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Machine Learning Models Illuminate Health Disparities in Regenerative Medicine Platelet-Rich Plasma Treatment for Knee Osteoarthritis

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Presenter: Mohamed Addani B.S. • Mayo Clinic Graduate School • PREP Scholar—IMSD Graduate Student—CTS Track

Background: Knee osteoarthritis (OA) is a widespread health concern impacting millions globally. Regenerative medicine (RM), particularly platelet-rich plasma (PRP) treatment, has demonstrated promise in addressing knee OA, according to multiple clinical trials. Limited data exist on PRP accessibility concerning demographic, clinical, and socioeconomic factors. This study aims to develop machine learning models to predict health disparities in PRP treatment for knee OA and explore potential disparities.

Method: In our cross-sectional study, we collected age, sex, race, ethnicity, area deprivation index (ADI), marital status, income, rurality, insurance, employment, language, religion, site, and region. We used logistic regression (LR) and random forest (RF) models to analyze a dataset of 3,600 samples with 14 predictors, comparing PRP to standard care. Models were evaluated using 10-fold cross-validation.

Results: Both models displayed strong predictive performance, with RF (ROC: 0.914, training accuracy: 0.869, testing accuracy: 0.828, AUC: 0.934) slightly outperforming LR (ROC: 0.913, training accuracy: 0.841, testing accuracy: 0.835, AUC: 0.920). Top predictors included religion, ADI, age, and site. PRP likelihood decreased by 4% with each year of age and 3% with each deprivation percentile increase. The odds of receiving PRP were 72% lower for black individuals, twice as high for married individuals, 95% lower for Roman Catholics, and 51% lower for not-employed individuals.

Conclusion: Our study successfully developed machine learning models to predict health disparities in PRP treatment for knee OA, filling the research gap. The models identified facilitators and barriers to PRP access, providing valuable insights for addressing health disparities in knee OA management.

Keywords: Regenerative Medicine, PRP, Knee Osteoarthritis, Machine Learning

MAYO CLINIC

The Molecular Mechanism of FAM111A: a Dimeric Protease Component of the DNA Damage Response

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Presenter: Julia Alvey • Mayo Clinic • GREP—Biochemistry and Molecular Biology

DNA-protein crosslinks (DPCs) are highly toxic DNA lesions that result from bulky protein adducts that are covalently or tightly bound to DNA. If left unresolved, these lesions cause replication fork stalling that can eventually lead to genomic instability and conditions such as premature aging, neurodegenerative disorders, or cancer. However, the toxicity of DPCs is also exploited as a molecular mechanism of action for various chemotherapeutic drugs. Due to their relevance in both the understanding and treatment of human health and disease, research has focused on understanding DPC repair mechanisms. We previously reported the roles of two proteases, SPRTN and FAM111A, in the promotion of replication at protein obstacles. However, despite similar structural features, our previous research suggested that FAM111A and SPRTN have some overlapping and some distinct functions, leading to the question of how FAM111A functions and is uniquely regulated on the molecular level. Given the clustering of mutations within the FAM111A serine protease domain (SPD) that occur in two rare diseases, Kenny-Caffey Syndrome and Gracile Bone Dysplasia, we performed a structural and biochemical analysis specifically on the FAM111A SPD. We found that FAM111A SPD forms a dimer via an alpha helical interface, and dimerization is important for FAM111A SPD protease activity. We also found that disordered regions are important regulators of FAM111A SPD protease activity. This structural analysis provides insight needed to guide the development of specific inhibitor therapies to enhance therapeutic efficacy or lead to the development of new therapeutic interventions for treatment of cancer and rare diseases.

Keywords: DNA Damage and Repair, DNA Protein Adducts, DNA Repair Enzymes

MAYO CLINIC

Characterization of a Transgenic Mouse Model Carrying Mutations Disrupting the Wave Regulatory Complex (WRC) Interacting Receptor Sequence (WIRS)

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Presenter: Theresa Benson • Northwestern University • PREP Scholar—Undergraduate Student

Autism Spectrum Disorder (ASD) is a neurodevelopmental disease characterized by abnormalities in social communication, cognitive functioning and other behavioral implications. Disruptions to the Wave Regulatory Complex (WRC) has been identified as a potential etiology for ASD, however the specific pathways within the complex that go awry are yet to be established. The WRC is a five-protein complex that mediates interactions of the actin cytoskeleton with membrane receptors that are crucial for cell development. The WRC is classified as a central hub that receives input from multiple risk genes of autism which allows for more efficient ways to study the disorder. The function of this complex is activated by different classes of ligands with this project focused specifically on the Wave Regulatory Complex Interacting Receptor Sequence (WIRS). We hypothesize that failure of the WRC's recruitment to the cell membrane during early stages of development disrupts downstream functioning integral to normal cell development and the production of autistic features. The goal of this project focuses on identifying if this specific mutation impedes normal behavior and produces autistic phenotypes within transgenic mouse models. Mouse behavioral assays include measures that test for impairments in social interaction, repetitive behaviors, anxiety and learning/memory, all known to be compromised with autistic onset. Evidence of abnormalities in transgenic mice behavior is suggestive of this pathway as a potential etiology for ASD, which can be beneficial for modeling more effective therapies to better manage the disorder.

Keywords: Neurodevelopment, WASF1, ASD

MAYO CLINIC

The G Protein-Coupled Receptor GPR31 Promotes Islet Inflammation and Diabetes

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Presenter: Christian Checkcinco • University of Chicago • IMSD Graduate Student—Molecular Metabolism and Nutrition

In type 1 diabetes a characteristic immune response targets insulin secreting β cells in pancreatic islets, resulting in their destruction. 12- Lipoxygenase (12-LOX) contributes to this pathogenesis by producing 12-HETE, which induces oxidative and ER stress resulting in cellular dysfunction. G-protein coupled receptor 31 (GPR31) was identified as a high affinity 12-HETE receptor and is increased in human islets after cytokine induced inflammation, but its unknown if GPR31 might mediate the downstream effects of 12-LOX. To investigate the role of GPR31 during diabetic inflammation, whole body GPR31 knockout mice were generated on the C57Bl/6J background. To mimic the setting of islet inflammation, WT and Gpr31b-/- mice were treated with streptozotocin (which induces beta cell death and macrophage influx). Compared to WT, Gpr31b-/- mice showed protection from development of hyperglycemia and glucose tolerance tests showed >30% protection, suggesting that GPR31 promotes islet dysfunction and hyperglycemia. We then isolated islets from WT and Gpr31b-/- mice and treated them with proinflammatory cytokines followed by RNA sequencing to interrogate gene expression pathways. Differentially expressed genes in Gpr31b-/- islets included markers for oxidative stress, MAPK signaling, and cellular homeostasis by Wnt/βcatenin , a finding reminiscent of 12-LOX inhibition. We then asked if GPR31 was involved in the mobility of macrophages using a transwell assay with chemoattractant. We saw a ~50% reduction in migrating bone marrow-derived macrophages in GPR31b-/- compared to WT. Taken together, these data suggest that GPR31 may be involved in mediating the proinflammatory responses of 12-HETE in both β cells and macrophages.

