosis, homologous deletion of the gene Ipr4 (homolog of human SP110) promotes TB disease progression. Many infected with HIV are also coinfected with Mycobacterium tuberculosis. Being these two infections potentiate and lead to the progression of one another, we are interested in understanding the role of SP110 in influencing HIV/TB co-infections and HIV disease progression. Since the function of protein Sp110 in HIV-1 infection of macrophages is yet to be clearly identified, this study looked to determine the effect of its depletion in human macrophages. We hypothesized that Sp110 expression in macrophages impedes HIV-1 infection.

Methods: In testing this hypothesis, we established two human THP1 monocytic cell lines, one line expressing shRNA against Sp110 (THP-1/Sp110; such that expression of Sp110 is diminished) and the other consisting of non-targeting shRNA (THP1/scramble) cells. THP-1 scramble cells serve as control as these cells express wild type levels of Sp110. THP1/scramble and THP1/Sp110 cells were infected with single cycle HIV-1 based lentivectors expressing GFP and TagRFP fluorescent proteins (HIV/TagRFP/GFP) as reporters of establishment of productive infection. The relative numbers of infected cells between cell lines were measured using flow cytometry as infected cells will fluoresce green and red due to presence of GFP and RFP. SP110 mRNA expression and protein content in THP1/scramble and THP1/Sp110 cells were measured by RT-qPCR and western blot analysis.

Results: While SP110 RNA expression was diminished in THP1/Sp110 cells compared to THP1/scramble cells, flow cytometry showed the THP1/Sp110 line to hold less infected cells. Western blot analysis was faulty as results showed the primary Sp110 antibody to fail and, as a result, no Sp110 protein concentration to be present in either of the cell lines.

Conclusion: These results do not conform to the hypothesis made in which decreased RNA expression in THP1/Sp110 would result in a greater quantity of these cells to be infected in comparison to wild type scramble cells. Correlation between SP110 expression and lower rates of infection are yet to be ruled out and further studies should evaluate the influence of this gene on the monocyte cell's susceptibility to HIV infection.

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



Effects of mesenchymal–derived extracellular vesicle treatment on distinct GABAergic cell types and receptors in perilesional premotor cortex

D. De Alba[1], D. Rosene[1], B. Buller[2], TL. Moore[1] and M. Medalla[1]
Department of Anatomy and Neurobiology, Boston University School of Medicine, Boston, MA[1]
Department of Neurology, Henry Ford Health System, Detroit, MI[2]

Understanding mechanisms of excitatory and inhibitory circuit plasticity that support functional recovery after cortical injury, such as stroke is necessary to develop novel therapeutics. Previous work from our lab has shown that intravenous infusion of mesenchymal-derived extracellular vesicles (EV)- circulating nanovesicles that mediate cell signaling - in a non-human primate (*Macaca mulatta*) model of cortical injury can enhance functional recovery of fine motor function (Moore et al., 2019) as well as normalize the injury-induced hyperexcitability and changes in the excitatory: inhibitory synaptic balance of layer 3 pyramidal neurons in the perilesional premotor cortex (PMC). Here, we assess the role that GABAergic inhibition plays in recovery after injury and how EV-treatment might alter expression of GABAergic synaptic receptor subunits and task-related activation of distinct inhibitory neurons. Once animals had reached an asymptotic level of motor function recovery, brains were fixed and processed for immunohistochemistry to label GABAA1 and GABAB2 receptor subunits and distinct inhibitory neuronal classes expressing the calcium-binding proteins parvalbumin (PV), calbindin (CB) and calretinin (CR) in dorsal and ventral perilesional PMC. To assess inhibitory cells activated during the motor task, brains were fixed 3 hours after a final testing session, and sections were processed for immunolabeling of c-fos, an intermediate early gene expressed in neurons activated during the task. Images were obtained using confocal microscopy and analyzed using cell counting and particle and co-localization analyses in ImageJ. Expression of GABAergic markers were compared between EV and vehicle treated monkeys. Optical density particle analysis of GABAergic receptor labeling showed no significant differences between treatment groups. Analysis of the density of PV, CB and CR and co-localization with c-fos to determine the activation of inhibitory cell populations recruited to during the motor task revealed a significantly greater density and proportion of CB interneurons that were colocalized with c-fos in the superficial layers of the PMC in EV-treated compared to vehicle-treated brains. The greater density of c-fos + neurons was significantly

correlated with a greater degree of recovery. Thus, based on the fact that CB interneurons target mainly the distal dendrites and spines of pyramidal neurons, our findings suggest that EV-treatment may facilitate recovery by enhancing distal dendritic inhibition to filter and refine inputs coming into the PMC. By assessing the possible mechanisms of EV-mediated enhancement of GABAergic plasticity following cortical injury, we will be better able to distil the neural circuit mechanisms underlying functional recovery and the viability of EVs as a clinical therapeutic for stroke and brain injury.

Dylan Dominguez
University of Arkansas
Class of 2020

Non-Operative Management of >80% Asymptomatic Carotid Artery Stenosis

Dylan Dominguez, Jeffrey Siracuse
Division of Vascular and Endovascular Surgery, Boston Medical Center Boston University School of Medicine, Boston, MA 02119

Background: Carotid artery stenosis is the condition where atherosclerotic plaque builds up within the arterial walls, causing a narrowing of the artery. This can ultimately lead to occlusion or stroke if left untreated. Treatments can be invasive; the plaque can be removed surgically via carotid endarterectomy (CEA) or revascularized through carotid angioplasty and stenting (CAS). Patients who are unable to undergo invasive procedure are treated using best medical therapy (BMT) which involves intensive use of blood thinners and cholesterol control. The data containing reasons that patients are not undergoing active treatment is currently lacking and further review is necessary. This data will ultimately help determine whether surgical intervention or BMT is optimal for treatment of asymptomatic carotid artery stenosis.

