2021 Multidisciplinary Summer Research Education Program for Health Professional Trainees

Final Presentations

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Multidisciplinary Summer Research Education Program for Health Professional Trainees

The Multidisciplinary Summer Research Education Program for Health Professional Trainees is please to present the 2021 Participants.

The Multidisciplinary Summer Research Education Program for Health Professional Trainees is a NIH-funded research/education program sponsored by the National Heart, Lung and Blood Institute (NHLBI). This program is intended for highly motivated students at New Jersey Medical School, Robert Wood Johnson Medical School, School of Dental Medicine and School of Health Profession interested in pursuing a career as a clinician scientist.

In this initial cohort, ten students from across the four schools participated in research in areas related to cardiology, pulmonology or hematology with faculty mentors with active, nationally recognized research programs. Numerous papers are in preparation based on this research, with multiple oral presentations accepted for prior or upcoming meetings. The participants are all to be commended.

In addition to their research experience, students continue to be involved in career development enrichment activities to foster their goals along the clinician scientist track. The training allows for a life-long commitment to scientific research, as well as to train the next generation of health professionals.

Highlights of the summer also included discussions with acclaimed clinician scientists across the country and a trip to Jansen Pharmaceuticals, where the students had the opportunity to learn about alternative clinician scientist career tracks.

We look forward to continued involvement in all our students' growth as they continue their pursuits as the next generation of clinician scientists,

Valerie Fitzhugh, MD *Program Director*
Diego Fraidenraich, PhD *Program Director*
Pranela Rameshwar, PhD *Program Director*
Lauren Sherman, MS *Program Coordinator*

Tripartite Motif-Containing 33 (TRIM33) Protein Functions in the Poly(ADP-ribose) Polymerase (PARP)-Dependent DNA Damage Response through Interaction with Amplified in Liver Cancer 1 (ALC1) Protein

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Activation of poly(ADP-ribose) polymerase (PARP) near sites of DNA breaks facilitates recruitment of DNA repair proteins and promotes chromatin relaxation in part through the action of chromatin-remodeling enzyme Amplified in Liver Cancer 1 (ALC1). Through proteomic analysis we find that ALC1 interacts after DNA damage with Tripartite Motif-containing 33 (TRIM33), a multifunctional protein implicated in transcriptional regulation, TGF-β signaling, and tumorigenesis. We demonstrate that TRIM33 is dynamically recruited to DNA damage sites in a PARP1- and ALC1-dependent manner. TRIM33-deficient cells show enhanced sensitivity to DNA damage and prolonged retention of ALC1 at sites of DNA breaks. Conversely, overexpression of TRIM33 alleviates the DNA repair defects conferred by ALC1 overexpression. Thus, TRIM33 plays a role in PARP-dependent DNA damage response and regulates ALC1 activity by promoting its timely removal from sites of DNA damage.

Role of Dental Pulp Mesenchymal Stem Cells in Progression of Hematopoietic Activity

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Many hematological malignancies show early manifestation within the oral cavity. Among these, acute myeloid leukemia (AML) is first diagnosed based on oral cavity infiltration in approximately 5% of cases. In order to better understand the clinical relevance of such presentation, it is important to understand the role and capacity for normal hematopoiesis within this region. The bone marrow is the most studied site of hematopoiesis, where it has been found that mesenchymal stem cells (MSCs) support normal and malignant hematopoietic stem and progenitor cell function and proliferation. MSCs have been identified in numerous other tissues including adipose, umbilical cord, dental pulp, and gingiva; MSCs from these various sources are reported to share many cell markers and functions, including the capacity to differentiate into various connective tissues and be licensed, or educated, to act as anti-inflammatory cells. During pathological conditions or inflammation, the process of extramedullary hematopoiesis can ensue at the specific afflicted location. The oral cavity is prone to inflammation, trauma, and disease, raising the question of whether the gingiva and pulp chamber can support extramedullary hematopoiesis and the potential involvement of the dental pulp MSCs (dpMSCs). Our group is interested in exploring how dpMSCs may support and progress normal and/or leukemic hematopoietic cells within the oral cavity. Specifically, if dpMSCs support the hematopoietic stem cell survival and induce proliferation within these sites, particularly in the case of inflammatory conditions. This was explored through coculture to establish direct interaction between the two cell types. Indirect interaction utilized a transwell system in which dpMSCs were added to the inner wells and hematopoietic cells to the outer wells. Patient-derived dpMSCs were isolated from healthy (healthy dpMSCs) and inflamed (periodontal dpMSCs) gingiva. Healthy HSCs were isolated from umbilical cord blood and leukemic cell lines (K562 and U937) were used for malignant hematopoietic stem/progenitor cells. The cells were assessed for maintenance of their stem or progenitor statuses through clonogenicity testing and changes in proliferation, viability, and metabolic activity were assessed using cell tracking metabolic dyes. Changes in hematopoietic cell stemness were assessed by changes in binding to the dpMSCs. Healthy dpMSCs showed greater adherence to hematopoietic cells as compared to dpMSCs isolated from a patient with periodontal disease. Both leukemic cell lines showed increased proliferation when cocultured with dpMSCs. However, the K562 leukemic cell line showed greater proliferation when cocultured with periodontal dpMSCS while the U937 leukemic cell line showed greater proliferation when cocultured with the healthy MSCs. Our findings indicate a role for dpMSCs in hematopoiesis and support the need to further elucidate the mechanism(s) by which dpMSCs and HSCs interact within the oral cavity.