Keywords: Diabetes, Islet, Inflammation, beta cell

MAYO CLINIC

Development of New COVID-19 Antigen Testing Protocol Increases Testing Access and Elucidates Sociodemographic Characteristics in the Community of Loiza, PR

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Presenter: Ana Mia Corujo Ramirez • Medical College of Wisconsin

The COVID-19 pandemic has disproportionately impacted underserved and vulnerable populations in the United States. However, Puerto Rico, a US territory, has received considerably less attention and resources during the pandemic due to infrastructure limitations and the long-term effects of Hurricane Maria. To address COVID-19 testing barriers in the under-resourced town of Loíza, a partnership between Yale University School of Medicine, Puerto Rico Public Health Trust, and the Parcelas Suarez Community Board implemented a COVID-19 surveillance program. The goal was to implement a new COVID-19 testing protocol using the BinaxNOW Antigen test and to understand sociodemographic characteristics in the community.

Asymptomatic participants from the municipality of Loiza were consented, and a pre-post study design was utilized to measure the number of tests performed during the implementation period of the new COVID-19 antigen test protocol. Participants auto-collected nasal swab samples and completed follow-up surveys every two weeks. Data collected and COVID-19 test results were stored in REDCap, and CDC guidelines and local resources were provided at each visit. Preliminary data shows an increase in the quantity of COVID tests performed and retention of participants during the implementation period of the new testing protocol. The study identified the BinaxNow COVID-19 antigen protocol as a potential alleviator of testing barriers in the community of Loiza.

The next steps include completing participant enrollment and continuing survey follow-ups using the new testing protocol. The ultimate goal is to ameliorate COVID testing for underserved and vulnerable populations, one community at a time. This study highlights the degree of disparity experienced by marginalized communities in Loiza in having reliable access to COVID-19 testing and underscores the importance of implementing testing protocols that can potentially alleviate testing barriers in under-resourced communities.

Keywords: COVID-19, SARS-CoV-2 diagnostics, community research, health equity, underserved populations, rapid acceleration of diagnostics

MAYO CLINIC

Survival and stress response of a human gut commensal Phocaeicola vulgatus

Nazik Elmekki, Leonor García-Bayona, Michael Coyne, and Laurie Comstock

Presenter: Nazik Elmekki • University of Chicago • IMSD Graduate Student—Microbiology

The human gut microbiota is a complex community including diverse bacterial taxa, which can often modulate host health. Among the most predominant bacterial phyla in the human gut is Bacteroidetes, which includes highly prevalent species from the genuses Bacteroides and Phocaeicola. One such species, Phocaeicola vulgatus, is relatively understudied compared to other gut-associated members of Bacteroidetes. In this study, we aim to characterize how P. vulgatus responds to and withstands the numerous stressors of the mammalian gut using a high-throughput genetic screening method, Barcoded Transposon-Insertion Sequencing (BarSeq). BarSeq allows us to calculate fitness scores for all genes in the bacterial genome under specific environmental conditions or stressors and determine which genes are conditionally essential for survival. First, we will use this genetic screen in mono-associated gnotobiotic C57/BL6 mice to capture which genes are important for surviving the mammalian gut. In this data set, we will compare gene fitness scores between young and aged mice, as the impact of host aging on gut commensal bacteria is poorly understood. Additionally, we will use the well-established Dextran Sodium Sulfate (DSS) colitis model in these mono-associated mice to further characterize which genes aid bacterial survival in an inflammatory environment. Based on our findings, we will design isogenic bacterial strains with single gene deletions to further characterize any fitness advantages, elucidate bacterial survival mechanisms, and determine any implications for host health. In summary, this study will provide insight into the mechanisms critical for commensal bacterial survival in the mammalian gut.

Keywords: gut microbiota, stress response, BarSeq, aging, colitis

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Using Biomaterials to Augment Head and Neck Regenerative Medicine

Jamie E. Ganem, BS; Dina M. Gadalla, PhD; Rachel M. Wells, CVT; David G. Lott, MD

Presenter: Jamie E. Ganem • Mayo Clinic-AZ

Head and neck injuries, surgeries, or diseases can have detrimental effects on patient outcomes. To improve clinical performance, tissue engineering efforts require a strong foundation in physiological repair processes. Although an increasingly common material for head and neck reconstruction, proper tissue integration and adequate angiogenesis in Medpor implants continues to challenge patient recovery. In this project, we incorporate different biomaterials onto Medpor scaffolds to determine which combinations promote angiogenesis and tissue healing.

Using a mouse subcutaneous implantation model, three different components were investigated for a total of 6 groups: plasma treated (PT) versus untreated (UT) Medpor; the use of fibrin glue (FG) versus collagen gel (CG) as a bioactive carrier; and the addition of purified exosomal product (PEP) versus no addition in fibrin glue (FG+Exo) and collagen gel (CG+Exo) implants.

Macroscopic H&E stainings demonstrated varying amounts of tissue integration in all treatment groups. Nevertheless, cells were able to proliferate throughout the entirety of each Medpor scaffold. Detection of angiogenic factors were significantly higher in PT than UT implants and were highest in the FG+Exo group. Yet there were no notable differences in angiogenic markers between PT, CG, or CG+Exo groups. Furthermore, although there was a significant increase in inflammatory markers between UT and PT implants, there was a significant decrease in these same markers in all groups that incorporated a bioactive carrier, matching UT implant levels.

Together, these findings suggest that plasma treating Medpor promotes angiogenesis, while incorporating fibrin glue or collagen gel can minimize host immune responses while delivering angiogenic biomaterials. Ultimately, improvements to this commonly used scaffold can improve tissue integration, angiogenesis, and patient outcomes in the long-term.

Keywords: Head & neck reconstruction, angiogenesis, tissue integration, plasma treatment, bioactive factors

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Viral Immune Antagonists: Enhancing Mengovirus Oncolytic Therapy for Pancreatic Cancer

Bellmary Garcia-Rodriguez; Kyla A. Boyd; Caitessa Venables; Autumn J. Schulze Ph.D

Presenter: Bellmary Garcia-Rodriquez • Mayo Clinic • PREP Scholar—VGT Track

Pancreatic cancer has a high mortality rate, and a lack of effective treatment options necessitates development of novel therapeutics. Immuno-oncolytic viruses have been of interest to treat pancreatic cancer, however clinical responses to monotherapies have been suboptimal. Resistance to oncolytic virotherapy may be due to pre-existing or upregulated antiviral immune responses following treatment, resulting in restricted virus amplification, spread, and cancer cell killing. While some cancer cells have defective antiviral signaling pathways that promote oncolytic virus replication, many have intact RNA recognition pathways that induce type I interferon production resulting in a cascade of interferon stimulated genes in surrounding cells limiting virus spread. Previously, we demonstrated the oncolytic potential of a genetically engineered Mengovirus, MC0R, wherein the polyC tract, a virulence factor, was replaced by microRNA target sequences that restrict virus replication in tissues associated with pathogenesis. However, its single-stranded RNA viral genome is susceptible to endogenous RNA recognition and interferon activation. To overcome this barrier, we propose a mechanism that utilizes viral immune antagonists to temporarily counteract the innate immune response. These antagonists target steps of RNA recognition and interferon signaling pathways that our immune response uses against viruses. Here, we demonstrate the oncolytic activity of MC0R against pancreatic cancer cell lines and characterize the effects of viral immune antagonists in vitro. Antagonists engineered as mRNA-based therapeutics with and without modified nucleotides are expressed in vitro at varying levels. Studies to determine their ability to restrict recognition and IFN activation by MC0R, enhance virus replication, and whether this correlates with enhanced therapeutic potency are ongoing. If successful, this project will create an effective and safe therapeutic for pancreatic cancer patients.