Objective: Form a characterization of >80% asymptomatic carotid artery stenosis patients that did not undergo CEA or CAS within 6 months of stenosis discovery. Also, to review outcomes in patients that proceeded to have a stroke or intervention (CEA or CAS) >6 months after discovery of stenosis.

Methods: In this retrospective case review study, 7405 patients were identified to have had carotid ultrasound imaging and carotid stenosis within the past 10 years. Patients with asymptomatic stenosis were defined as having atherosclerotic extracranial internal carotid artery narrowing and lack of stroke or transient ischemic attack (TIA) 6 months prior to the imaging. Stenosis was determined using ultrasound imaging to record systolic and diastolic velocities, a systolic of >125 cm/s and a diastolic of >140 cm/s were necessary to define as >80%. Using the eligible cohort (n=34) data such as age, race, gender, comorbidities, medications, intervention, and progression to stroke or TIA were recorded.

Results: Our data indicates reasons for patients to not undergo initial CEA or CAS included factors such as severe comorbidities (50%), delayed/ no referral (14.7%), advanced age (11.7%), patient refusal (8.8%), other cerebrovascular issues (8.8%), and active/advanced cancer diagnosis (8.8%). Patients who went on to have an intervention were relatively low (11.7%), with CEA (8.8%) being more common than CAS (2.9%). The data also shows a high occurrence of stroke (11.8%) and TIA (11.8%) >6 months after onset of BMT, with 17.6% of patients presenting with a stroke or stroke-like symptoms. 17.6% of patients died after onset of BMT with a 4-year survival rate of 79%, though no deaths were related to stroke or the carotid artery.

Conclusion: Patients with severe asymptomatic carotid stenosis were not able to undergo invasive intervention for a variety of reasons, most of the time because of severe comorbidities. Stroke and TIA rates were high, indicating that if comorbidities could be managed, CEA and CAS could be the best option for management of severe asymptomatic carotid artery stenosis.

Sojourna Ferguson
University of the Virgin Islands, Class of 2019
Boston University School of Medicine, Class of 2023

Identifying the Etiology of Squamous Cell Carcinoma

Sojourna Ferguson, Andrew Tilston-Lunel, and Xaralabos Varelas
Department of Biochemistry, Boston University School of Medicine, Boston, MA 02118

Squamous cell carcinoma represents an area of lung cancer that has not been effectively studied. Consequentially, the therapeutic options are quite limited. It is hypothesized that this carcinoma emerges from the airway epithelia, but the origin of the disease is poorly understood. Knowledge into the mechanisms contributing to diseases onset may therefore offer directions for developing effective treatments for patients. The goal of the project was to identify factors that maintain airway epithelial homeostasis by screening gene deletions that drive precancerous airway phenotypes in mouse primary tracheas. We started by conditional knock outs of the polarity regulator Crumbs3 (Crb3) in the luminal cells of the airway epithelia contributes to precancerous cell growth. We also attempted to identify additional factors important for maintaining airway epithelial homeostasis by generating and screening specific gene knockouts that have been implicated in the lung cancer using ex vivo CRISPR/Cas9 strategies. For this, primary mouse tracheas were isolated then treated with viruses transducing unique guides that promote candidate gene knockout (CDKN2A, PTEN, ARID1A, and FAT1). Tissues for each knockout and controls were monitored for the expression of the markers of the normal epithelium to look for imbalances in cell fate outcomes. Our overall goal was to identify perturbations that contribute to precancerous phenotypes that associate with squamous cell carcinoma development. Such knowledge will improve our understanding of the etiology of squamous lung cancers and ideally direct therapeutic opportunities for patients with this disease.

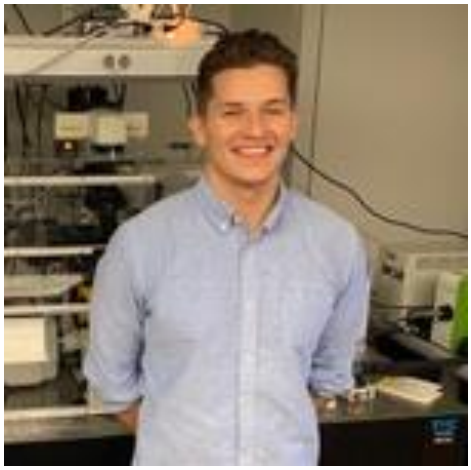
Cynthia Flores
Mount Saint Mary's University
Class of 2021

Point Mutations in Surface-Exposed Tyrosines of the Adeno-Associated Virus DJ Capsid to Improve Transduction Efficiency *In Vivo*

Cynthia Flores[1], Elisabeth Smith[2], Beatriz Ferran Perez[2], and Markus Bachschmid[2] Mount Saint Mary's University[1], Los Angeles CA, Boston University[2], Boston, MA

Gene therapy using adeno associated virus (AAV) is becoming a popular method of introducing healthy or modified genes into animals and patients. Adeno-associated viruses, from the parvovirus family are preferred vectors due to their high transduction efficiency, safety, negligible immune-response, and extended stable gene expression. Recent phase I/II clinical trials with AAV2 are promising but require high vectors doses to achieve sufficient gene expression. A 2008 study found that changing specific surface-exposed tyrosines to phenylalanine on the AAV2 capsid significantly increased viral transduction efficiency. Our study aims to improve further the transduction efficiency of the AAV-DJ capsid, a vector engineered for liver gene therapy. Using PyMOL, we performed molecular modeling of the viral DJ capsid structure and identified surface-exposed tyrosines. We mutated potential tyrosine residues to phenylalanine with site-directed mutagenesis and confirmed the clones by sequencing. To produce the wildtype and mutant AAVs, we transfected HEK293T and HepG2 cells with pHelper, a viral expression plasmid encoding green fluorescent proteins (pAAV-IRES-EGFP), and the respective replication/ capsid vectors (pRep/Cap). We purified the virus by PEG-precipitation and determined the titer with quantitative real-time PCR. We cloned and verified DJ capsid mutants with Y443F, Y445F, and Y732F. Unexpectedly, preliminary data has shown that mutant capsids generated higher titers than wild type AAV-DJ.

