# Abstract Program

*Postdoc Poster Session &*
*Graduate Student Poster Session*

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# Role of Exonuclease 1 Protein-Protein Interactions in Human Mismatch Repair

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DNA repair is the process by which cells identify and correct mutations present within the genome. These correction mechanisms maintain genomic stability and prevent the development of cancer. The DNA mismatch repair (MMR) pathway identifies and corrects small insertions, deletions, and misincorporations that arise within the genome as a result of errors during DNA replication. MMR is also critical for inducing apoptosis after chemically induced mispairs, such as those from environmental alkylating agents. The process by which MMR corrects these mispairs includes recognition of the mispair by the MutS complex, recruitment of the MutL complex to the mutation site, excision of the mispair, and gap filling by DNA polymerase. Exonuclease 1 (Exo1) is the protein primarily responsible for the excision step of MMR. In Exo1-dependent MMR, Exo-1 binds to both the MutS heteroduplex (MSH2-MSH6) and MutL heteroduplex (MLH1-PMS2). The MutL interaction with Exo1 in budding yeast is facilitated by an Mlh1 interaction peptide (MIP) box. We recently identified a Msh2 interaction peptide (SHIP) box in yeast Exo1. This project aims to understand how human MIP and putative human SHIP box motifs influence human Exo1 recruitment to MMR processes. We have created point mutations within the predicted binding domains of human Exo1. We observe changes in localization of Exo1-mutant proteins within the cell, suggesting that changes in the overall MMR process may be present. We also observe changes in MMR-mediated apoptotic response when a subset of Exo1-mutations are expressed in the presence of endogenous wildtype Exo1, indicating a potential for a dominant negative interaction. Alterations in Chk1 phosphorylation suggest the Exo1 mutations alter DNA damage response pathways. Our ongoing studies are expected to shed more light upon how the mechanisms of human MMR process and overall genomic stability. This study will have important implications on human cancer development and treatment.

**Impact of intestinal microbiota on Campylobacter jejuni pathogenesis**

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Campylobacter jejuni is the primary pathogen responsible for bacterial gastrointestinal infections in children and adults worldwide, and the leading cause of diarrheal diseases in the United States. The mechanism of how commensal microbiota and their metabolic products affect the pathogenesis and establishment of infection of C. jejuni are poorly understood. Combining in silico, immunoblotting, chemotaxis and bioinformatic analyses, we have looked for potential target gut microbial metabolites affecting the pathogenesis of C. jejuni in a high protein diet (HPD) mouse model. For this project, we focused on trimethylamine oxide (TMAO), a metabolite significantly associated with our infectible HPD mouse model, and a known alternative electron acceptor for C. jejuni respiration in a limited oxygen environment. We confirmed the chemotaxis of C. jejuni towards TMAO with stab-agar assays, and began investigations of effect of TMAO on intestinal epithelial barrier functions in vitro, using our CaCo-2 cell culture model via immunoblotting for tight junction proteins. We also have determined potential C. jejuni chemosensors and utilizing enzymes of TMAO via in silico and bioinformatic analysis of protein-ligand interactions. Next, we began investigating the impact of TMAO on C. jejuni into colonic epithelial cell (CaCo-2) monolayer. This work is the building block for determining how C. jejuni interacts and utilizes the intestinal microbiota and the intestinal metabolome in respiration and further pathogenesis.

**The first Glucocorticoid Receptor-beta (GRb) knockout mice display reduced adiposity via lower PPAR-gamma activity in adipose tissue.**

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Obesity and its associated comorbidities are major contributors to deaths worldwide. This has led to increasing rates of obesity-associated comorbidities, such as type II diabetes, non-alcoholic fatty and liver disease. A conundrum exists in that short-term glucocorticoid therapy reduces body weight, but the long-term chronic treatment causes weight gain and adiposity. Because of the observed effects, we are interested in better understanding the complex role of glucocorticoid receptor (GR) isoforms in lipid and glucose metabolism. We hypothesized that the conundrum is related to an imbalance of the GRa and GRb isoforms. They arise from a single GR gene (Nr3c1) due to the alternative splicing of exon 9 and are antagonists to one another. To test this hypothesis, we developed specialized GRb CRISPR knockout (KO) technology to KO GRb in mice; therefore, only expressing GRa. The GRb homozygous KO mice seem to be lethal. However, the heterozygous KO (GRbHetKO) mice are viable, with no observable phenotypes on standard chow. Therefore, we used GRbHetKO mice to study the effects of this isoform on adiposity. We placed the GRbHetKO and GRWT mice on a high-fat diet (HFD) for 8 weeks. We found that the GRbHetKO mice fed HFD had significantly reduced body weights and adiposity compared to their littermates, which was paralleled with significantly reduced PPARg expression in inguinal white adipose tissue (iWAT) but not in eWAT or liver tissues. The liver histology sections showed decreased lipid accumulation. Molecular analysis of GRb-PPARg signaling indicates that GRb positively affects PPARg transcriptional activity. We conclude that GRb drives PPARg expression and activity in adipose to induce adiposity in iWAT. Our studies here indicate that GRb might explain differences observed for short- and long-term glucocorticoid therapies, and a better understanding of the GR isoforms could benefit patients with obesity and possibly improve obesity-associated comorbidities.

**Cardiomyocyte-Restricted Deletion of RAD Improves Heart Failure**

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Background: Heart failure (HF) is the second leading cause of hospitalization and represents a third of cardiovascular disease deaths. Dilated cardiomyopathy (DCM) is the most common cardiomyopathy resulting in systolic heart failure with reduced ejection fraction. Current therapies fail to address a principal issue: loss of contractile force. Targeting dysfunctional Ca2+ handling proteins involved in excitation-contraction, such as Cav1.2 may offer a means to improve quality of life and attenuate adverse cardiac remodeling. Cardiomyocyte-restricted deletion of RAD, a Cav1.2 regulatory unit that mediates beta-adrenergic receptor (beta-AR) signaling has been shown to stably improve cardiac function in healthy mice.

Hypothesis: RAD ablation—after disease onset—improves systolic cardiac function by increasing trigger Ca2+. Methods: A DCM model of HF, the muscle lim protein knockout mouse (MLPKO) was used to test if tamoxifen-inducible, cardiomyocyte restricted RAD deletion in 10-week-old mice improved function and attenuated pathology relative to MLPKO mice with RAD still present. Longitudinal echocardiography for in vivo assessment was performed, bulk RNAseq of hearts, isolated live cell ventricular cardiomyocyte Ca2+ imaging and sarcomere function in addition to whole-cell patch clamp recordings of Cav1.2 current. Beta-AR agonist was used to test cellular beta-AR responsiveness in isolated cells.