Keywords: Oncolytic Viruses, Immune Antagonists, Mengovirus, Pancreatic Cancer

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Not All Tauopathies are Created Equal

Donte Lorenzo Garcia, Sara Rose Dunlop, Yasushi Nishihira*, Ivan Ayala, Eileen Bigio, Margaret E. Flanagan, Tamar D. Gefen, M.-Marsel Mesulam, Changiz Geula

Presenter: Donte Garcia • Northwestern University • PREP Scholar—Undergraduate Student

Cortical Basal Degeneration (CBD), a subtype of Frontotemporal Dementia, is a neurodegenerative disease in which pathology and neuronal loss is seen in the frontal and temporal lobes and subcortical brain structures. This results in progressive loss of verbal communication ability, other cognitive deficits, and motor abnormalities. While clinical and behavioral symptoms differentiate CBD from other neurodegenerative diseases and dementias, the unique underlying pathobiology of CBD is not fully understood. This lack of insight in CBD has led to a lack of disease modifying therapeutic treatment options and poor preventative mechanisms. To help resolve these issues, we studied basal forebrain cholinergic neuron (BFCN) vulnerability to the hallmark tau protein pathology in human CBD postmortem brain tissue. In Alzheimer's Disease (AD), one of the most prevalent neurodegenerative dementias, the BFCNs are a primary target for tau protein inclusions which lead to neurofibrillary tangles and cell death. Using immunohistochemistry and unbiased stereology to quantify BFCN density and tau protein inclusions, we investigated whether the BFCNs are also vulnerable to degeneration in CBD. We found that while BFCNs in CBD displayed tau protein inclusions, these inclusions did not seem to lead to degeneration, in contrast to that seen in AD. This suggests that cholinergic neurons in CBD may be resilient to tau protein induced neurodegeneration, and that the species of pathologic tau may be different in AD when compared with CBD. This also implies that cholinergic based therapies used for treatment of AD are likely to be ineffective in treating CBD. Further research is needed to understand the characteristics of tau pathology in CBD and identify new therapeutic targets.

Key Words: Cortical Basal Degeneration (CBD), Basal Forebrain Cholinergic Neurons (BFCN), Tau Protein, Alzheimer's Disease (AD)

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Using AAV-Mediated Overexpression of Nrf2 to Prevent Alzheimer's Neuropathology

Karen P. Gomez, Katherine R. Sadleir, Yunlu Xue, Connie Cepko, Robert Vassar

Presenter: Karen P. Gomez • Northwestern University • PREP Scholar

Background: Oxidative stress has been observed in degenerative conditions and normal aging, leading to damaged proteins, nucleic acids, and lipids. Nrf2 is a protein that regulates antioxidant function and prevents oxidative damage by boosting the expression of various detoxifying and anti-inflammatory genes. Prior research found that Nrf2 mediated neuroprotective effects in photoreceptors and retinal ganglion cells, suggesting it may be an effective treatment for multiple cell types in diseases that involve oxidation. Additionally, other studies showed that deletion of Nrf2 increased cognitive deficits in AD model mice. Few studies have tested Nrf2 activation by gene delivery via viral vectors in animal models, which may prevent early pathogenic processes in Alzheimer's. We hypothesized that neuronal AAV-mediated Nrf2 overexpression would exhibit neuroprotective effects.

Methodology: To overexpress Nrf2 in 5XFAD and non-Tg mouse brains, we had four conditions: an uninjected group, GFP, GFP+low Nrf2, and GFP+high Nrf2. The three injection groups received a ventricular injection within 24 hours of birth. The mice were perfused at 9.5 months of age, where half of the brain was fixed for sectioning and immunofluorescent analysis, while the other half was processed for immunoblotting. Sections were stained with antibodies to quantify neuronal loss, neuroinflammation, plaque load, BACE1 expression, and dystrophic neurites.

Findings/Future Directions: We found that high Nrf2 overexpression had adverse effects as it increased plaques and neuronal loss. The low dose of Nrf2 overexpression showed beneficial effects as it reduced BACE1 in the hippocampus and dystrophic neurites. Future experiments include performing bulk mRNA seq from hippocampi.

Keywords: Alzheimer's Disease, Nrf2, AAV-mediated overexpression

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The Effect of Sarcopenia on Power and Endurance of Diaphragm Muscle Fibers

Genesis A. Hernandez-Vizcarrondo, Alyssa D. Brown, Leah A. Davis, Humzah Abdulka

Presenter: Genesis A. Hernandez-Vizcarrondo • Mayo Clinic • PREP Scholar

Sarcopenia is defined as age-related atrophy of muscle fibers as well as a decrease in specific force (force per cross-sectional area). In the rat diaphragm muscle (DIAm), sarcopenia primarily affects type IIx/IIb fibers that generate greater specific force, have the fastest maximum shortening velocities, but display greater fatigue with repeated activation. We hypothesized that 6-month-old (young) rats will be able to generate more power and have decreased endurance compared to the 24-month-old (old) rats because of sarcopenia's effect on IIx/IIb fibers. DIAm strips ~ 2 -3 mm wide were excised and suspended in a tissue chamber, with Rees–Simpson solution, with the costal margin clamped and the central tendon tied with silk and attached to a force transducer. The isometric DIAm force was identified by stimulating the muscle strip via platinum plate electrodes at supramaximal (~250 mA) pulses to 75 Hz delivered in 1-s trains. Once the maximum force was identified, the muscle strip was stimulated at different percentages of the maximum isometric load and allowed to shorten over a 30-ms period to measure velocity throughout a 600 ms train to identify the peak power output and maximum loaded shortening velocity (Vmax). In a subsequent train stimulation, the muscle was stimulated at 75 Hz in a 330 ms train repeated each second for a 2-minute period. During the initial 225 ms of the train the muscle was isometrically activated at and then allowed to shorten at a constant velocity of 30% Vmax for the subsequent 105 ms. At the end, an additional DIAm strip was excised, stretched to ~150% its resting length, and was frozen in melting isopentane. Subsequently, transverse serial sections of muscle fibers were cut to identify muscle fiber by immunoreactivity to specific myosin heavy chain (MyHC) isoform antibodies, to label mitochondria using MitoTracker Green, and measure maximum velocity of the succinate dehydrogenase reaction (SDHmax). Maximum isometric force and power for the 6-month-old rats was much larger compared to those of the 24-month-old rats. However, no significant difference in the maximum shortening velocity between the young and old age groups were found. During the 120 second repeated stimulation train, it was observed that approximately after 60 seconds, the endurance of the muscle maintaining its force measurements was maintained in 6-month-old rats. On the other hand, for the 24-month-old rats, this endurance measurements were not observed until after the muscle was continuously stimulated for 80 seconds. When analyzing the energetic demands between the old and young age groups, the MVD and SDHmax for the 6-month-old were greater for in type IIx/IIb fibers compared to the 24-month-old. However, the mitochondrial volume density and SDHmax of type slow and IIa were comparable between both age groups. These results support how intrinsic differences of muscle fibers size and energetic demands can provide a functional measure for the ability of the DIAm to perform different motor behaviors.