Braden Garland
Northwest University
Class of



Exploring UPP inhibitors as a potential method to decrease CPT resistance in the treatment of CRC.

Ajit K. Bharti, Braden A. Garland, Elizabeth C. Unan, Koji Ando, Julian Taylor-Parker, Ankur K Shah

1.Department of Medicine, Division of Hematology Oncology, Boston University School of Medicine, 88 East Newton Street, Boston, MA 02118 USA

2. Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku Fukuoka City, Fukuoka, Japan 8128582

Topoisomerase I (topoI) inhibitors (camptothecin and its analogues, CPTs) represent a highly potent class of anticancer drugs. Two FDA approved CPT analogues, topotecan and irinotecan, are used in treating cancer patients. Irinotecan is used as the first line of therapy in metastatic colon cancer, and both are used as the second or third line of therapy in many other solid tumors, including pancreatic, gastric and small cell lung cancer. Several third generation topoI inhibitors are in different phases of clinical development and have shown significantly better pharmacokinetics and toxicity profiles. However, only 13 to 32% of patients respond to these drugs. The mechanism of resistance is not well understood, but in previous years, the Bharti lab has discovered BRCA1 as the ubiquitinating enzyme to TOPO1. With this knowledge, the lab set out on a mission to discover a drug which can inhibit BRCA1 at the RING domain and therefore reduce TOPO1 degradation. Using CRISPR/CAS genome editing, we have integrated GFP with a topoI gene in two CRC cell lines HCT15 (CPT resistant) and HCT116 (CPT sensitive). Upon observing the CPT sensitive cell line, TOPO1 shows rapid localization and co-localization with BRCA1. This was visualized using the SIM (Super-resolution Microscope) at UMASS in Amherst, MA. Our objective was to then identify drugs that have a synergistic effect with CPT, that result in an increased sensitivity cell death. After using these resistant HCT cells and a small molecule library, we have identified a handful of potential analogs that may increase the effectiveness of CPT. Of these lead compounds, we tested D2 and PD901 for synergy and viewed the colocalization of TOPO1 and BRCA1 within the resistant HCT cell line. This data is an exciting possibility for improved cancer treatments, especially CRC. Further research is required to see if there are any other more effective UPP inhibitors and the possibility of purifying already known UPP inhibitors to increase the synergistic effects with CPT.

Nina Goodson
American University
Class of 2020

Treating Lung Cancer through Inhibition of the Aryl Hydrocarbon Receptor Nina Goodson[1], Megan Snyder[2] and David Sherr[2]

[1]American University, Washington, D.C.,

[2]Department of Environmental Health, Boston University School of Medicine, Boston, MA

Background: Lung cancer is the leading cause of cancer death in both males and females and is projected to affect over 228,000 Americans in 2019. Lung cancer is typically caused by inhalation of carcinogens, but is also a result of abnormally activated signaling pathways mutated to promote tumorigenicity. The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that is constitutively activated by an amplification loop involving the enzymes, indole 2,3-dioxygenase (IDO) and tryptophan dioxygenase (TDO), and the AHR-ligand, kynurenine. When AHR is activated by kynurenine, it translocates to the nucleus to transcribe cytochrome P450 family member genes, *CYP1A1* and *CYP1B1*. It also transcribes genes encoding for IDO and TDO enzymes that metabolize tryptophan into kynurenine. The continuous production of the endogenous ligand leads to immunosuppression and cancer stem cell progression. Previous studies show that there is heightened activation of AHR in glioblastoma, melanoma, oral and breast carcinomas, and that knocking out the AHR has enhanced the immune response. The goal is to treat lung cancer by blocking the AHR-driven amplification loop with AHR inhibitors; thus, inhibiting immunosuppression and tumorigenesis.

Methods: The mRNA expression of *CYP1A1* and *CYP1B1* is analyzed by RT-qPCR to determine whether the inhibitors are effective in lowering the activity of AHR. CMT mouse and A549 human tumor lines are plated separately, and dosed with the inhibitors, CB113, HP163, Drug A, or a combination of the treatments for 4 hours. A replicate cell plate for each tumor line is also conditioned with kynurenine to amplify the effects of the ligand on the AHR pathway. After treatment, the RNA is isolated from the cells, made into cDNA, and the relative expression is measured by qPCR. The mRNA expression of the transcripts is compared to housekeeping gene, GAPDH and the diluent, DMSO.

Results: The cells that received treatment expressed lowered levels of *CYP1A1* and *CYP1B1* mRNA than the cells that did not receive inhibitors. The combination treatments with Drug A and one of the antagonists, appeared to be more effective than the drugs alone in lowering the expression of the transcripts in both cell lines, a relationship further amplified by kynurenine. Cells conditioned in only CB113 or HP163 showed mRNA expression that was significantly less than the cells conditioned only in DMSO. Drug A in the presence of kynurenine exhibited inconsistent results where 1A1 and 1B1 expression increased in CMT cells, but drastically decreased in A549 cells.

Conclusions: The combination of AHR inhibitors effectively lowered the mRNA expression of *CYP1A1*
at the inhibitors can hinder the AHR-driven loop, and show promise iable target for treating lung cancer

Jacob Hernandez
University of Texas at El Paso, Class of 2018
Boston University School of Medicine, Class of 2022

Effects of Thrombospondin-1 on Outflow Facility and Hydrodynamics of the Aqueous Outflow Pathway in the Porcine Eye

Jacob Hernandez, David L. Swain, Lucia Yang, Haiyan Gong

Background: Glaucoma is a leading cause of blindness worldwide. Elevated intraocular pressure (IOP) is a major risk factor for primary open-angle glaucoma (POAG). IOP is maintained by dynamic balance between aqueous humor (AH) production and drainage. If AH drainage pathway through the trabecular meshwork (TM) and Schlemm's canal (SC) is impaired, AH can build up and increase IOP. Thrombospondin-1 (TSP-1) is an extracellular glycoprotein and expressed within the eye, including the TM and SC. TSP-1 expression has found increased in 1/3 of POAG patients compared to normal. Deletion of TSP-1 in mice resulted in lower IOP than control mice. These studies suggest that TSP-1 may play a role in regulating AH outflow and IOP. However, the mechanism through which TSP-1 regulates IOP is still unknown.