Results: After tamoxifen-induced cRADKO of MLPKO mice, ejection fraction % significantly increased versus single-knockout MLPKO mice with RAD present after 1 month in both males and females. Transcripts associated with HF (Nppb, Acta1, Myh7, Xirp2) were downregulated significantly in cRADKO MLPKO. Electrophysiological whole-cell patch clamp recordings of cardiac Cav1.2 demonstrated increased current in cRADKO MLPKO. Ex vivo live cell measurements showed improvement of excitation-contraction. This ongoing study implicates a new therapeutic target for HFrEF and DCM.

**Fatty acid synthase regulates Notum expression in colorectal cancer**

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Upregulation of lipid synthesis has been associated with a poor clinical outcome in colorectal cancer (CRC). Fatty acid synthase (FASN) synthesizes 16-carbon fatty acid palmitate which can be utilized for post-translational modifications of various proteins. Notum, a palmitoleoyl-protein carboxylesterase, is involved in the negative regulation of Wnt signaling pathway via its role in de-palmitoylation of Wnt ligands and has been identified as a marker for poor prognosis in CRC. However, the crosstalk between FASN and Notum has not been reported. Tumor and normal intestinal organoids were established from transgenic mice models, ApcMin and Apc/VillinCre-ERT2, with inducible hetero- and homozygous deletion of FASN. LIVE/DEAD™ Viability/Cytotoxicity Cell Viability Kit and Cell Titer-Glo® 3D Cell Viability Assays were used for quantitative analysis of organoids. HCT116, NTC and FASN shRNA, and SW480, control and FASN overexpression, cells were used for analysis. ERT2-mediated deletion of Apc leads to upregulation of FASN and Notum expression in mouse intestinal tissues and organoids. RNA-seq analysis of adenomas from Apc/VillinCre mice showed that hetero- and homozygous germline deletion of FASN is associated with a significant decrease in number of adenomas, expression of Notum and CRC stem cell markers. We further confirmed that FASN downregulation is associated with a decrease in active β-catenin, Notum and stem cell markers. Additionally, downregulation of FASN results in a decrease in bud formation in Apc/VillinCre-ERT2 organoids, and viability and size in ApcMin organoids. Furthermore, overexpression of FASN increases the levels of active and total β-catenin, Notum and stem cell markers expression in SW480 cells. However, FASN deletion decreases Notum expression in HCT116 cells. Delineating the role of FASN regulation of stemness via β-catenin/Notum signaling and other stem cell markers will provide the rationale for targeting FASN/Notum axis in CRC.

**Does a closed head injury early in life have lasting effect on microglia and astrocytes in an Alzheimer's disease-relevant mouse?**

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Traumatic Brain Injury (TBI) is a common neurological injury caused by falls, vehicle collisions, war-sustained injuries, sports accidents, and intimate partner violence. TBI causes a cascade of pathophysiological changes, including hyperexcitability, excitotoxicity, and neuroinflammation. TBI can also cause increase the risk of long-term neurological impairments and neurodegenerative diseases such as Alzheimer's (AD). Microglia and astrocytes respond within minutes to hours after a traumatic brain injury. However, it is unclear whether an injury early in life will have a lasting effect on microglia and astrocytes in old age that may influence AD-related amyloid pathology. To test this hypothesis, we induced a closed head injury (CHI) around 5 months of age in APP/PS1 KI and wild-type mice. We found that microglia in young adult mice are highly plastic, and injury-induced changes in microglia observed during the first-week post-injury resolved over time. In contrast, APP/PS1 KI mice had elevated immunopositivity for GFAP 1- and 4-months post-injury. By 13 months of age, a genotype effect was seen with the primary driver of GFAP immunostaining, with a significant number of GFAP+ cells around amyloid plaques. These findings indicate an interaction between TBI and AD-related changes during the first 4-months after injury. However, 8 months post-injury, a TBI-related effect on GFAP, IBA1, or 6E10 is not observable. This could suggest that the TBI-related changes have resolved over time, or that the significant amyloid-beta-related changes in the APP/PS1 KI mice create a ceiling effect that is masking TBI-related changes.

**Targeting Leukemia initiating cells in T-cell acute lymphoblastic leukemia (T-ALL) through the β-catenin pathway: Repositioning of Erlotinib**

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This study aimed to identify a small molecule that inhibit the Leukemia Initiating Cells (LIC) in T-cell acute lymphoblastic leukemia (T-ALL) by targeting the β-Catenin signaling cascade. The Wnt/β-Catenin signaling axis plays an important role in development of the cancer initiating cells in multiple cancers including T-ALL. Since this pathway is involved in normal tissue regeneration, available inhibitors are associated with significant side effect that limit their clinical utility. There is an un-met need of Wnt/β-Catenin pathway inhibitors with an acceptable safety profile. We employed a TCF/LEF transgenic zebrafish with GFP reporter to interrogate a library of over than 770 FDA approved drugs for their effect on this pathway. We identified Erlotinib as a hit compound. Erlotinib is a small molecule tyrosine kinase inhibitor that targets the epidermal growth factor receptor (EGFR), that is commonly expressed in many cancer types. We found that Erlotinib is able to significantly reduce the number of colonies formed in vitro (P value= 0.0076) using the 3D sphere formation assay. Using zebrafish T-ALL models and the limiting dilution method, Erlotinib resulted in three-fold decrease in frequency of T-ALL leukemia initiating cells (P value= 0.0352) and about 60% decrease in leukemia burden in vivo. Erlotinib treatment significantly inhibited the expression of Wnt/β-Catenin target genes possibly through modulating the cell's ATP metabolism.