Endothelial Mechanisms for Downregulation of Stimulated Hyperpermeability

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The mechanisms that initiate microvascular hyperpermeability in response to inflammation are relatively well known. However, little or nothing is known about the endothelial mechanisms that terminate microvascular hyperpermeability. Our laboratory demonstrated a few years ago that translocation of eNOS from cell membrane to cytosol and release of eNOS-derived NO are fundamental to initiate hyperpermeability. We propose to investigate the endothelial signaling that returns endothelial permeability to control levels. We study the role of Epac1 (exchange protein activated by cAMP) and VASP (vasodilator stimulated phosphoprotein) in this process. We assess endothelial permeability using human microvascular endothelial cells (HMVEC) seeded onto a Navicyte diffusion chamber. We apply platelet-activating factor (PAF) and vascular endothelial growth factor (VEGF) as agonists and determine the endothelial layer permeability to FITC-dextran-70 (a mimic of albumin). We also analyze the location of the proteins of interest using microscopy and proximity ligation assay.

Hyperphosphorylation of Cx43 is Protective Against Arrhythmias and Preserves Gap Junctions in Mice with Duchenne Muscular Dystrophy

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Duchenne muscular dystrophy (DMD) is a degenerative muscular disease that typically leads to dilated cardiomyopathy. Connexin-43 (Cx43) is upregulated and remodeled in the hearts of both dystrophic (mdx) mice and human patients with DMD. We hypothesize that aberrant remodeling of Cx43 promotes dysfunction in DMD hearts and that hyperphosphorylation of serine residues at positions 325, 328, and 330 (S3E) of Cx43 in mdx mice is protective against Cx43 remodeling, thus limiting pathology in cardiac myocytes. We mimicked phosphorylation of the serine residues with glutamic acid residues, crossed Cx43 (S3E) mice with mdx mice, and blinded functional and histological analysis of Cx43 in mdx mice (mdxS3E). Hyperphosphorylation of Cx43 at residues 325, 328, and 330 was found to be cardioprotective against inducible arrhythmias and preserves gap junctions. These findings can open an avenue for pharmaceutical intervention that could be preventative of cardiomyopathy in DMD, as well as contribute to knowledge about the mechanisms in which Cx43 contributes to cardiac pathology in DMD.

Exposure to Bone Marrow-Derived Mesenchymal Stem Cell Secretome Alters Gene Expression in Human Endometrial Stromal Cells

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Human endometrial stromal cell (HESC) migration and invasion are essential for endometrial regeneration following parturition and menstruation. Previous studies have shown that bone marrow-derived mesenchymal stem cells (BM-MSCs) secrete biological products involved in the regulation of HESC cell migration and invasion. We have shown that exposure to multiple sources of BM-MSCs increases HESC migration and invasion, but the underlying mechanisms remain poorly understood. We hypothesize that exposure to the secretome of BM-MSCs activates genes involved in cell motility and survival.

BM-MSCs were cultured from two sources: the bone marrow aspirate of a healthy female donor (BM-MSC-1) and ATCC commercially available MSCs (BM-MSC-2). HESCs were indirectly co-cultured with the BM-MSC secretome for 24hrs. RNA was then extracted, and mRNA sequencing was performed to identify differentially expressed genes (DEGs) and altered pathways. MSigDB was used to identify the top 15 enriched biological pathways ($padj<0.05$). RT-qPCR was performed to validate changes in mRNA expression of DEG common to both BM-MSC exposures and statistical significance was defined as $p<0.05$.