Objective: This study aims to determine the effects of TSP-1 on outflow facility and hydrodynamics of AH outflow pathway in *ex vivo* porcine eyes using ocular perfusion and confocal microscopy.

Study Design and Research Methods: 8 pairs of normal porcine eyes were perfused with glucose-phosphate buffer (GPBS) to measure baseline OF and then with GPBS or TSP-1 in GPBS (850 ng/mL) for 3 hours at 15 mmHg. Anterior chamber contents of each eye were exchanged and perfused with a fixed-volume of red fluorescent tracers to label AH outflow pattern. Eyes were then perfusion-fixed. Change in outflow facility over time and compared to baseline was determined. Each eye was dissected into 12 radial wedges circumferentially. Each wedge was then cut frontally into anterior/posterior pieces, including the region of interest: TM and aqueous plexus (AP, analogous to human SC). Images were taken on both pieces of each wedge using confocal microscopy. Effective filtration length (EFL), one-dimensional equivalent to effective filtration area, was measured per eye and calculated as the

fluorescent-decorated length (FL) / total length (TL) of the inner wall of AP. FL is denoted as length where the red tracers reach the inner wall of AP. Average percent EFL (∑FL/ ∑TL) in each eye was calculated. A minimum of 3 images per wedge from all 12 wedges were measured and analyzed for each eye (total: 553). Statistical analysis was performed with R.

Results: Outflow facility increased significantly compared to baseline ($p < 0.01$) in control eyes due to washout effect at each time point, but did not increase as much in TSP-1 treated eyes, ($p < 0.05$) compared to controls at each time point ($N = 8$ eyes per group). The percentage change in outflow facility was significantly less in TSP-1 treated eyes compared to control at all time points. ($p < 0.05$). The percentage EFL was not significantly different between groups ($N = 4$ eye each group).

Conclusions: TSP-1 treated eyes had reduced outflow facility compared to GPBS control, through inhibiting the washout effect. Whether EFA is related to this change in OF needs further investigation with a larger sample size.

Shantae Lewis
University of the Virgin Islands
Class of 2020

The Role of Cancer Associated Fibroblasts in Maintaining Cancer Stem Cells

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Background: Breast cancer is one of the leading causes of cancer related deaths in females. Treatment options include surgery, radiation, chemotherapy, targeted therapy, and hormone therapy. However, these treatments ultimately fail, resulting in cancer relapse. One explanation for recurrence after treatment is the presence of cancer stem cells (CSCs). CSCs have high proliferative potential and are able to initiate tumor growth. In order to target CSCs, it is important to understand how CSCs are maintained. Recent studies have shown that cancer-associated fibroblasts (CAFs) aid in the maintenance of CSCs. CAFs are an abundant cell type in the tumor microenvironment and have been found to promote tumor growth and confer resistance to anti-cancer drugs by supporting cancer stem cells.

Hypothesis: We hypothesized that CAFs promote and maintain the growth of cancer stem cells in a novel mouse tumor model (MCaP).

Methods: Mammary carcinoma cells (MCaP) were propagated in culture from a tumor that developed spontaneously in an aged mouse. Cancer cells, along with CAFs were expanded from the isolated tumor. After identifying CSCs in the MCaP culture, we asked whether the CAFs were critical for maintaining the CSCs. We used several assays to test our hypothesis. Cancer cells were grown as a mixed culture with CAFs or alone after enrichment. First, we used a colony growth assay to assess the formation of CSCs, which grow as colonies. We fixed and stained cultures with the cancer marker cytokeratin. Colonies were manually counted using a fluorescence microscope. The size of the colonies was quantified using ImageJ. Next, we performed flow cytometry, to detect and quantify surface expression of CSC markers CD44 and CD24 in mixed cultures and isolated cancer cells. To investigate whether CAFs enable cancer cell resistance to chemotherapy, we measured the dose response to cyclophosphamide or paclitaxel. Finally, immunofluorescence was performed on tissue sections of metastatic lymph node and primary tumor. Tissues were stained with cytokeratin,

aldehyde dehydrogenase (ALDH), and alpha-sma (SMA) and visualized with fluorescence microscopy.

Results: From the colony growth assay, colony formation and the size of colonies were both increased when MCaP cells were cultured with CAFs. Flow cytometry showed that MCaP cancer cells grown with CAFs resulted in the appearance of a CD44 high/CD24low population. This CSC population was not seen in cancer cells grown alone. After treating cells with paclitaxel or cyclophosphamide, we saw no significant difference in the viability of cancer cells in the presence or absence of CAFs. Immunofluorescence staining showed ALDH was expressed in SMA-positive CAFs, but not in cytokeratin-positive cancer cells.

Conclusion: Although we did not find that CAFS protected cancer cells from chemotherapy, CAFs appear to have a positive effect on the size and quantity of CSC colonies. In addition, we detected a CD44 high, CD24 low CSC population in mixed cultures. Finally, with immunofluorescence, CAFs were found to express the CSC marker ALDH, with expression increasing as the size of the tumor increased. This finding could mean that CAFs may provide a niche for CSCs. In conclusion, these results suggest that CAFs play an important role in maintaining CSCs in the MCaP mouse model.

Antonio Lopez
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Characterizing hippocampal projections to the thalamic reticular nucleus

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Background: Schizophrenia is a mental disorder known for disorganized thought and issues with attention and memory. A hallmark of schizophrenia is the inability to maintain and properly switch attention. Implicated in shifts of attention is the thalamic reticular nucleus (TRN), a collection of inhibitory neurons that surrounds the thalamus and filters incoming information to the thalamus. The TRN also plays a role in the production of sleep spindles during second stage NREM sleep, which is thought to contribute to neuronal plasticity and memory consolidation. The TRN has been shown to connect with hippocampus (HPC), the memory center of the brain. The HPC is thought to provide contextual and experiential information to the TRN that is incorporated with sensory information from other brain areas (e.g. amygdala, prefrontal cortex) to decide whether to change attentional focus. Although we understand some of the roles the two regions have with attention and memory, the interplay between the two has not been explored in depth, especially in primates.