**Molecular Mechanisms of the Activity-Driven Plasticity of the Auditory Stereocilia Cytoskeleton: Role of Myosin 15 Isoforms**

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Stereocilia in the inner ear detect sound waves through the opening of mechano-electrical transduction (MET) channels. Even at rest, a basal MET channel current results in a calcium influx into the cell. It has been previously demonstrated that this influx at rest is essential for the stability of the stereocilia cytoskeleton. However, the molecular mechanisms involved in this phenomenon are still unknown. Given that the non-conventional myosin 15 is required for the normal elongation and maintenance of the stereocilia bundle, we wondered whether the myosin 15 isoforms are also involved in this calcium-dependent remodeling of the stereocilia cytoskeleton. Myo15sh2/sh2 mice have a missense mutation which prevents all myosin 15 isoforms from reaching the stereocilia. Hair cells in Myo15sh2/sh2 mice have abnormally short stereocilia but still exhibit MET currents. We found that the blockage of the MET channels in Myo15sh2/sh2 cochlear explants does not lead to visible stereocilia remodeling. Myo15ΔN/ΔN mice lack the long isoform of myosin 15, they develop stereocilia bundles of normal heights and staircase arrangements with normal MET currents but exhibit stereocilia degeneration. After MET channel blockage, hair cells from Myo15ΔN/ΔN mice exhibit greater stereocilia shortening than heterozygous or wild-type controls. Finally, after MET channel blockage, auditory hair cells from Myo15ΔN/ΔN mice exhibit shortening in the tallest row of stereocilia, which normally do not exhibit MET-dependent cytoskeleton remodeling. Thus, our data suggest that the lack of a long isoform of myosin 15 could change the row identity of the stereocilia. In summary, our data indicate that the molecular machinery involved in the calcium-dependent stability of the stereocilia cytoskeleton relies on myosin 15. In addition, the lack of the long isoform of myosin 15 could change the insensitivity of the tallest row of stereocilia and mislocate proteins that may be required for this insensitivity.

**APOE genotype modifies microglial immunometabolism**

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Metabolic dysfunction and neuroinflammation characterize Alzheimer's disease (AD), but it is unclear if these two facets of the disease are linked. The E4 allele of Apolipoprotein E (APOE) is the strongest genetic risk factor for late-onset AD and is associated with increased neuroinflammation. Recent data show that E4 is also associated with increased aerobic glycolysis. These two findings may be intrinsically linked through the concept of 'immunometabolism' - an emerging paradigm that implicates increased glycolysis during pro-inflammatory activation, whereas increased oxidative phosphorylation is required for anti-inflammatory responses. Primary microglia were isolated from mice expressing human APOE isoforms and metabolic responses to inflammatory stimuli were measured using the Seahorse platform and metabolomics. We also performed single-cell and spatial RNA sequencing on brains from APOE targeted replacement mice at 3-, 12-, and 24 months of age, as well as those crossed to the 5xFAD amyloid model. Seahorse revealed increased glycolysis and decreased mitochondrial respiration in E4 microglia. Targeted metabolomics revealed increased lactate and succinate in E4 microglia. Aged E4 brains were found to harbor a metabolically distinct cluster of microglia that expressed a signature similar to the established 'disease associated microglia' (DAM) phenotype, even in the absence of neuropathology. SCENIC regulon analysis linked this cluster to the transcription network of HIF1a. Our findings reveal that inflammatory stimulation, age, and amyloid each induce a distinct metabolic response in E4 microglia conducive to pro-inflammatory activation, whereas decreased mitochondrial respiration may preclude effective anti-inflammatory responses. We propose that this altered metabolism skews E4 microglia towards a a phenotype that favors chronic neuroinflammation. Thus, reprogramming metabolism in E4 microglia may provide a novel therapeutic avenue for the treatment of AD.

**Hepatocyte-specific deletion of angiotensinogen reduces Western Diet-induced hepatic steatosis with a suppression of complement component C4**

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Background: Western diet (WD) causes liver steatosis resulting in chronic liver disease. WD-induced steatosis has been shown to be abated by hepatocyte-specific angiotensinogen (AGT) deficiency, a precursor of angiotensin peptides. However, the mechanism by which AGT facilitates WD-induced steatosis is unknown. This study aims to determine the contribution of AGT to WD-induced transcriptomic changes in the liver.

Methods and Results: We first performed sequential bulk RNA sequencing in mice fed WD for different intervals. Livers from low-density lipoprotein receptor-deficient (LDLR-/-) mice fed WD for 5, 14, or 42 days were evaluated against mice fed normal diet. Gene ontology analysis revealed that upregulated genes were related to inflammation at each interval. Liver transcriptomes underwent a transition at 14 days WD that endured at 42 days, evidenced by principal component analysis. To explore the role of AGT in steatosis, we performed additional liver transcriptomic analyses comparing hepatocyte-specific AGT deficient (hepAGT-/-) mice and wild-type (hepAGT+/+) littermates at 14 and 42 days of WD. Compared to hepAGT+/+ littermates, there were 128 and 23 differentially expressed genes (DEGs) in hepAGT-/- mice at 14 and 42 days of WD, respectively. Interestingly, four DEGs were overlapped between intervals. The four DEGs were Agt, Mup21 ¬– a urinary protein with no human homolog, Moxd1 – a predicted enzyme with no known substrate, and C4a – a pro-inflammatory zymogen critical in complement pathways. Since the gene ontology analysis identified multiple pathways related to inflammation, we focused on C4a. RT-qPCR detected a 2.7-fold decrease in C4a mRNA expression in hepAGT-/- mice at 42 days of WD.

Conclusions: Inflammatory genes were upregulated in development of steatosis. Hepatocyte-specific AGT deficiency suppressed C4a mRNA. Future studies will determine which complement pathways are activated by WD and determine the spatial distribution of C4a.

**Retention of PF4 by Megakaryocytes Depends on Serglycin, an Intracellular Proteoglycan**

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Background/Objectives: Platelets are produced by megakaryocytes (MK) in the bone marrow. Disorders affecting the development of platelet granules are well documented. These storage pool disorders (SPDs) lead to disrupted trafficking of granule cargo. The trafficking of cargo to these granules is integrated with the development of MK, and the α-granule stores several growth factors that affect the HSC niche and the bone marrow microenvironment, such as PF4 and TGF-β respectively. Many SPDs also exhibit myelofibrosis in the bone marrow, indicating that the aberrant trafficking of α-granule cargo is affecting the surrounding milieu of the bone marrow and the HSC niche. The purpose of this study was to assess the release of PF4 from MKs in vivo and in vitro in knockout mice that lack two key a α-granule packaging proteins: Serglycin and NBEAL2, and how alterations in extramedullary PF4 affect MK development and the bone marrow microenvironment

Methods: To investigate this relationship, we have utilized primary MKs from Serglycin-/- and NBEAL2-/- mice. Whole bone marrow was isolated from euthanized mice, mature cells were removed by magnetic separation with lineage depletion kit (Miltenyi Biotec), and immature cells were then cultured in 50 ng/ml TPO. Samples for western blot and ELISA were taken daily. PF4 levels were then assessed by western blot and ELISA (R&D systems). Daily samples were taken for FACS analysis to assess DNA content. To address in vivo levels of PF4 we isolated bone marrow plasma by centrifugation.