Keywords: Diaphragm, sarcopenia, force, endurance

MAYO CLINIC

Investigating Dose-Dependent Expression of Pentatricopeptide Repeat (PPR) Proteins and their RNA-Binding Efficacy

Patricia Hernandez, Kavini Nanayakkara, Stephen C. Ekker, PhD

Presenter: Patricia Hernandez • University of Minnesota-Rochester • Undergraduate Student—Health Sciences

Pentatricopeptide repeat (PPR) proteins play a crucial role in post-transcriptional gene regulation through RNA manipulation. PPRs have shown promise as a gene editing tool because of their RNA nucleotide binding specificity. Our studies have shown that transient exogenous expression of PPR proteins knockdown target proteins, but there is little known about endogenous designer PPR expression and subsequent protein knockdown in vitro. Here, we investigate the dose-dependent expression of endogenous PPR proteins by expanding the range of doxycycline concentrations to elucidate PPR ability to bind specific RNA in the context of knocking down protein targets. Flp In T-Rex HEK293T cells were transfected with a plasmid containing a PPR construct that binds the mRNA 5' UTR region of target proteins, which is under the control of a dox-inducible promoter. Following selection with hygromycin B, the cells were treated with various concentrations of dox for 24, 48, and 72 hours. Cell lysates were prepared for western blotting, and protein target bands were compared against reference bands for quantifying protein expression and analysis. By expanding the numerical window of doxycycline treatments, we hope to capture a dose-dependent curve where increased doxycyline correlates to decreased protein target expression. Future directions of this research include applying this methodology to other protein targets and replicating the dose-dependent curve, along with using qPCR to investigate how other genes are upregulated or downregulated as a result of protein knockdown.

Keywords: Pentatricopeptide Repeat Protein, Dose-Dependent Curve, Genetic Engineering, RNA binding

MAYO CLINIC

Measurement of maximum velocity of the succinate dehydrogenase reaction in isolated cardiomyocytes

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Presenter: Cole W. Jensen • Mayo Clinic • GREP—Physiology & Biomedical Engineering

Succinate dehydrogenase (SDH) is a key enzyme in the tricarboxylic acid (TCA) cycle, as well as Complex II of the electron transport chain (ETC). Thus, measurement of the maximum velocity of the SDH reaction (SDHmax) provides insight into both TCA cycle and ETC contributions to mitochondrial function. In the present study, a quantitative histochemical technique to quantify SDHmax in permeabilized cardiomyocytes isolated from male Sprague-Dawley rats was explored. In this assay, the oxidation of succinate to fumarate is coupled to the reduction of nitroblue tetrazolium to its diformazan (NBTdfz). The progressive accumulation of NBTdfz is quantified by repeated densitometric measurements at 570 nm. The optical density (OD) measurements are converted to mM fumarate using the Beer-Lambert-Bouger equation. Changes in OD within a series of 10 optical slices were measured every 15 s over a 5 min period and expressed as mM fumarate/min using the Beer-Lambert- Bouger equation. We found that the SDH reaction was linear across 5 min, and that the change in OD was consistent throughout all slices. SDHmax values decreased in response to ETC inhibition, while there was no change following FCCP treatment. Our results confirm that this assay of SDHmax is reproducible in cardiomyocytes and offers a single cell alternative to high-resolution respirometry. The ability to measure SDHmax in single cells is in contrast to respirometry, which only measures the average O2 consumption of aggregates of cells. Thus, a direct measurement of SDH max can provide valuable physiological insight into mitochondrial variation across a population of cardiomyocytes.

Keywords: Succinate dehydrogenase, mitochondrial respiration, single cell

MAYO CLINIC

A BIBLIOMETRIC CENSUS OF THE BRAIN

Richanne Matthews, Reese Richardson, Thomas Stoeger, Luis Amaral

Presenter: Richanne Matthews • Northwestern University • PREP Scholar—Department of Chemical and Biological Engineering

Mental illness is a dominating concern globally with one in eight people reporting to have at least one. Depression alone is a leading cause of disability, and death by suicide is one of the most common contributors to mortality worldwide. As such, there is a pressing demand for novel, effective treatment for all forms of mental illness. The advent of new techniques in gene research (e.g. CRISPR and next generation sequencing) has enabled biomedical researchers to investigate the genetic or transcriptomic mechanisms of virtually any disease. While some medical conditions have been given great attention in genetic research, little is known about the popularity of gene-based methods while studying the brain and behavior, including how common gene-based methods have become in comparison to traditional methods like behavioral testing or brain imaging. In this study, we use bibliometric data to quantitatively characterize trends in gene-based research on mental illnesses. We compare these patterns to other neurological, neurodevelopmental, and neurodegenerative diseases, including cancers of the nervous system. We then quantify the degree of inequality of research attention to genes across all included fields. While trends across all fields are highly heterogeneous, our overall findings suggest increasing attention toward the role of genes and gene products in mental health research. However, inequality of attention to genes reflect pre-existing patterns of bias and provide insight on how a broader set of less-investigated genes could characterized functionally in a mental health context.

MAYO CLINIC

Sensation by free pectoral fin rays of the hawkfish and its role in benthic station holding

Ramses Ngachoko, Florence Li, Adam Hardy, Melina Hale

Presenter: Ramses Ngachoko • University of Chicago • IMSD Graduate Student—Computational Neuroscience PhD

Examination of limb function most often focuses on the limbs' movement; however, standing, perching, and otherwise maintaining posture without movement are also critical roles of the limbs. We investigated the role of proprioception in the function of free pectoral fin rays of dwarf hawkfish (Cirrhiticthys falco), a benthic marine reef fish adept at maintaining stable posture on surfaces, often in flow. To examine the mechanosensory signals that hawkfish receive from their fin rays, we applied ramp-and-hold bend stimulation to individual fin rays while recording from sensory nerves innervating free fin rays. Rapid burst firing of mechanosensory afferents occurs during the ramp on and off periods, while the fin is being bent. Tonic firing of some afferents was observed during the hold period; this activity is different from that previously reported in membrane-connected fin rays used in swimming of other species. To investigate potential roles of sensation in these free fin rays we recorded the ability of hawkfish to maintain position in flow. We compared performance before and after bilateral transection of sensory nerves innervating the free fin rays. Hawkfish with intact nerves are expert at maintaining position even at high flows. Hawkfish with transected nerves could station hold as well, but with more difficulty at high flow rates. These experiments indicate similarities in mechanosensory physiology and function among very different vertebrate taxa and environments.

Keywords: Proprioception, Mechanosensation, Station-Holding

MAYO CLINIC

Count Consistency Between PanCancer Pathways and Immunology Gene Expression Panels in NanoString Platform

Jon Ocal, MS; Colleen Ramsower, MS; Yuan Xiao, PhD; Lisa Rimsza, MD

Presenter: Jon Ocal, MS• Mayo Clinic-AZ • GREP

Hypothesis/statement about problem under investigation: This analysis studied the similarity and comparability of gene expression counts for a subset of overlapping probes present in two distinct genetic panels used on the same 28-sample set.