Objective: To understand how the HPC modulates neuronal processes in the TRN. This will be done by studying HPC boutons synapsing on TRN dendrites and by studying whether calcium binding proteins, specifically calretinin (CR), are present in labeled boutons or synapsing dendrites.

Methods: Three rhesus macaque monkeys were stereotactically injected with neuronal tracers into the CA1 and CA3 regions of the HPC to label for HPC boutons. Afterwards, the animals were perfused and their brain tissue fixed. TRN tissues were then immunolabeled with gold, TMB, and DAB, which labeled calcium-binding proteins, and our tracer. The tissue was run through electron microscopy processing involving heavy metal infiltration and afterwards the tissue was viewed under a transmission electron microscope and a scanning electron microscope.

Results: Three labeled boutons were found, all synapsing on dendrites with mitochondria. Only one dendrite had a label for CB, others had no labeling. These boutons were not adequate for quantitatively describing synaptic surface area or bouton volume. Finding this few boutons makes the pathway sparser than we initially thought.

Conclusion: More work must be done to describe hippocampal projections to the TRN, including finding enough boutons to calculate synapse size and bouton volume. Future work involves neuropil analysis of CR marked dendrites, which have yet to be studied.

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Adipo C Inhibits Acyl-CoA Synthetase and Reduces Lipid incorporation into Clonal Pancreatic ß-Cells (INS-1).

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Background: Type 2 Diabetes (T2D) is a metabolic disease in which the body becomes resistant to the insulin that it naturally produces. Basal insulin hypersecretion resulting from chronic exposure to excess nutrients (glucose and fatty acid (FA)) is believed to contribute to the development of this state of insulin resistance. Transcription Factor 7-Like-2 (TCF7L2) is the strongest genetic risk factor for T2D that is currently known. It has been shown that deletion of TCF7L2 results in reduced long-chain acyl-CoA synthetase 5 (ACSL5) mRNA. Therefore, it is also thought that ACSL5, an enzyme that converts FA to its CoA ester required for FA metabolism, also plays a role in the occurrence or progression of T2D. Adipo C is a putative ACSL5 inhibitor. The goal of our research is to determine the effect of Adipo C on ß-cell lipid metabolism.

Methods: Insulin secretion was measured using an HTRF insulin immunoassay (CisBio). Fatty acid (FA) incorporation into lipid was measured by fluorescence imaging after thin layer chromatography (TLC) of chloroform:methanol (2:1) lipid extracts from INS-1 cells incubated with fluorescent bodipy-FA (BFA) with and without Adipo C (10 µM). ACS activity was measured from INS-1 cell homogenates incubated with BFA with and without Adipo C (10-100 µM) after organic separation of the bodipy-FA-CoA product.

Results: Chronic exposure to excess nutrients resulted in lipid accumulation and basal insulin hypersecretion. Adipo C reduced BFA incorporation into neutral lipids of INS-1 cells including mono- and diacylglycerides. Adipo C inhibited ACS activity in extracts from INS-1 cells cultured in excess nutrients.

Conclusion: Our results confirm Adipo C inhibits ACS activity in INS-1 cells and reduces FA incorporation into lipid. Adipo C may help to prevent, delay, or reverse lipid accumulation leading to basal insulin hypersecretion and T2D.

Sabreea Parnell
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Apoptosis and neutrophil recruitment in aortas of vascular smooth muscle Bcl11b knockout mice

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BACKGROUND and SIGNIFICANCE: 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 vasculature. When BCL11B is knocked out of the vascular smooth muscle of mice treated with the potent hypertensive agent angiotensin II for 2 weeks, 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. After validation of RNA sequencing results using qRT-PCR with specific Taqman probes to measure gene expression of 15 selected genes and statistical analysis by ANOVA, 2 genes (CASP8 and PPBP), which displayed the highest mRNA fold change, were selected for further studies. BSMKO (n=4) and WT (n=4) mice were treated with angiotensin II (1mg/kg/day) for 7 days to further study protein expression of CASP8 and PPBP. Downstream targets of CASP8, including cleaved caspase-3 and apoptosis, were assessed via immunofluorescence staining of aortic sections (10μm) with cleaved caspase-3 antibody and a TUNEL assay, respectively. NIMP-R14, a neuthrophil cell surface marker, and anti-neutrophil elastase antibodies were utilized through immunofluorescence as downstream indicators of PPBP protein expression.

RESULTS: Cleaved caspase-3 expression in BSMKO/angII mice increased when compared to WT/angII mice. TUNEL staining showed similar instance of apoptosis between BSMKO/angII and WT/angII mice after 7 days treatment. Neutrophil recruitment was observed within the wall

of the aorta with increased presence in BSMKO/angII mice, corresponding to an average comparative increase of neutrophil elastase.

CONCLUSIONS: Our results suggest that activation of caspase 8 and 3 are early molecular events preceeding overt apoptosis, which may contribute to the development of aortic aneurysms in BSMKO/angII mice. Blocking the caspase cascade could represent an early stage therapeutic target to prevent aneurysm formation by apoptosis in at risk individuals. Evidence of an early inflammatory response warrants further investigation into its implications on aortic aneurysm development when BCL11B is absent from vascular smooth muscle.