Results and Conclusions: The results of these experiments showed that PF4 is secreted by MKs normally during their development as shown in WT mice. However, the deletion of Serglycin led to increased secretion of PF4 during earlier stages of MK development, and a near complete depletion of internal PF4 by day 5. Comparison to NBEAL2-/- MKs showed a lack of internal PF4 and very low levels of secreted PF4. These results are supported by the in vivo measure

**Understanding the Role of Interleukin-1 in Regulating Neuroinflammation after Closed-Head Injury in Mice**

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Neuroinflammation is a complex cascade of responses to neural insults that, when dysregulated, can lead to a host of detrimental effects in the brain. A single mild traumatic brain injury (mTBI) can trigger neuroinflammation, which, if unresolved, can lead to persistent cognitive and behavioral impairments. Interleukin-1 (IL-1), a key molecule involved in this process, increases in the brain following mTBI and significantly drives the inflammatory response. However, it is unknown if IL-1 contributes to long-term deficits after mTBI. We found persistent cognitive deficits in the active avoidance task and immunohistochemical evidence of persistent reactive glia at 14 weeks post-mild closed head injury (CHI), which improved in global IL-1R1 KO mice. We then hypothesized that the acute elevation of IL-1 during the first hours to days after the injury might be critical to the protective phenotype in the IL-1R1 KO mice. To test our hypothesis, we subjected wild-type (WT) mice (n=56) and global IL-1R1 KO mice (n=51) to either a CHI or sham procedure. We sacrificed the mice at 3hrs, 9hrs, 24hrs, and 72hrs post-injury and extracted RNA from the neocortex. We measured gene expression changes using the Nanostring neuroinflammatory panel. Our results show that IL-1R1 signaling significantly alters gene expression at 3hrs and 9hrs post-injury, with pathway enrichment analysis highlighting the most significant changes to genes related to astrocyte functioning following CHI. Overall, our study provides evidence that IL-1 has a specific, time-dependent function in regulating the expression of neuroinflammatory genes following CHI in mice, contributing to prolonged neurological impairments.

**Urobilin, formed by the microbiome catabolism of bilirubin, is positively associated with adiposity and insulin resistance in humans**

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For unknown reasons, plasma bilirubin levels are lower in obese humans and rodents. One possible explanation is that the hepatic expression of enzymes that regulate its half-life, such as the UGT1A1 UDP-glucuronosyltransferase, is higher in the obese compared to lean persons. Bilirubin is marked for excretion within the liver by the conjugation by UGT1A1, where it is then transported to the intestines by the biliary system. Once in the intestines, the gut microbiota containing bilirubin reductase remove the glucornyl groups and modify the structure to produce urobilin which can be absorbed via the hepatic portal vein and enter the systemic circulation. However, the physiological function of urobilin is unknown. The purpose of our study was to determine if there is a correlation between bilirubin and urobilin compared to adiposity and insulin resistance in lean and obese women and men. We hypothesized that bilirubin would be lower and urobilin higher in the plasma of obese humans compared to lean. We found that the plasma levels of urobilin and bilirubin are inversely correlated in women and men. Urobilin levels were positively associated with adiposity and insulin resistance in obese women. Urobilin was also positively correlated with adiposity in males. However, the males were not insulin resistant and had no significant difference in HOMA IR compared to lean men. In both women and men, plasma bilirubin levels were negatively associated with adiposity. We observed that plasma bilirubin levels are negatively associated with adiposity, which has been previously reported. Based on these data, we propose that bilirubin and urobilin are maintained at inverse levels in humans via the UGT1A1 axis and that activity is higher in the obese, reducing plasma bilirubin and providing more substrates to produce urobilin. These events cause an elevation in plasma urobilin levels that commence obesity-associated comorbidities.

## ABL1 Mediates MLH1 Regulation and DNA Mismatch Repair

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The DNA mismatch repair (MMR) pathway and its regulation are critical for genomic stability. MMR repairs misincorporated bases that are not recognized and removed during DNA replication. MMR is also critical for initiating apoptosis after select DNA damaging agents. MMR defects are seen in a large portion of sporadic colorectal, endometrial, and gastric cancers. Cells with defective MMR (dMMR) exhibit an increased mutation rate and unrepaired expansion and contraction of repetitive sequences termed, microsatellite instability (MSI). MSI-high/dMMR tumors respond well to immunotherapy, presumably due to a high tumor mutation burden and increased neoantigen production. Loss or mutation of critical MMR protein MLH1 leads to defective MMR, increased mutation frequency, and MSI-high phenotypes. In this study, we report that 2 tyrosine kinase inhibitors (TKIs) lead to decreased MLH1 protein expression and that the target responsible is the ABL1 kinase. TKI treatment or ABL1 knockdown result in decreased apoptosis after treatment with alkylating agents, suggesting the level of MLH1 reduction is sufficient to disrupt MMR function. We demonstrate that MLH1 downregulation requires Hsp70 and the lysosomal degradation pathway. We also observe that MLH1 can be directly tyrosine phosphorylated by ABL1. Taken together, we propose that ABL1 prevents MLH1 from being targeted for degradation by the Hsp70 chaperone and that in the absence of ABL1 activity, a subset of MLH1 is degraded through the lysosome. A promising MLH1 mutant disrupting a predicted phosphorylation site is under further investigation. This study represents an advance in understanding regulation of the MMR pathway and has interesting and important clinical implications, given the rise of immunotherapy use in MSI-high/dMMR tumors. We are currently pursuing this work as a strategy for sensitizing MSI-low/MMR proficient tumors to immunotherapy.

**Concurrent microRNA and flow-cytometry UMAP analysis identify cell-type specific correlations in the cerebrospinal fluid of patients with aneurysmal subarachnoid hemorrhage**

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Background:
Prior clinical research used flow cytometry to identify an active presence of innate and adaptive immune cells in cerebrospinal fluid (CSF) of patients with aneurysmal subarachnoid hemorrhage (aSAH). The purpose of this study was to expand on this work to identify longitudinal novel immune cell subsets using an un-biased algorithm that self-identifies unique populations through clustering in high-dimensional space. Moreover, we analyzed cell-free CSF microRNA (miRNA) changes using the same specimens to study the correlation of miRNAs with specific immune cell populations in the CSF.

Methods:
CSF samples (n=30) were collected from an extra ventricular drain (EVD) placed in aSAH patients (n=8). Samples were obtained at days 3, 5, 7, and 10 post-aSAH. All samples were processed and stained using a general immunophenotyping panel. Gating was performed in FlowJov10 and analyzed using uniform manifold approximation projection (UMAP). For miRNA a customized TaqMan Low-Density Array (TLDA) was employed to analyze the levels of 48 selected miRNAs and controls in the CSF.