HESCs exposed to BM-MSC-1 resulted in the differential expression of 6099 genes and 8281 genes for the BM-MSC-2 exposure ($padj<0.05$), with 4351 DEGs overlapping in both treatment groups. Pathway analysis showed 9 of the top 15 significantly enriched pathways in HESCs exposed to BM-MSC were shared between treatment groups. *CCL-2* and *HGF* (up) and *GDNF* (down), cell motility genes, were common to both BM-MSC-1 and BM-MSC-2 exposures ($padj<0.05$). RT-qPCR confirmed that *HGF* and *CCL-2* were significantly increased in BM-MSC-1 (1.8-fold and 7.7-fold respectively) and BM-MSC-2 (1.3-fold and 5-fold respectively), while *GDNF* remained unchanged. Our data supports the role of upregulated genes, *CCL-2 and HGF*, in a mechanism stimulating endometrial stromal cell motility.

Blood Viscosity as a Biomarker for Vascular Homeostasis and Machine Learning Clinical Assistance

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Scientific discoveries on the relationship between blood viscosity and how the body transduces that information into neuronal signals have allowed for a reimagining of how clinicians assess hemodynamic status. Obtaining a clinical snapshot of a patient's blood viscosity has become increasingly relevant as it changes during chronic diseases such as diabetes and cancer. Previous guidelines pertaining to accurate measurement of blood viscosity and its impact on flow were restricted to invasive procedures such as catheterization, which carry the risk of infection and are seldom performed in hemodynamically unstable patients. We aim to build on a theoretical framework that has established the feasibility of utilizing a noninvasive ultrasound exam as a safer and more effective means of obtaining blood viscosity estimates. Burn patients were chosen as the clinical population due to their extensive documentation following admission and the need to closely monitor fluid administration as part of the treatment plan. Despite improved survival following severe burn injury, patients with multiple comorbidities that disproportionately affect communities of color continue to have worse medical outcomes. We believe this worsened prognosis is a consequence of a decreased capacity to clear excess volume and alters blood viscosity measurements as measured on ultrasound exams. The impact of applying novel biomarkers, including blood viscosity, when treating severely burned patients can be exponentially magnified when coupled with the concurrent development of machine learning software that will inform clinical interventions of the future.

Exosomes: The Key to Neural-Hematopoeitic Axis

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The bone marrow (BM) is innervated by different types of neurons. The experimental and clinical evidence indicated crosstalk between the BM and brain. Since the BM is the site of hematopoiesis that generate blood and immune cells, an understanding of the innervated BM system is important to get insights into hematopoietic functions. These studies could also be relevant to the brain thereby impacting neurodegenerative diseases, such as Parkinson's Disease and Alzheimer's disease with neuronal degeneration and death in the brain and/or spinal cord. BM-derived human mesenchymal stem cells (MSCs) can generate electrophysiological functional neurons, including peptidergic and cholerinergic neurons. These *in vitro* methods require the use of a differentiation media which would prove ineffective as a treatment *in vivo* due to the inherent toxicity of the reagents. Here, we tested an alternate approach for regenerating neurons endogenously by differentiating MSCs into peptidergic neurons via use of exosomes, which microvesicles (MV) (40-150 nm in diameter). The MV can deliver functional proteins and RNA to neighboring cells. We tested the hypothesis that MVs released from neuronally differentiating MSCs can in turn induce the differentiation of fresh MSCs into neurons in a standard growth media. First, we determined the timeline release and analyses of neuron-derived MVs. We observed optimum release on day 6 neuronal induced MSCs. We added the MVs differentiating MSCs (days 2, 6, and 12) to MSCs and then study their development to neurons. Fluorescence imaging for neuronal markers at days 2, 4 and 12 indicated that MVs collected from induced MSCs before day 6 were capable of differentiating the neurons early as compared to the use of induced media. We also concluded that the more efficient media during MV-mediated formation of neurons contained Dulbecco's Modified Eagle Media (DMEM) as compared to the previous use of Ham's F12 media. Use of a scratch assay indicated that the neuron-derived MVs were able to enhance wound repair. Evaluating the data in the context of the BM, it appears that induced MSCs with MVs from endogenous neurons might indicate a new concept of a third brain, following the enteric region. The data also suggest neuron-mediated increase in BM fibrosis. In total, these findings indicate the potential for therapy in BM functions as well as for neural disorders.

Comparison of Macrophage Phagocytosis of Mycobacterium tuberculosis Strains

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Mycobacterium tuberculosis (Mtb) infection can lead to the development of tuberculosis, the leading killer among global infectious disease. Although Mtb is one of the most successful pathogens known, mechanisms underlying its variability in transmission remain poorly understood. Using mice models, our lab recently probed that the transmission potential of High (HT) and Low (LT) transmission Mtb strains differed by bacterial burden and lung pathology. This study aims to better understand the mechanistic basis of infection by looking at phagocytosis of LT-Mtb and HT-Mtb by THP-1 macrophages in vitro. Ziehl-Neelsen Acid Fast Bacterial staining was used to visualize and determine presence of intracellular bacteria. Although no difference in percent infectivity was observed between cells infected with LT-Mtb and HT-Mtb strains, a difference in bacterial distribution was detected. These results suggest bacterial replication may play a critical role in the difference seen in transmission potential between LT-Mtb and HT-Mtb strains.