Description of experimental methods/materials: Using Excel and nSolver, we identified 100 specific genetic probes present in both gene panels. Each probe was averaged across the 28-samples, creating a single value from each panel that could subsequently be analyzed. A variety of cut-points for minimum count thresholds were tested for both raw probe counts and counts normalized to panel-specific housekeeping genes, below which a probe would be excluded from further analysis.

Results/expected outcomes: Raw counts were tested at cutoffs of 20 and 50, removing 10 and 17 probes from consideration, respectively. Normalized values were cutoff at 25 and 60, removing 10 and 18 probes. After removal of outliers, raw counts had correlations between the panels of 94.3129% without QC, 94.1560% at cutoff 20, and 94.0196% at cutoff 50. Normalized data showed correlations of 94.1159% without QC, 93.9361% at cutoff 25, and 93.7512% at cutoff 60.

Explanation of significance: As individualized medicine continues to evolve, so does our reliance on gene expression profiling technologies. Improving our understanding of this platform will build a foundation for more advanced clinical integration in the future.
Conclusion: In conclusion, probe counts can confidently be compared between separate unique gene testing panels when used on the same samples. Additionally, the consistently high correlation values, regardless of QC cutoff, demonstrates an extremely high level of count accuracy, precision, and consistency down to even the lowest of gene expression levels.

Keywords: NanoString, gene expression profiling, biostatistics

MAYO CLINIC

α-PD-1 and extended half-life IL-2 synergize for treatment of preclinical murine glioblastoma models

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Presenter: Carley A. Owens • Mayo Clinic • PREP scholar—Neuroscience

Glioblastoma (GBM) is the most prominent malignant brain tumor diagnosed in adults, resulting in 220,000 deaths annually. Despite numerous attempts to increase treatment options, GBM patient survival has only increased by months. Immune Checkpoint Blockade (ICB) strategies, including administration of anti-PD-1 antibody, are an increasingly common therapy used to treat other forms of malignant tumors. However, ICB therapy has not been found to effective in GBM patients. This lack of therapeutic response is potentially due to local and peripheral immune suppression that occurs in GBM patients. In this study, we sought to determine the extent in which addition of the lymphoproliferative cytokine, IL-2, could enhance ICB therapy through overcoming the immunodeficiencies associated with GBM.

Methods: Young Female mice were inoculated with either CT2A or GL261 gliomas intracranially. Two treatments of combination therapy consisting of α-PD-1 and bioengineered extended-half life IL-2 was administered singularly and in combination at 14- and 21-days post inoculation. Tumor volume was assessed every 3-4 days via either MRI for the CT2A model or Bioluminescant imaging for the GL261 glioma model.

Results: The synergistic combination of α-PD-1 and bioengineered extended-half life IL-2 therapy was highly effective against gliomas, with approximately 50% of GL261 tumor bearing mice being eradicated of tumor. A similar response rate was observed in the CT2A glioma model.

Conclusions: The “enhanced checkpoint blockade” (ECB) strategy, combining extended half-life IL-2 with ICB, is effective in multiple glioma models. These preclinical findings set the stage for clinical translation combining extended half-life cytokine therapy with ICB for human GBM.

Keywords: Glioblastoma, Immune-Checkpoint Blockade, Immunotherapy, IL-2, α-PD-1

MAYO CLINIC

MDM2-p53 antagonist triggers DNA damage signaling in GBM and sarcoma models

Karolina Pellot Ortiz, Ann C. Mladek, Danielle Burgenske, Jann Sarkaria

Presenter: Karolina Pellot Ortiz • Mayo Clinic • PREP Scholar

MDM2 is an E3 ubiquitin ligase that binds to and targets the tumor suppressor p53 for degradation. However, under cellular stress such as DNA damage, MDM2 binding to p53 is disrupted, resulting in p53 accumulation and activation of downstream genes. To investigate the effects of an MDM2 inhibitor (MDM2i) on tumor cells, in vitro studies were conducted using MDM2-amplified GBM and sarcoma cell lines (GBM108, SJSA) and corresponding MDM2-nonamplified lines (GBM14, U2OS). Cell proliferation and cell death data were collected after MDM2i treatment. A significantly greater reduction in proliferation and increase in cell death was observed in MDM2-amplified lines compared to non-amplified lines following MDM2i treatment. Protein expression of p53 and MDM2 was also evaluated, and results indicate a significant increase in p53 in both MDM2-amplified and non-amplified lines. MDM2 expression also increased significantly with MDM2i treatment in MDM2-amplified lines, but only moderately in non-amplified lines. Additionally, MDM2i treatment induced phosphorylation of DNA damage response proteins ATM at Ser-1981, MDM2 (Ser395), and Chk2 (Thr68), which was blocked by co-treatment with an ATM inhibitor. These findings suggest that MDM2 inhibitor therapy in MDM2-amplified tumors causes significant stress, specifically associated with the activation of ATM-mediated DNA damage signaling. This study is the first to demonstrate this association, which could be useful in the development of more effective treatments in MDM2-amplified cancers.

Keywords: MDM2, p53, Cellular stress, MDM2 inhibitor (MDM2i), DNA damage response, ATM-mediated DNA damage signaling

MAYO CLINIC

Mixed-methods Approaches to Evaluating Brain Tumor Reddit Content

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Presenter: Karolina Pellot Ortiz • Mayo Clinic • PREP Scholar

The occurrence of brain tumors, which can affect up to 40% of cancer patients, has a high psychosocial burden for patients and caregivers. With the growth of technology, social media platforms have become a useful tool for patients and caregivers to seek information and support. As current social platforms such as Reddit grow, patients and caregivers have more access to search for and share information. This work aims to shed light on the use of Reddit for patients and caregivers accessing brain tumor content. Earlier phases of this project looked to comprehend the unmet needs for patients and caregivers. The earlier project used a qualitative descriptive design and findings revealed how Reddit users predominantly accessed the community to process the feelings associated with receiving a brain tumor diagnosis. In the current phase of this project, we are applying machine learning and natural language processing techniques to extract common themes from a larger set of /r/braincancer posts. The results of this analysis will then be aligned and compared with the themes identified in our 2022 analysis. Student researchers with subject matter expertise will play a key role aligning these two analyses. The work provided by this study could provide additional insight from the original project by sampling more posts and content. We anticipate the results will show how social platforms have a niche for information seeking and a way of finding support from people going through similar situations. Topics identified by our analysis could potentially be integrated into clinical practice.

Keywords: Brain tumors, cancer patients, caregivers, Reddit, machine learning, natural language processing

MAYO CLINIC

Contemporary Trends in Minimally Invasive Sacroiliac Joint Fusion Utilization in the Medicare Population by Specialty

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Presenter: Karolina Pellot Ortiz • Mayo Clinic • PREP Scholar

Sacroiliac joint dysfunction is a significant cause of axial pain and disability, and some patients may require surgical arthrodesis for refractory pain. While open approaches have traditionally been used for this surgery, minimally invasive surgical (MIS) techniques and new devices approved for MIS approaches have become more popular over the past decade. This study aimed to analyze trends in SI joint fusions performed by different provider groups, along with the charges billed and reimbursement provided by Medicare. The study analyzed data from the Centers for Medicare and Medicaid Services' yearly data sets from 2015 to 2020, finding that a total of 12,978 SI joint fusion procedures were performed, with 76.5% being MIS procedures. Non-surgical specialists performed most MIS procedures (52.1%), while spine surgeons performed most open fusions (71%). The study noted rapid growth in MIS procedures across all specialties and an increased number of procedures in outpatient settings and ambulatory surgical centers. The reimbursement-to-charge (RCR) ratios were similar between spine surgeons and non-surgical specialists performing MIS procedures, and the overall RCR increased over the study period. The study suggests that non-surgical specialists' adoption has led to substantial growth in the number of MIS procedures for SI pathology, with reimbursement and RCR increasing for these specialists. Further research is needed to better understand the impact of these trends on patient outcomes and healthcare costs.