Results:
Neutrophil populations trended upward, especially from days 3 and 5. We observed a significant increase in CD4 T cells from day 5 to day 7 ($p=0.0145$, Tukey's HSD test). CD8 T cells showed significant differences across post bleed days ($p=0.34$, Mixed-effect analysis) and significantly decreased from day 3 to 5 across all patients ($p=0.046$, Tukey's HSD test). CD19 B cells significantly tended downward over 10 days ($p=0.0169$, Mixed-effect analysis). We identified a unique CD4+ CD161+ T cell subset. This data shows how UMAP analysis of longitudinal CSF sampling can identify unique subpopulations of immune cells that may be lost using traditional gating analyses. Patients 4 and 6 exhibited high neutrophils levels and also exhibited the largest increased levels of inflammatory miRNAs (miR-142-3p, miR-146a, miR-146b, miR-150, and miR-155) in the CSF.

**Association of Baseline Cerebrovascular Reactivity and Longitudinal Development of Enlarged Perivascular Spaces in the Basal Ganglia**

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Background: Increasing evidence suggests that enlarged perivascular spaces (ePVS) are associated with cognitive dysfunction in aging. However, the etiology of ePVS remains unknown. Here we tested the possibility that cerebrovascular dysfunction, as measured by an MRI measure of cerebrovascular reactivity (CVR), contributes to ePVS development.

Methods: A total of 79 cognitively normal, older adults (46 women, age range 60-84) were recruited to undergo MRI scanning at baseline and 50 participants returned for a follow-up scan approximately 2.5 years later. ePVS were counted in the basal ganglia, centrum semiovale, midbrain, and hippocampus. CVR, an index of the vasodilatory capacity of cerebral small vessels, was assessed using carbon-dioxide inhalation while acquiring blood oxygen-level dependent (BOLD) MR images.

Results: Low baseline CVR values in the basal ganglia were associated with increased follow-up ePVS counts in the basal ganglia after controlling for age, sex, and baseline ePVS values (coefficient estimate (SE) -15.87 (3.92), $p < 0.001$, 95% confidence interval [CI] -23.68 to -8.05). This effect remained significant after accounting for self-reported risk factors of cerebral small vessel disease (cSVD) (coefficient estimate (SE) -15.03 (4.00), $p < 0.001$, CI -23.02 to -7.05) and neuroimaging markers of cSVD (coefficient estimate (SE) -13.99 (4.02), $p < 0.001$, CI -22.03 to -5.95).

Conclusion: Our results demonstrate that low baseline CVR is a risk factor for later development of ePVS. MRI-based CVR may represent a promising biomarker of cSVD.

**Amylin, a diabetes-associated amyloid-forming peptide, accumulates in thrombi and on red blood cells - new biomarker for stroke?**

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Emergent large vessel occlusions result in severe ischemic stroke without appropriate treatment with thrombolysis and/or mechanical thrombectomy. Type-2 diabetes mellitus (T2DM) is a major risk factor in stroke, with 25% of ischemic attacks occurring in individuals with T2DM and T2DM diagnosis is associated with poorer functional outcomes and increased risk of recurrent stroke. Amylin, a peptide co-secreted with insulin from pancreatic Î²-cells, is hypersecreted in T2DM and readily forms neurotoxic oligomers which deposit in brain parenchyma.

Due to amylin's role in T2DM and T2DM's relationship to stroke, we anticipated an increased level of amylin would be deposited on red blood cells (RBCs) of stroke patients when compared to non-stroke patients. Additionally, we anticipated an increased level of amylin immunoreactivity (AIR) in clot lysates when compared to RBC lysates and plasma. Blood samples and thrombi (n=47) were collected from patients undergoing mechanical thrombectomies for stroke while blood samples (n=21) were collected from patients with non-stroke neurological conditions. Samples were lysed and assayed for total protein concentration and intensity of AIR. Amylin uptake coefficients (AUCs) demonstrating the proportionality of amylin deposited on RBCs compared to total circulating amylin were calculated.

After normalizing to total protein concentration, analysis revealed a significantly increased level of AIR in stroke clots when compared to stroke and non-stroke plasma and RBC lysates ($p<0.001$ for each). Additionally, a significant increase ($p<0.0073$) in AUC was found in stroke versus non-stroke.
In summary, amylin accumulates in thrombi and deposits on RBCs of stroke patients. Further research into amylin's potential role in thrombus formation is justified. Future studies are also needed to determine if stroke severity is associated with amylin level in thrombi and if T2DM exacerbates amylin-stroke pathology.

**PFOS exposure upregulates CD36 expression and induces translocation of CD36 to the plasma membrane of CD4+ T cells**

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Perflurooctane sulfonate (PFOS, an 8-carbon PFAS) is an environmental pollutant that has been detected frequently in the environment. Scientific literature suggests that PFOS exposure can have a negative impact on human health. According to published research, PFOS can promote immunotoxicity in the spleen and alter immune response. Although PFOS are regarded as immunological hazards for people, it is still unclear how they cause immunotoxicity. Our preliminary data showed that PFOS exposure increases the expression of the CD36 gene in CD4+ T cells in mice. CD36 is a scavenger receptor and an essential metabolic regulator of T cell metabolism in immune response. Therefore, the goal of the current study is to investigate if PFOS-induced alterations in the CD36-lipid metabolism axis contributes to immunotoxicity.

In this study, in vitro and in vivo settings were used to examine the involvement of a CD36-lipid metabolism axis after PFOS exposure in inducing immunotoxicity. In in vivo studies, C57BL/6 mice were given PFOS-contaminated water for 7 weeks. CD4+ T-cells were then isolated from splenic tissue of control and PFOS treated mice and qRT-PCR was used to measure expression of CD36. In vitro studies included T cell isolation and PFOS treatment in cell culture. Splenic CD4+ T cells were stimulated with anti-CD3+/CD28+ activation beads, treated with PFOS, and then analyzed by qRT-PCR and flow cytometry analysis. In vivo and in vitro data demonstrate the increase in CD36 mRNA expression in splenic CD4+ T cells from PFOS treated mice as compared to control C57BL/6 mice. Consistently, flow cytometry analysis suggests that exposure to PFOS leads to an increase in the CD36 level on the cell surface of CD4+ T cells. In summary, our studies demonstrate that PFOS exposure can contribute to increased CD36 expression in splenic CD4+ T cells. (Supported in part by NIEHS/NIH grant P42ES007380 and by UK-CARES grant P30ES026529)

**Activated skeletal muscle stem cells fuse to myofibers without first proliferating during hypertrophy**

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Background: Current dogma dictates that skeletal muscle stem cells (MuSCs) must first proliferate prior to fusing to myofibers during hypertrophic growth. In an effort to reevaluate this dogma, we generated an inducible transgenic mouse model that allowed us to definitively track MuSC following fusion to the myofiber together EdU labeling to detect MuSC proliferation. Single-cell RNA-sequencing (scRNA-seq) was also performed to investigate MuSC heterogeneity and dynamics during hypertrophy.