Kayley Pate
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A Deep Learning Framework to Combine Multimodal Data and Predict Recurrent Stroke Risk

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Approximately one in four stroke victims will have another stroke within their lifetime, known as a recurrent stroke. These strokes usually have a higher rate of death and disability, because parts of the brain that are previously injured may not be resilient to a second attack. However, it is thought that approximately 80% of recurrent stroke cases can be prevented through lifestyle changes and medical interventions. Advanced machine learning models, such as deep learning neural networks, could be used to assess recurrent stroke risk. The goal of this project is to gather first-time and recurrent stroke data of Boston Medical Center patients and use it to develop a deep learning model to predict the risk of recurrent stroke. This is a cross-sectional study on BMC patients with first-time and recurrent stroke at year 1, year 3, or year 5. Of these patients, MR images, demographic data, and clinical data were gathered from the BMC Clinical Data Warehouse. This multimodal data will be assigned a label of 'recurrent stroke' or 'no stroke' and then fed into a neural network computer to train the model to predict the risk of recurrent stroke, based on the information. Accuracy and precision of the test model will be cross-validated by comparing the test predictions against the predictions of the trained model with labels. Preliminary data shows that 1,765 patients between 2009-2019 had a first-time stroke primary diagnosis. Of those, 72 had a recurrent stroke within year 1, 104 within year 3, and 122 within year 5. The next step of the project is to train the computer model using this data. This project will be the first effort to build and use a multimodal fusion model using deep learning to predict recurrent stroke risk. If the computer model can be used to create an accurate stroke prediction tool, this will result in early and life-saving risk prediction modeling of recurrent stroke.

Moises Ramirez
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A Quantitative Study of Antibodies 1558 and 1168 Against Anthrax

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Background: B lymphocytes are able to produce a diverse antibody repertoire through a process called recombination, in which the distinct immunoglobulin V, D, and J segments are rearranged during the B cell's development. As the immune response progresses, antibody affinity for the antigen increases through somatic hypermutation, while natural selection serves as the impetus towards the most effective VDJ combination. The result ends with the expansion of B cells bearing high affinity antibodies, ready to combat the same invading pathogen if there is another encounter. Research is being done with efforts to exploit the process of affinity maturation using computer-based methods in order to create a new approach towards highly effective vaccines against diseases such as the gram-positive Bacillus Anthracis (Anthrax).

Hypothesis: In this project, the B cell dynamics and immunoglobulin affinity maturation are studied in human subjects following the Anthrax Vaccine Adsorbed (AVA) administration. The Anthrax Vaccine Adsorbed (AVA) administration protocol is inefficient compared to other vaccines (3 injections at 0, 1, and 6 months, booster injections at 12 and 18 months, and a yearly booster for those who are still at risk).

Methods: Previous work through next generation sequencing has identified candidate anthrax-reactive antibodies, among which two antibodies, 1168 (high affinity) and 1558 (medium affinity), are selected for synthesis in order to further evaluate their function. The antibody DNAs were synthesized, cloned into vector pcDNA3.3, and amplified in a DH5-α E. coli strain. Restriction mapping, together with a basic gel electrophoresis procedure, will be performed to confirm the acquisition of the correct antibodies. Mammalian cells (293F) were cultured for > 5 passages, and the amplified recombinant DNA was transformed into the cultured mammalian cells. Antibodies 1168 and 1558 are expected to be expressed from the 293F cells. Their affinity for Anthrax is to be evaluated via Surface Plasmon Resonance (SPR) analysis, mass spectrometry analysis will assist in determining antibody molecular weight (kD), and western-blotting will be used to determine if antibodies are intact.

Results: Due to the promising data acquired during the AVA administration tests, antibodies 1168 and 1558 are expected to show a significant level of affinity towards the anthrax strain, post-SPR analysis. Mass spectrometry is expected to allow the determination of heavy chain and light chain masses of the studied antibodies.

Conclusion: Based on the expected data to be determined after SPR analysis, it will then be known whether antibodies 1168 and 1558 actually have a high level of affinity for the anthrax strain. If so, this study will confirm the efficacy of the utilized methods, and the studied antibodies will be used to further advance the development of an improved vaccine for anthrax. Characterization of the antibodies will assist in the elucidation of the immunological response to anthrax.

Erlin Ravariere
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Examining the Difference Between B-Cell Specific IRF5 Knockout and Wild-Type Mice with Lupus

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Background: Systemic Lupus Erythematosus (SLE) is a progressive, chronic autoimmune inflammatory disease that can affect any part of the body. SLE is characterized by flares, spontaneous remission, relapses and is also the most common type of lupus. This disease is also a type of glomerulonephritis in which the glomeruli, which are found in the cortex of the kidney, become inflamed. SLE can affect any part of the body however often damages the skin, joints, heart, kidneys, lungs, and nervous system. Patients with SLE are also known to be at a 50% risk of developing organ-threatening symptoms of this disease whereas the other half of patients may not. Also, 85% of recorded patients have been noted to be women; Native Americans are also the most susceptible. The exact cause of SLE is unknown however Interferon Regulatory Factor 5(IRF5) has been a focus during the study of this disease due to its potential involvement however how this occurs is uncertain.

Goals/Hypothesis: There were three goals of this project. The first was to discover which IRF5 presenting cells are important. Secondly, we wanted to generate conditional knockout mice IRF5 and delete IRF5 in B cells only. Lastly, to examine the difference between IRF5 B-cell knockout and wild-type mice with lupus based on Immunoglobulin G (IgG) and Complement Cascade 3 (C3) deposition in kidneys.

Methods: Wild-type and IRF5 B-cell knockout mice were sacrificed and the kidneys of these mice were extracted. Kidneys were then frozen, sliced, and placed on a slide for staining. Kidney slides were stained with FitC antibody for C3 and Alexa 568 for IgG. Upon being stained, slides were then imaged using a deconvoluted imaging microscope. Images would then be analyzed using Fiji in order to measure the cell fluorescence of 10 glomeruli per slide.

Results: When comparing the staining results of C3 in knockout mice samples in comparison to wild-type mice samples, it was observed that knockout samples did not stain. However, wild-type samples did stain. For IgG, both knockout and wild-type samples both stained however wild-type samples were more fluorescent not only in glomeruli but also within the tissue itself.