Keywords: Sacroiliac joint dysfunction, minimally invasive surgical techniques, reimbursement-to-charge ratios, non-surgical specialists, spine surgeons

MAYO CLINIC

Cohousing C57BL/6 Mice with Dirty Mice or Dirty Bedding

Donna Roscoe, Sara Dresler, Dr. John Fryer, PhD

Presenter: Donna Roscoe • Mayo Clinic Graduate School of Biomedical Sciences • IMSD Graduate Student—BMEP

The abnormally clean facilities in which normal laboratory mice are kept act as an essential part of making research more controlled and replicable. They also cause major concerns regarding translatability to humans. The clean facilities in which they are kept, although not germ-free, lack normal exposures to diverse commensal and environmental microorganisms. Dirty mice, as those found in the wild or in pet stores are referred to, have much more diverse microbiomes and immune systems that mirror those of adult humans more closely. It is possible to turn lab mice dirty by co-housing C57BL/6, or other genetically inbred mouse strains, with pet store mice. Our initial aim is to determine what method of co-housing lab mice best transfers the microbiomes and immune systems of the dirty mice by doing a direct head-to-head comparison of co-housing lab mice for 8 weeks with dirty mice bedding or the dirty mice themselves. We expect co-housing with live dirty mice to be the superior method. We will collect fur and cage swabs for PCR pathogen screening, as well as fecal, skin, and vaginal samples from each mouse at the end of the 8 weeks. We will perform 16s and ITS sequencing on the samples to compare the microbiomes and mycobiomes, and use flow cytometry to examine the immune cell populations of multiple organs, paying special attention to immune cell populations of brain tissue. This will set the stage for future studies examining the influence of dirty microbiomes on neurodegeneration.

Keywords: Cohousing, dirty mice

MAYO CLINIC

Growth and Viability of Female Reproductive Tract Microbiome upon Exposure to Endometrial Cancer Secretome

J. Schneider; J. Yao; A. Asangba, PhD; E. Whittle, PhD; M. Walther-Antonio, PhD

Presenter: Jessica Schneider • Mayo Clinic • GREP

Endometrial cancer (EC) is the sixth most commonly diagnosed cancer among women worldwide, with rates on the rise. However, the molecular mechanism by which EC emerges remains elusive. Recent studies identifying distinct differences in female reproductive tract microbiome (FRTM) composition between patients with benign uterine conditions and those with EC identified Porphyromonas somerae as the most predictive microbial biomarker of the disease and the species was later shown to exhibit pathogenic behavior. Here we investigate the relationship between the EC secretome and FRTM, particularly P. somerae, to further our understanding of EC progression. Bacteria exposed to conditioned media from EC line KLE, benign menstrual stem cells, and benign stromal THESC cells are assessed with optical density readings and host-invasion assays. Results showing statistically significant increase in P. somerae replication upon exposure to the KLE secretome warrants further investigation into FRTM interaction with EC secretome.

Keywords: Endometrial Cancer, Microbiome, Women's Health, Reproductive Cancer, Bacteria

MAYO CLINIC

SeqStain based spatialomic profiling of human kidney tissues identifies cellular neighborhoods

Ishwarya Venkatesh, Anugraha Rajagopalan, Disha Varma, Ameera Shaw, Anum Awais, David J. Cimbaluk, Vineet Gupta

Presenter: Ameera Shaw • Rush University • PREP Scholar-IMSD Graduate Student—Integrated Biomedical Sciences PhD

Background: An improved understanding of the underlying cellular heterogeneity of human kidney tissue is essential in providing an accurate disease diagnosis, rate of progression and potential therapeutic avenues for a variety of chronic kidney disease pathologies. Newer multiplex imaging-based methods are providing such tools for implementation in the clinical setting in the future. We recently developed a novel tissue imaging method, termed SeqStain, for immunofluorescence based multiplexed tissue imaging and analyses. Here, we describe utility of this approach to improve understanding of kidney tissue samples from healthy subjects and patients with various glomerular diseases.

Methods: SeqStain utilizes fluorescent DNA-tagged antibodies and antibody-fragments for analyzing tens of kidney-specific analytes in a single tissue section. We designed and optimized SeqStain multiplex panels with sets of antibodies to probe different histological regions relevant to the kidney and used conventional fluorescence microscopy set-up for imaging and analyses.

Results: We show that SeqStain is an efficient method for multiplex imaging of both paraffin-fixed and frozen tissue sections. We were able to accurately image tens of antigens on single tissue specimens for healthy subjects and patients with lupus nephritis (LN) or diabetic nephropathy (DN). Automated analysis of the aligned tissue images showed enrichment of specific cellular clusters into distinct neighborhoods.
Conclusions: This newly developed imaging method, SeqStain, provides an easy to use and robust platform for deep profiling of kidney tissue specimen. The generated spatial maps will provide important new insights about the disease pathobiology and improve future diagnostics and therapeutics for LN and DN.

Keywords: kidney, multiplex imaging, glomerular diseases, pathobiology

MAYO CLINIC

Investigating Microglial Calcium Responses to Purine Signals in Epilepsy Development

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Presenter: Grace Thyen • University of Minnesota Rochester • Health Sciences—Undergraduate Student

We have recently discovered that microglia exhibit elevated calcium activity preceding epilepsy development in the systemic kainate model. In the naive state, calcium activity is rarely observed in microglia. The cause and purpose of this calcium signaling is unknown. Damaged neurons can release purines such as ATP and ADP. We recently discovered that ADP and ATP can activate calcium signaling in microglia through the P2Y12 pathway; however, this mechanism is novel and requires further study. P2Y12 is a unique purinergic receptor to microglia, which is best known to regulate process motility. The goal of this project is to evaluate ATP release and P2Y12 receptors as a unique component of epilepsy development mediating calcium activity in vivo. We have utilized a novel ATP sensor with two-photon microscopy to discover an increase in neuronal ATP release 2-24 hours after kainate. We also characterized P2Y12 expression over epilepsy development and found that one day after kainate administration, there is also a coordinated increase in microglial P2Y12 expression. As epilepsy development progresses, microglial P2Y12 expression later drops below baseline, suggesting down-regulation. We are currently characterizing a Cx3Cr1GCaMP7s-tdTomato mouse line to observe microglial calcium activity (GCaMP7s) and process movement (td-Tomato) in vivo. We find that the Cx3Cr1 promoter effectively and specifically drives GCaMP and tdTomato expression in microglia, and will use this mouse in combination with our ATP biosensor to longitudinally image how ATP regulates microglial calcium and motility in the living brain.