Methods: To track MuSCs following myofiber fusion, we crossed the satellite cell-specific Tet-ON mouse (Pax7-rtTA/+) with a tetracycline-response reporter mouse, the TRE-H2B-GFP mouse, to generate the Pax7rtTA/; TRE-H2B-GFP mouse designated the Pax7-GFP mouse. Following doxycycline administration (2mg/ml for 14 days) to GFP-label MuSC nuclei, Pax7-GFP mice underwent myotenotomy surgery to place a mechanical overload (MOV) on the plantaris muscle to induce MuSCs activation. Fluorescent-activated cell sorting was used to isolated MuSCs after 5 days of MOV for scRNA-seq analysis. To detect MuSC proliferation, EdU (25mg/kg/day) was continuously delivered via mini osmotic pump for the entire period of MOV. Immunohistochemistry (IHC) analysis was used to visualize MuSC derived GFP labeled myonuclei and EdU colocalization.

Results: Trajectory inference analyses of scRNA-seq results showed a trifurcating trajectory after MuSCs activation during MOV with one path predicted to bypass proliferation towards direct differentiation. IHC analyses show GFP-positive MuSCs derived myonuclei with no EdU incorporation at day 5 and 7, indicating that MuSCs are able to fuse to the myofiber without having to first proliferate.

Conclusion: Through scRNA-seq and IHC analyses, we have acquired the first evidence showing that MuSCs can directly fuse to myofibers in response to MOV independent of cell proliferation.

**Suppression of UGT1A1 in Obese Mice Using A Liver-Specific GalNAc-RNAi Improves Metabolic-Associated Fatty Liver Disease (MAFLD)**

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Several population studies have found lower serum bilirubin levels to be associated with the pathology of metabolic-associated fatty liver disease (MAFLD). Yet, treatments to target this metabolic trend have not been explored. In our study, we hypothesized that inhibition of hepatic UGT1A1 would increase serum bilirubin levels and improve conditions like MAFLD and hepatic insulin resistance. Therefore, we designed an RNAi targeting murine Ugt1a1 expression in the liver. The RNAi molecule was constructed by conjugating it with an amino sugar derivative of galactose, N-Acetylgalactosamine (GalNAc) [GalNAc-UGT1A1-RNAi]. GalNAc covalently linked to the siRNA enables delivery through the asialoglycoprotein receptor (ASGPR)-mediated targeting to hepatocytes in vivo. Hence, the treatment of the GalNAc-UGT1A1-RNAi primarily targets the liver. In this study, male C57BL/6J mice were fed a high-fat diet (HFD, 60%) for 30 weeks for diet-induced obesity (DIO) and were treated subcutaneously with GalNAc-UGT1A1-RNAi or sham control (n=6) once weekly the final 4 weeks while on the HFD. The results show that GalNAc-UGT1A1-RNAi treatment significantly raised serum bilirubin levels compared to sham control-treated, decreased hepatic fat content and hepatic triglycerides, and improved fasting blood glucose and insulin levels. We performed extensive kinase activity analyses using PamGene PamStation technology and found a significant increase in insulin receptor downstream mediators such as SRC, AKT, and ERK kinase families with the GalNAc-UGT1A1-RNAi treatment, indicating improved hepatic insulin sensitivity. These results show that GalNAc-UGT1A1-RNAi improves MAFLD, increases serum bilirubin, reduces its catabolized produce urobilin in the plasma, and reduces hyperglycemia and hyperinsulinemia. These indicate that UGT1A1 antagonism might serve as a treatment for MAFLD and hepatic insulin resistance, which could improve obesity-associated comorbidities.

**Glucocorticoid Receptor (NR3C1): A Crucial Mediator of the Human Periovulatory Process**

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Glucocorticoids are suggested to play a role in multiple aspects of reproductive functions through acting on its receptor, NR3C1. However, the function of glucocorticoids/NR3C1 in the ovary remains largely elusive. Our recent study demonstrated that the levels of cortisol, an active glucocorticoid, and the expression of NR3C1 were markedly increased after human chorionic gonadotropin (hCG) administration in human periovulatory follicles and forming corpus luteum in vivo and primary human granulosa/lutein cells (hGLC) in vitro. To further determine the function of NR3C1 in the periovulatory process, in the present study, we utilized an hGLC model that mimics in vivo upregulation of cortisol production and NR3C1 expression by hCG and a selective NR3C1 antagonist, CORT125281. The cells were cultured with or without CORT125281 in the absence and presence of hCG for 24 or 36 h. At the end of the culture, the cells and conditioned media were used for further analyses. Because the LH surge/hCG is known to increase steroid production and the expression of many genes in granulosa cells of preovulatory follicles, we measured the levels of cholesterol (a substrate for steroids), progesterone, and cortisol as well as the expression of key genes known to be critical for ovulation and luteinization. Our data revealed that antagonizing the activity of NR3C1 with CORT125281 reduced hCG-induced increases in the level of cholesterol, progesterone, and cortisol in conditioned media. Corresponding with these reductions, CORT125281 downregulated the expression of genes associated with cholesterol biosynthesis and steroidogenesis. In addition, the expression of key ovulatory genes was also reduced in hGLC treated with CORT125281. Together, these data revealed for the first time that NR3C1 plays a crucial role in the human periovulatory process by functioning as a critical transcription factor regulating the expression of genes involved in ovulation and luteinization in the human ovary.

**Glucocorticoid-driven Sgk1 signaling properties in young and aged dorsal and ventral hippocampus**

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Objective & Rationale

Glucocorticoid (GC) signaling is thought to play a key role in stress' negative impact on brain aging (BA). Several lines of evidence indicate that stress and stress hormone exposure appear to accelerate BA, yet the mechanistic interaction between the two remains unclear. The GC-dependent downstream effector molecule, Sgk1, is increased with both stress and BA. Sgk1's response to GC appears to be enriched in white matter, and may play a role in oligodendrocytic differentiation and morphological changes following stress exposure. Although, the organization of the hippocampus (HIP) is conserved throughout its dorsal-ventral (D-V) axis, the DHIP is thought to play a more prominent role in spatial navigation and short-term memory, while the VHIP provides feedback inhibition of the stress response. However, little work has examined Sgk1 expression across the D-V axis. Here, an ex vivo HIP slice preparation is used to test the hypotheses that: Sgk1 levels are elevated by age and GC exposure, this elevation is stronger in VHIP, aged animals show higher baseline Sgk1 than young, and young will show a greater dynamic response to GC exposure than aged.