The role of YAP in modulating inflammation in neutrophils

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Cardiovascular disease is the #1 killer worldwide. Ischemic heart injuries, such as myocardial infarction, are typically caused by an occlusion in the coronary arteries that supply the heart with blood and results in a massive loss of myocytes nearest the occlusion (infarct) surrounded by injured but salvageable myocytes known as the area at risk. The infarct thins, and therefore weakens the ventricle wall and as cardiomyocytes are terminally differentiated, they cannot replace themselves. Instead, the heart undergoes remodeling in which myocytes hypertrophy and resident fibroblasts are activated to prevent rupture and keep up with cardiac demand. Eventually, this remodeling becomes pathological and when the heart can no longer keep up with this demand, it progresses into heart failure. The current gold standard treatment for MI is reperfusion of the afflicted artery, but reperfusion itself paradoxically causes additional myocyte loss in the area at risk, and is now thought to account for up to 50% of the final infarct size. These two injuries, collectively referred to as ischemia/reperfusion injury, not only result in infarction and reduced cardiac output but trigger a synergistic inflammatory immune response.

Inflammation is a well-established driver of worsened cardiac outcomes and has been extensively implicated in ischemic heart injuries. Necrotic myocytes act as damage-associated molecular patterns (DAMPs) that attract large numbers of inflammatory innate immune cells to the infarct. Of these innate cells, we are focusing on the neutrophil because they are the first cells recruited to the heart, are short lived (<24h), and influence downstream immunity, making them good targets to suppress inflammation. Although neutrophils have been shown to contribute to infarct expansion, elimination of neutrophils prior to injury interferes with the overall repair process. Thus, we are looking at the underlying mechanisms in this response to modulate inflammation without impacting repair and improve cardiac outcomes after therapeutic reperfusion.

Yes-Associated Protein (YAP) Facilitates Pressure Overload-Induced Dysfunction in the Diabetic Heart

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Patients with diabetes are more prone to developing heart failure in the presence of high blood pressure than those without diabetes. Yes-associated protein (YAP), a key effector of the Hippo signaling pathway, is persistently activated in diabetic hearts, and YAP plays an essential role in mediating the exacerbation of heart failure in response to pressure overload in the hearts of mice fed a high-fat diet. In this experiment, we explore whether YAP promotes cardiac dysfunction in response to PO in mice fed a high fat diet (HFD), a mouse model of Type 2 Diabetes and whether the exacerbation of cardiomyopathy in HFD-fed mice in the presence of pO is accompanied by cardiomyocyte dedifferentiation.

In order to analyze the biochemical pathways that may influence the progression of heart failure, twelve week old mice models underwent a sham operation or transverse aortic constriction; at the same time, the arterial pressure gradients will be measured using a high-fidelity micromanometer catheter. In addition, human samples from explanted hearts were preserved and underwent immunostaining; biopsy samples were fixed with 10% paraformaldehyde in phosphate-buffered saline (7.4), paraffin embedded, and sectioned. Real-time quantitative polymerase chain reaction was performed in order to evaluate the presence of biological markers such as YAP.

YAP was elevated in the hearts of HFD-Fed mice. Immunostaining of heart sections showed that consumption of HFD significantly increased the number of cardiomyocytes with YAP-positive nuclei. Immunoblot analyses confirmed that the level of total YAP protein was also increased in the hearts of mice fed HFD. Furthermore, consumption of HFD for 8 weeks significantly sown-regulated the level of phosphor-Lats2, which suggests that this model mimics Hippo deficiency. In addition, PO induced left ventricular dysfunction with cardiomyocyte de-differentiation in HFD-fed mice. YAP was elevated with or without transverse aortic constriction, and expr3ession of MYH7, ACTA2, and RUNX1, markers of cardiomyocyte dedifferentiation, was increased in the presence of TAC. Finally, YAP expression as elevated in the hearts of patients with HF with diabetic cardiomyopathy. Immunostaining and immunoblot analyses showed that both nuclear expression of YAP in cardiomyocytes and the level of YAP protein expression in the heart were significantly greater in patients with HF with diabetes than in HF patients without diabetes. The level of YAP may correlated with the severity of diabetes.

We have demonstrated that YAP is activated in the heart in response to HFD consumption, where persistent activation of YAP exacerbates cardiac dysfunction in the presence of PO. Importantly, both pharmacological and genetic interventions to inhibit the YAP pathway attenuate the PO-induced exacerbation of HF in the heart in the presence of HFD consumption