Keywords: microglia, calcium activity, epilepsy development, ATP sensor, and P2Y12

MAYO CLINIC

Nuclear Envelope Reformation is Linked to Centromere Assembly

Destiny Wallace*, Adriana Landeros*, Amit Rahi, Christine Magdongon, Dileep Varma

Presenter: Destiny Wallace • Northwestern University • PREP Scholar—Biology and Chemistry

It is known that many Lamin A/C and Lamin A/C associated proteins (LAAPs), BANF1 and EMD, are key constituents of the nuclear envelope and assemble at the “core" region on chromosomes during Telophase. Prior research suggested that core formation was the first step of Nuclear envelope reformation. However, the identity and function of the chromosomal core regions has not been explored outside of Nuclear envelope reformation. Furthermore, the core's contribution to mitosis remained unknown. It has been shown that the expression of a mutant Lamin A/C cause defective distribution of the centromeres within interphase nuclei in cells. Prior research has supported genomic instability in LAAP inhibited cells such as micronuclei formation and microtubule-spindle defects. Here, we show that a distinct section of the core region overlaps with the centromeric/kinetochore regions of the chromosomes during mitotic telophase. We tested whether centromere assembly is connected to nuclear envelope re-formation. We find that centromere assembly is markedly perturbed after the inhibition of function of Lamin A/C and the core-localized LAAPs, BANF1 and Emerin. We also find that the LAAPs exhibit a biochemical interaction with centromere and inner kinetochore proteins. Consistent with these observations, normal mitotic progression and chromosome segregation was severely impeded after this inhibition of LAAP function. Surprisingly, the inhibition of centromere function also interferes with the assembly of certain LAAP components at the core region, which suggests a mutual dependence of LAAP and centromeres for their assembly at the core region. Our evidence points to a model where LAAPs might serve a key function in loading new centromeric proteins onto this site via the core regions during mitotic exit.

Keywords: Nuclear Envelope Reformation, Centromere assembly, Core, Lamin A/C, BANF1, EMD, LAAPs

MAYO CLINIC

Flow augmentation after occlusion maintains functional connectivity and mean diffusivity

C.S. Warioba, M. Liu, S. Foxley, G.A. Christoforidis, T.J. Carroll

Presenter: Chisondi S. Warioba • University of Chicago • IMSD Graduate Student—Medical Physics

We compare the use of two flow augmentation therapies (NEH and Sanguinate). Functional and structural therapeutic effects of both treatments in acute stroke were studied through resting-state functional magnetic resonance imaging (fMRI) and diffusion tensor imaging (DTI) analysis in a two day pre- and post-occlusion canine model.

Two experimental groups and a control group consisting of ~10 canines per group were imaged on a 3T Philips Achieva dStream scanner with resting state fMRI (BOLD-sensitive EPI, TR/TE = 1400/20 ms, voxelsize/ Temporal positions = 2.5mm/300) and structural DTI (SE-EPI, TR/TE = 2993/83 ms, slice thickness/directions = 2 mm/33)on Day 1. On Day 2 the MCA was permanently occluded; images were re-acquired. Data was pre and post-processed using FMRIB Software Library tools. Functional connectivity analysis was conducted through Z-score comparison of the anterior Default Mode Network. Scalar maps of the principle direction of diffusion, fractional anisotropy (FA), and mean diffusivity (MD) were generated and compared for Day 1 and Day 2 of a representative subject in each experimental group (Fig 3).Functional connectivity and comparison of the average T2* signal intensity over time was conducted through regional analysis ipsilateral and contralateral to the region of occlusion in the anterior MCA region avoiding ventricles for each subject. Pial collateral score was considered in all analyses where the mean/standard deviation for each group were control = 9.6/1.5, NE&H = 8.7/2.2, and Sanguinate = 9.43/0.98.1,2. The Sanguinate group maintained functional connectivity as well as T2* signal intensity and had minimal loss in MD in the occluded region.

Keywords: resting state fMRI, DTI, stroke

MAYO CLINIC

Exploring Differences in Tissue Composition for Women with Benign Breast Disease That Do and Do Not Progress to Breast Cancer

William Jons, Jun Jiang, PhD; Thomas de Bel, MS; Mark Sherman, MD; Amy Degnim, MD; Chen Wang, PhD; Stacey Winham, PhD

Presenter: William Jons • Mayo Clinic • IMSD Graduate Student—BMEP

Background: Benign breast disease (BBD) is diagnosed in women when a palpable or mammographically suspicious lesion results in a biopsy with benign findings. Our goal is to identify what tissue composition differences exist at the time of biopsy between women with BBD that remain cancer free or alternatively develop ER+ or ER- breast cancer (BC).

Methods: A total of 130 patients diagnosed with BBD from the Mayo Clinic Benign Breast Disease Cohort were selected for this study. We conducted bulk RNA-sequencing and H/E staining of FFPE-preserved biopsy tissue obtained from the index biopsy that led to their BBD diagnosis. Using a previously validated convolutional neural network for tissue segmentation trained on H/E images of women with BBD, digitized H/E slide images of the benign biopsy tissue are segmented into various tissue regions (epithelium, intralobular stroma, extralobular stroma, lumen, adipose tissues, vessels, and borders of acini). We compare tissue composition proportions between BBD individuals that later develop BC and those that do not, as well as tissue compositions between those that develop ER+ versus ER- BC.

Results: Preliminary results from a small group of patients (N=21) suggest BBD patients progressing to ER+ BC have a greater proportion of luminal tissue (4.74 vs. 2.45%; P=0.219) and adipose tissue compared to BBD patients remaining cancer free (33.45 vs. 21.08%, P=0.2469), although differences were not significant in the small sample size.

Conclusion: This work will improve understanding of the role of tissue composition differences in the progression to future BC among women with BBD.

Keywords: digital pathology, convolutional neural network, benign breast disease

MAYO CLINIC

An in Vitro Functional Assay Using Raw Blue Cells to Predict in Vivo Efficacy of Rapamycin-loaded Nanoparticles

Deborah Wood, Natalie Klug, Sultan Almunif, Evan Scott

Presenter: Deborah Wood • Northwestern University • PREP Scholar

Pancreatic islet transplantation presents a promising alternative to exogenous insulin treatment for Type 1 Diabetes. Orally administered rapamycin (RAPA) has been used as an immunosuppressant to protect islets from the host immune system during this procedure. The mechanism of RAPA lies in its ability to directly halt immune response through inhibition of the mammalian target of rapamycin (mTOR) kinase and subsequent downstream T cell inactivation. Despite this, oral RAPA is plagued by its insufficient bioavailability and widespread biodistribution to off-target organs. Hence, we have developed poly(ethylene glycol)-b-poly(propylene sulfide (PEG-b-PPS) polymersomes loaded with RAPA (rPS) to improve these outcomes. While RAPA biodistribution and bioavailability showed marked increase when subcutaneously delivered in a diabetic mouse model, we determined that rPS modulates antigen presenting cells in place of inhibiting T cell proliferation, which shifts RAPA function towards tolerance over immunosuppression. Given these unprecedented outcomes, we explore RAPA behavior through a colorimetric assay using Raw Blue cells with the aim to design a tool that rapidly predicts drug response across free and rPS conditions. We demonstrate that rPS more readily mitigates inflammatory response in Raw Blue cells induced towards inflammation with lipopolysaccharide. By studying RAPA function across various biologically relevant conditions, predictions can be made regarding RAPA function prior to using in vivo models.