Methods

HIP slices were mapped according to their position in the D-V axis and incubated in an interface chamber in either 0.1% DMSO or 3.5 ÂµM corticosterone for 2 hrs. RNA was then isolated, and concentration and integrity were assessed. Real-time PCR was then performed with forward and reverse primers for Sgk1. Whole transcriptome RNA sequencing was then performed using a dual indexed, paired end, strand-specific read-strategy.

Results & Conclusions

The well-established slice preparation also is appropriate for investigating age-related, GC-driven Sgk1 signaling. In this preparation, GC exposure is sufficient to drive Sgk1 mRNA expression in DHIP and VHIP in young and aged subjects. Further, adjustments to dose, duration, number of samples, as well as pre-vetting with EP may be appropriate.

**Nanopore long-read RNAseq enables precise RNA isoform discovery and quantification in human brains, with potential to reveal previously hidden transcriptomic signatures in Alzheimer's disease**

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Intro: Short-read sequencing studies identified genes that are differentially expressed in human Alzheimer's Disease (AD) brains, but due to technical limitations these studies did not perform differential RNA isoform expression. Long-read sequencing overcomes this limitation by sequencing entire RNA molecules in a single read, providing accurate quantification of each isoform within a given gene, including de novo RNA isoforms. With this technology researchers can now perform differential isoform expression analysis to determine if specific RNA isoforms within a given gene are associated with AD status or phenotypes. Methods: We sequenced 12 aged human frontal cortex samples (six controls and six AD) from postmortem tissue using nanopore long-read sequencing (Kit PCS111, Flow Cell 9.4.1). Each sample was sequenced with one PromethION flow cell. Data were base called using Guppy (v6.0.7). Reads were trimmed and oriented using Pychopper (2.7.1) and then aligned to the GRCh38 human reference genome using minimap2 (2.24). Transcripts were assembled and quantified with the Bambu R package (3.0.5). GitHub: https://github.com/UK-SBCoA-EbbertLab/brain_cDNA_discovery.

Results: We discovered 245 new gene-bodies and 428 new RNA isoforms in annotated gene-bodies. Of these 428 new isoforms, 49 are from medically-relevant genes such as MAOB. We identified 3309 gene-bodies expressing multiple RNA isoforms in a human frontal cortex, including genes implicated in Alzheimer's Disease such as MAPT(4), CLU(4), APP(5), PSEN1(5), and BIN1(7). These data demonstrate that AD risk genes are actively transcribing multiple RNA isoforms in one tissue, suggesting that the "single gene" could be performing multiple functions. Conclusions: Being able to perform differential isoform expression analysis in AD samples will be critical to understanding the role of each individual RNA isoform for a given gene. These findings could provide more precise molecular targets for future AD treatments.

**Rad5 and Its Human Homologs, HLTF and SHPRH, are Novel Interactors of Mismatch Repair**

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DNA mismatch repair (MMR) is the DNA repair mechanism that repairs base-base mispairs and small insertions or deletions remaining after replication. MMR is also required for apoptosis after certain types of exogenous DNA damage that result in damage-associated mispairs. The basic MMR mechanism is well understood; however, proteins associated with MMR continue to be identified, and the roles of these proteins in MMR are largely unknown. We have identified the yeast protein Rad5 as a novel interactor with the critical MMR proteins, Msh2 and Mlh1. Rad5 is a DNA helicase and E3 ubiquitin ligase involved in post-replicative repair. However, to date, Rad5 has no known role in MMR despite interacting with both two MMR factors. We show that deletion of yeast RAD5 does not have the mutation rate or mutation spectrum associated with defective canonical MMR. Rad5â€™s interactions with MMR are conserved throughout evolution and split between its two human homologs, HLTF and SHPRH, with human MSH2 interacting with HLTF and human MLH1 interacting with SHPRH. Loss of HLTF, SHPRH, or both does not affect canonical MMR. SHPRH knockdown with siRNA or knockout with CRISPR/cas9 induces moderate resistance to MMR-mediated apoptosis. We recently confirmed that our HLTF and SHPRH knockout cells affect survival after exposure to DNA damage that is a substrate for post-replicative repair versus MMR and are currently investigating whether the interactions between HLTF, SHPRH, and the MMR proteins influence post-replicative repair pathways or pathway choice. This study defines a novel accessory factor that binds with MMR proteins and is conserved throughout evolution. Additionally, this study provides a deeper understanding of how accessory proteins factors may provide a mechanistic distinction between canonical and non-canonical MMR and how MMR influences post replicative repair pathways.

**Nuances of Vertical Agarose Gel Electrophoresis and Resolving High-Molecular-Weight Proteins**

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The separation of high-molecular-weight proteins (>200 kDa) using polyacrylamide electrophoresis is difficult because gels with a large enough pore size for adequate protein mobility are mechanically unstable. A 1% vertical sodium-dodecyl sulfate-agarose gel electrophoresis system has been developed that allows Titin (a protein with the largest known isoform of 3,000–4,000 kDa) to migrate over >5 cm in a 10 cm resolving gel. Such migration gives clear and reproducible separation of Titin isoforms. Proteins ranging in size from myosin heavy chain (~220 kDa) up to Titin can be resolved on this gel system. We show this vertical agarose gel electrophoresis system has revealed three Titin size variants, one in rabbit soleus muscle (N2A) and two in human cardiac muscle (N2BA and N2B). Agarose electrophoresis should be the method of choice for separating and blotting proteins with very large isoform sizes. Our results indicated that this electrophoresis method efficiently studies the transitions in Titin isoforms. This method provides efficient protein extraction with urea-thiourea-glycerol buffer from hard tissues such as striated muscles. This method provides an efficient way to separate large proteins ranging from 200 – 4,000 kDa. Our method combines with silver staining to detect large protein isoforms and quantify the separated protein bands. This poster particularly focuses on the nuances of the technique, including sample preparation, incubation steps, gel assembly, and staining methods so that high molecular weight isoforms can be visualized.