Conclusion: Previous research has shown that IRF5 is causing SLE. The goal of this research is to discover which IRF5 cells are important. To investigate this, we generated conditional knockout mice of IRF5, and deleted IRF5 in B-cells only. From the results of both C3 and IgG it can be suggested that by knocking out IRF5 in B-cells that they play a vital role in increasing the levels of IgG and C3 in the kidneys of lupus mice. For future work, images that were procured from the deconvoluted microscope will be analyzed using Fiji in order to peruse the cell fluorescence of the glomeruli.

Gerardo Sequen Rivera
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Ultrastructural Analysis of Dorsal Striatum in the Q175 Model of Huntington's Disease

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Huntington's Disease (HD) is an inherited autosomal disorder caused by the excessive elongation of CAG repeats (>39) on exon 1 of the Huntingtin (Htt) gene. This trinucleotide expansion leads to the production of the mutant Htt protein. HD is a progressive neurodegenerative disorder that affects the thalamus, cortex, and predominantly, the striatum, which is comprised of 90-95% of GABAergic Medium Spiny Neurons (MSNs). As a consequence of the degeneration of cortico- thalamo-striatal brain regions, individuals experience progressive motor, cognitive, and psychiatric deficits that compromise their activities of daily life. In HD, synapses degenerate via a poorly understood mechanism. The present study is derived from our previous confocal microscopy studies that revealed an overlap of vesicular glutamate transport subtypes (Vglut1 and Vglut2), and Parvalbumin (PV) and Vesicular GABA Transporter (Vgat) at the dendritic spines of direct (D1) and indirect (D2) MSNs in the $Q175^{+/-}$ mouse model suggesting the presence of inhibitory and excitatory appositions or putative synapses that may change during disease progression. Electron microscopy (EM) serves as a tool to validate appositions seen at the light level as definite synapses. Thus, we compared the dorsal striatum of $Q175^{+/-}$ mice, which closely mimics the genetic context of patients with HD, and Wild Type (WT) mice at 12 months of age, using EM. We quantified the nature of excitatory and inhibitory synapses on the somata and dendrites of MSNs in $Q175^{+/-}$ and WT mice at the ultrastructural level. A total of six one-year old mouse subjects (3 WT and 3 $Q175^{+/-}$ mouse models) were used for purposes of this study. Subjects' brain tissues underwent EM processing, serial-blockface imaging, and 3D reconstruction. Reconstruct -a 3D reconstruction software- was used to trace regions of interest (ROIs) in order to quantify postsynaptic density, presynaptic bouton volume, number of vesicles, and spine volume data on MSNs. MSNs were classified by

type (excitatory vs. inhibitory), by target (on spine vs. on shaft) and by morphology (e.g. perforation). Preliminary linear regression results show no significant difference in density of inhibitory synapses along dendrites and soma. However, Student's t tests revealed: significantly lower vesicle number in inhibitory presynaptic boutons apposed to somata ($p<0.049$), significantly higher vesicle number in inhibitory presynaptic boutons apposed to dendrites ($p<0.101$), and significantly reduced number of vesicles in excitatory boutons apposed to dendrites ($p<0.014$) in $Q175^{+/-}$ compared to WT mice. These preliminary findings suggest an imbalance of excitation and inhibition in the dorsal striatum of $Q175^{+/-}$ mice.

Chelsey Skeete
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The Role of LOX-1 in Epithelial Cells during Pneumonia

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Background: Pneumonia is an acute lower respiratory condition often caused by a bacterial, viral or fungal infection. Treatment options include antimicrobial strategies such as antibiotics. However, there is often an accumulation of lung inflammation and cell death caused by the patient's immune response which needs to be resolved to resume normal lung function. Therefore, it is essential to identify the biological pathways under which tissue damage either worsens or improves the following infection. Previously, we have shown that LOX-1, a class E scavenger receptor, increases in lung epithelial cells during pneumonia. LOX-1 has been shown to worsen inflammation in vascular tissue, however, there is scarce information on the receptor's effect on the lungs.

Hypothesis: Our objective is to explore how activation of the LOX-1 receptor impacts inflammatory responses during pneumonia. We hypothesized that direct activation of the LOX-1 in this cell type would induce inflammatory gene expression and cellular injury.

Methods: To test our hypothesis, a mouse epithelial cell line (MLE12) was stimulated with *Escherichia coli* and/or 100µg of the main ligand for LOX-1, oxidized low-density lipoprotein (oxLDL). After stimulation, inflammatory cytokine expression was measured using qRT-PCR. In addition, an intratracheal injection with 100 ug of the main ligand for LOX-1 was administered to mice to stimulate LOX-1 activity in the left lobe of the lungs. Mice were euthanized 24 hours after oxLDL installation and bronchoalveolar lavage fluid (BALF) samples were collected. BALF from treated mice was compared to saline controls were analyzed for changes in cytokine expression. The LDH assay was conducted on supernatant in vitro samples to measure cell death.

Results and Conclusion: As expected, in vitro *E. coli* was sufficient to elicit induction of several cytokine transcripts. While oxLDL combined with *E. coli* did not further elevate this response, treatment of cells with oxLDL alone was sufficient to increase cytokine expression for the gene encoding CXCL5, a known epithelial-specific chemokine for neutrophils. In conclusion, oxLDL is sufficient to induce inflammatory signaling in vitro. Analysis of specimens collected from oxLDL treated mice remain ongoing, but initial results have not indicated an effect on cytokine expression. In summary, it is possible that stimulation of the LOX-1 receptor during pneumonia induce inflammation in mouse epithelial cells. If this interaction with the receptor increases inflammation, then treatment for pneumonia should also include treating epithelial tissue damage.