Keywords: Rapamycin, PEG-b-PPS, polymersomes, Raw Blue cells

MAYO CLINIC

Prolactin Receptor (PRLR) is required for maintaining liver homeostasis

Jennifer Yanum, Guoli Dai

Presenter: Jennifer Yanum • Indiana University-Purdue University Indianapolis (IUPUI) • IMSD Graduate Student – Biology PhD

PRLR is abundantly expressed in the liver with little known functions. The exclusive expression of PRLR mRNA in hepatocytes suggests that it may directly regulate the property of these epithelial cells. To investigate the role of Prlr in the liver, we deleted the Prlr gene specifically in hepatocytes of adult female virgin mice using a virus-based approach and evaluated liver transcriptome, function, and signaling molecules. The absence of Prlr in hepatocytes did not alter liver histology and size. However, the lack of Prlr resulted in differential expression of only 133 genes largely associated with hepatocyte structure, metabolic function, and inflammatory response. Blood biochemistry assessments revealed excessive accumulation of urea in the blood due to the absence of Prlr. Expression of the genes encoding the enzymes controlling urea cycle did not show PRLR-dependent changes however, the loss of function of Prlr in hepatocytes caused increased expression of ASL. The results demonstrate that PRLR modulates the expression of ASL post-transcriptionally and thus ureagenesis. Also, it has been considered that STAT5 largely mediates PRLR signaling in the liver. However, we found that Prlr deletion profoundly reduced the activity of STAT3, but mildly inhibited that of STAT5. The results suggests that in homeostatic conditions, STAT3 may transduce PRLR signaling dominantly over STAT5 in the liver. Taken together, we demonstrate that PRLR contributes to the maintenance of liver homeostasis partially via regulating the basal expression of a limited number of genes and is essential for urea homeostasis by post-transcriptionally modulating the expression of hepatic ASL.

Keywords: Liver, Prolactin receptor, homeostasis, signaling, STAT3

MAYO CLINIC

Alleviating the Unfolded Protein Response in Human Airway Smooth Muscle Cells Utilizing ROS Scavengers & Chemical Chaperones

Jane Q. Yap, Debanjali Dasgupta, Philippe Delmotte, and Gary C. Sieck

Presenter: Jane Q. Yap • Mayo Clinic • GREP Scholar

Airway inflammation is involved in many respiratory diseases, such as asthma. Pro-inflammatory cytokines such as tumor necrosis factor α (TNFα) mediate airway inflammation and induce endoplasmic reticulum (ER) stress in human airway smooth muscle (hASM) cells. Previously, we showed that TNFα selectively activates the inositol-requiring enzyme 1α (IRE1α)/X-box binding protein 1 splicing (XBP1s) ER stress (unfolded protein response) pathway. This selective activation of the IRE1α/XBP1 ER stress pathway targets several genes that promote mitochondrial fragmentation and biogenesis. Most likely, an increase in reactive oxygen species (ROS) formation and the continuation/ accumulation of unfolded proteins is responsible. We hypothesize that ROS scavenger TEMPOL or 4-phenylbutyrate (4-PBA), a chemical chaperone, will alleviate TNFα mediated ER stress in hASM cells. Bronchiolar tissues were obtained from 5 healthy. hASM cells were dissociated and serum-deprived for 48 h and phenotype was confirmed. hASM cells from the same donor were then divided in 4 12-h treatment groups: 1) TNFα (20 ng/mL), 2) TNFα + TEMPOL (30 min pretreatment), 3) TNFα + 4-PBA (30 min pretreatment), and 4) untreated control. Phosphorylation of IRE1α was evaluated by Western blot. PCR was used to quantify XBP1s mRNA. We found that TNFα induced IRE1α phosphorylation and XBP1 splicing (ER stress) in hASM cells were significantly reduced with ROS scavenging using TEMPOL or chemical chaperone 4-PBA. Our results indicate that ROS scavenging and chemical chaperone treatment can reduce ER stress induced by TNFα and thus alleviate the effect of TNFα on mitochondrial fragmentation and biogenesis in hASM cells.

Keywords: TEMPOL, 4-PBA, airway inflammation, ROS, scavengers, chemical chaperones

MAYO CLINIC

Metabolic consequences of SDH loss in a mouse chromaffin cell model of paraganglioma

Sherry Zhou; Jim Maher, Ph.D.

Presenter: Sherry Zhou • Mayo Clinic • MSTP Student—BMB Track

Succinate dehydrogenase (SDH), also known as Complex II in the electron transport chain, is a key mitochondrial enzyme involved in aerobic respiration through both the tricarboxylic acid cycle and the electron transport chain. Deleterious mutations in subunits of SDH can result in hereditary tumors such as paraganglioma and pheochromocytoma, rare neuroendocrine tumors of chromaffin cells in the nerve ganglia and the adrenal gland, respectively. While mutations in the B subunit of SDH are the second most common hereditary mutation in paraganglioma and pheochromocytoma (VHL being the most common), SDHB-mutant tumors are strongly associated with more aggressive and metastatic disease. Previous studies have shown many consequences of succinate accumulation from SDH loss and possible mechanisms of tumorigenesis in various alternative cell models of paraganglioma, including DNA hypermethylation, pseudohypoxia, and protein hyperacylation, though the exact mechanism of tumorigenesis remains unclear. We seek to validate and better characterize SDHB-loss induced protein hyperacylation and explore the metabolic consequences of protein hyperacylation in an SDHB-mutant immortalized mouse chromaffin cell model of paraganglioma.

Keywords: paraganglioma, pheochromocytoma, chromaffin, succinate dehydrogenase (SDH)

MAYO CLINIC

Role of Thermoregulatory Sweat Test in the Diagnosis of Facial Flushing: A Retrospective Case Series

Rachel L. Ziebart, Elizabeth Coon, Julio C. Sartori-Valinotti

Presenter: Rachel L. Ziebart • Mayo Clinic Alix School of Medicine • PREP Scholar—MD

Hypothesis: Many patients with unexplained facial flushing will have anhidrosis as evidenced by an abnormal thermoregulatory sweat test result.

Methods: We completed a retrospective case series at a tertiary institution with consecutive patients who presented with facial flushing and underwent a thermoregulatory sweat test.

Significance: Facial flushing is a common condition that negatively impacts quality of life and may be caused by many different conditions, requiring a broad differential diagnosis. Evaluation is typically directed at identifying endocrinologic causes. One contributing factor to facial flushing that is rarely evaluated for is anhidrosis.

Results: From a total 36 patients, 28 (78%) were women. The majority of patients (N=25; 69%) had an abnormal thermoregulatory sweat test. Patients aged 40 and older were statistically more likely to have an abnormal sweat test.

Conclusion: Greater than two thirds of patients presenting with unexplained facial flushing had an abnormal sweat test. These novel results support the use of thermoregulatory sweat testing in the diagnosis of facial flushing, and it should be considered as part of the diagnostic approach, especially in the older adults and female patients.

Keywords: Medical dermatology; flushing; facial flushing; thermoregulatory sweat test; anhidrosis; facial erythema