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The Role of Protein Stability in Leukocyte Chemotactic Factor 2 (LECT2) Amyloidosis

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Amyloidosis is a rare disease in which insoluble extracellular protein fibrils in beta-pleated sheets infiltrate multiple organs, causing organ dysfunction and failure. There are many different classifications of amyloidosis, including light chain, transthyretin, amyloid A component, and gelsolin amyloidosis. Our research focuses on a form of the disease known as LECT2 amyloidosis. LECT2 amyloidosis is the latest systemic type of amyloidosis to be described, discovered only 10 years ago. In this disease, amyloid fibrils are formed from the misfolding of the LECT2 protein. This is a multifunctional protein predominantly expressed in the liver and acts as a signaling agent to regulate the function of other cells. LECT2 amyloidosis now seems to be the third most common cause of acquired renal amyloidosis, occurring predominantly in patients from South Asia, North Africa, the Middle East, and Mexico.

LECT2 has a protein polymorphism. About 1/3 of the population has valine at the position 40 as opposed to the rest of the population that has isoleucine. This is important because all patients with LECT2 Amyloidosis have been found to have the valine variant of the protein. This study is aimed toward determining if there is a stability change associated with the two variants of LECT2 If so, we hope to determine whether this change in stability has an effect in the process of amyloid formation.

We hypothesized that the valine form of LECT2 is less stable than the isoleucine form and the less stable protein will form amyloid fibrils more readily. To test this hypothesis, we will

measure the stability of both forms of the protein. Gene cloning was used to create the isoleucine form of LECT2. Side-directed mutagenesis was then used to create the valine form of LECT2. We then worked on expressing and purifying the protein. Because LECT2 has disulfide bonds that aid in its proper folding, we first tried purifying the protein from the periplasm of E. coli cells where disulfide bonds can form. When this method failed, the signal sequence used to send the protein to the periplasm was removed, causing the protein to be sent to the cytosol. We used E. coli cells which can form disulfide bonds in their cytosol. We were able to successfully extract LECT2 protein from the cytosol in a soluble form. As a result of these experiments, we concluded that periplasmic expression of LECT2 does not yield soluble protein but cytosolic expression does give soluble protein. Our next step is to express and purify both variants of LECT2. Once we have enough soluble protein, we can use proteolysis to determine which form of the protein is more stable.

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Gene overexpression effects on hippo pathway activated RPE cells.

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The hippo pathway is a regulator for cell proliferation by deactivating the oncogenic transcriptional factors YAP and TAZ. When active, YAP and TAZ are present in the nucleus and they lead to cell proliferation unless the Hippo pathway is activated and then YAP and TAZ get degraded in the nucleus. Mutations on the hippo pathway that lead to the hipper activation of YAP/TAZ have been identified to be very common in cancer cells, but mutations in the regulators of YAP/TAZ are rare. The understanding and discovery of new YAP/TAZ regulators can lead to the discovery of new targets for cancer treatment. Performing a wide gene overexpression screening on RPE CRISPRa cells, 39 genes were identified to be overexpressed in cells that can bypass the hippo pathway when this was activated in these cells. To this day, there are very few know components that can turn off the hippo pathway by activating YAP and TAZ proteins in the cells. The idea that there might be unknown regulators of the Hippo pathway that can archive this by overexpressing certain genes was the main subject of matter for this project. We generated 39 RPE cell lines, each overexpressing one of these 39 gene hits from the screening. We tested for YAP/TAZ nuclear and cytoplasmic levels in the cells using immunofluorescence and cell imaging and we also identified YAP/TAZ levels and phosphorylation by performing Western blotting on these cells. This analysis revealed that several genes significantly increase the nuclear localization of YAP/TAZ and their phosphorylation levels. These genes include several known novel genes like GPR35, PRDM16, MPND, and BCAR1. This increase of YAP/TAZ levels suggests that some of these genes might be directly regulating the activation of YAP/TAZ in the cells when they are overexpressed and they can also help the cells to overcome the effects of the hippo pathway through mechanisms that are still to be explored and could eventually lead to new therapeutic targets.

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Longitudinal Changes on Metabolic Heart Disease in Young, Obese Patients

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Background: Heart failure with preserved ejection fraction (HFpEF) is a progressive cardiovascular disorder with no current therapies. Thus, early identification of preclinical disease in high-risk individuals is warranted. Metabolic heart diseases (MHD) or preclinical heart failure, characterized by abnormal changes in the heart structure and function (specifically left ventricular hypertrophy (LVH), diastolic dysfunction, and pulmonary hypertension) are seen in obese individuals. The goal of this study is to understand and characterize how changes in weight in young obese patients affect the presence and severity of MHD

Methods: The longitudinal study consists of Visit 1 and Visit 2. In Visit 1, patients (n=250) with the Boston Medical Center records were recruited during 2010-2014. An echocardiogram, electrocardiogram, physical examination, and patient history were obtained during their visit. Patients categorized into three groups: (a) non-obese (controls) (b) obese, MHD-negative, and (c) obese, MHD-positive. Obesity classification was assigned if subjects had a BMI of at least ≥ 30 kg/m^2. MHD-positive classification was assigned if subjects had LVH or diastolic dysfunction and pulmonary hypertension. Visit 2 was comprised of the same four procedures.

Results: Among our Visit 2 subjects (n=50), 65% were African-American, 85% female, and 14% undergoing bariatric surgery since Visit 1. Comparing the 50 individuals from Visit 1 and Visit 2, mean BMI of Visit 1 was 38.9 kg/m^2 compared to Visit 2 BMI of 36.9 kg/m^2. Diabetes was higher in Visit 2 compared to Visit 1 (45% vs. 40%) but with similar rates of hypertension between visits. 20% of Visit 2 individuals increased their BMI by 5 kg/m^2. PASP and diastolic dysfunction was similar across the groups that gained and lost weight in the interval time between Visit 1 and Visit 2. Furthermore, patients who were considered severely obese displayed higher LV mass, increased pulmonary arterial systolic pressure, and worse diastolic dysfunction than nonobese patients.

Conclusions: MHD represents preclinical disease for HFpEF in young, obese individuals, and an important fraction of these individuals appear to have worsened metabolic health status. In addition to their metabolic risk factors, they continue to have ongoing evidence of multiple components of MHD. Further understanding of longitudinal changes of obesity on MHD in these high-risk individuals is needed to help guide preventative efforts.