**The Effects of Secretion Regulators, α-Synuclein and Cysteine String Protein-α, on Thrombosis and Hemostasis**

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Platelets use SNARE-mediated exocytosis to maintain vascular integrity via the release of three types of granules: dense, α, and lysosomal. To understand how the process of exocytosis is regulated, we probed the roles of α-synuclein and its binding partner Cysteine String Protein-α (CSPα) and assessed how they affect thrombosis and hemostasis. These abundant proteins are the only detectible isoform from its respective family in platelets. To address the role(s) of α-synuclein and CPSα in platelets. We examined the hemostatic phenotypes of α-synuclein-/- and CSPα-/- mice. The levels of platelet secretory machinery were assessed by quantitative western blotting. Secretion by platelets from these mice was measured using kinetic assays. Platelet aggregation and ADP release were examined with Lumi-aggregometry. Platelet activation was examined using cytometry. Localization of proteins in platelets was assessed by Super-Res Immunofluorescence microscopy. Hemostasis was evaluated in different injury contexts using tail-bleeding, FeCl3-induced carotid injury, and jugular vein puncture models. α-Synuclein-/- platelets had a mild dense granule secretion defect while α- and lysosomal granule release were unaffected. Tail-bleeding times for α-synuclein-/- mice were slightly increased compared to wild-type mice, but bleeding from CSPα-/- mice was significantly prolonged. Occlusion times in the FeCl3 carotid injury model and cessation of bleeding in the jugular vein puncture model were similar between α-synuclein-/- and wild-type mice. Localization studies indicate that α-synuclein and CSPα are both cytoplasmic and granular. These experiments demonstrate roles for both α-synuclein and CSPα in platelet secretion and hemostasis. These data fill gaps in our knowledge of both α-synuclein's and CSPα's physiological function and in our understanding of how platelet exocytosis is regulated. Funding: HL56652, HL 138179, HL150818, VA, and an NSF HRD 2004710.

**Human metapneumovirus recruits purine biosynthesis enzymes to phase-separated compartments to regulate viral replication**

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Human metapneumovirus (HMPV) is a respiratory virus that causes severe pathology in children, elderly, and immunocompromised individuals. It is a member of the Mononegavirales order, which contains non-segmented negative-stranded RNA viruses (NNSV). Replication and transcription of NNSVs occur in phase-separated compartments termed inclusion bodies (IBs). Formation of these phase-separated regions is driven by the phosphoprotein (P), which in combination with the nucleoprotein (N) and the viral RNA polymerase (L), carries out replication and transcription. L, N, and P are sufficient to induce viral RNA replication and transcription in-vitro and in-situ. However, it is not known how NNSVs switch between replication or transcription. Previous work shows that the pneumovirus polymerase L utilizes local GTP and ATP levels to switch between replication and transcription in-vitro. Therefore, HMPV's IBs may allow for local changes in nucleotide concentrations through the use of purine biosynthesis enzymes to regulate replication and transcription. Our data shows that GTP concentrations increase during the early stages of infection. Metabolomics analysis of infected cells also shows increased metabolic substrates for purine synthesis. Additionally, IMPDH and ADSS were found in proximity or colocalized in HMPV IBs using immunofluorescence microscopy, alluding to a proteomic switch of local nucleotide concentrations. IMPDH and ADSS knockdown using shRNAs slowed viral replication and transcription in cell culture. Additionally, replication and transcription were also inhibited in a dose-dependent manner by ADSS and IMPDH inhibitors when a minigenome system was used. The use of inhibitors for IMPDH also showed increased viral replication and decreased viral transcription in a dose-dependent manner. Together, these findings suggest that the recruitment of purine biosynthesis enzymes to IBs may be a key component for modulating viral replication and transcription.

**Sex-based differences in B cell subsets & functional recovery after stroke**

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Background: Brain-localized age-associated B cells (ABCs) occur during multiple sclerosis, performing pro- or anti-inflammatory roles depending on context, but their role(s) during stroke remain unclear.

Aim: To classify ABCs in the context of stroke using mouse and human tissue.

Method: Mice (n=60;19-28 mos) were randomly assigned to 30-min middle cerebral artery occlusion surgery or uninjured controls for either flow cytometry or histology. Mice underwent rotarod (motor coordination), open field (anxiety), and catwalk (gait) behavioral assays to examine functional recovery. Flow cytometry antibodies CD45, CXCR5, CD23, TCRÎ², CD27, CD11b, CXCR4, IgG2a/c, IL-21R, CD11c, CD80, CCR7, Mouse IgD, IgM, MHC II, CD138, and CD21/25 identified ABCs in splenic or brain-derived samples using high-dimensional clustering (UMAP). For histology, mouse (40Âµm coronal) or post-stroke human cortical tissue (20 µm) was stained with B220 (B cells), T-Bet (ABCs), and DAPI. Confocal/epifluorescent imaging occurred on a Nikon Ti2 or Zeiss Axioscan Z.1. Statistics performed with GraphPad Prism.

Results: Aged male mice (n=15) demonstrated motor coordination deficits (p=0.0029, 0.0006, and 0.0010) but no gait deficits through 3 weeks post-stroke. Aged females (n=15) showed no post-stroke deficits in either motor coordination or gait, but pre-stroke baseline was already worse (p=0.0001) than young controls (n=15). Clustering analysis comparing males vs. females showed varying B cell subsets (p=0.0001), including CD11bhi ABCs in both males and females, though females demonstrated higher subset variability. Histology confirmed T-Bet+ ABCs in cortical, hippocampal, and cerebellar regions post-stroke. ABCs were also in the brains of healthy aged mice (n=15), though without discernable patterns of diapedesis. Cortical ABCs were present in post-stroke in human parenchyma.

Conclusion: Our data are the first histological confirmation of ABCs in the aged brain pre- and post-stroke, with potential.

**Early life stress induces MR-dependent increases in circulating and adipose tissue-derived IL17a in female mice fed a high fat diet**

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Increased levels of pro-inflammatory cytokine Interleukin 17a (IL17) from adipose tissue (AT) is linked to hypertension, metabolic syndrome, and early life stress (ELS) in humans and mice. IL17 is secreted by T-helper 17 (Th17) cells downstream of mineralocorticoid receptor (MR) activation on dendritic cells (DC). In obesity, Th17 infiltrate and proliferate in AT. Factors secreted from perivascular AT (PVAT) directly modulate vascular function. Our previous data show that female mice exposed to maternal separation and early weaning (MS), a model of ELS, display exacerbated obesogenic response to high fat diet (HF) and the metabolic phenotype that is attenuated by MR antagonist treatment. Therefore, we aimed to determine if there is an MR-dependent role of IL17 from PVAT on endothelial function in obese female MSEW mice. Female MS and control (C) mice were weaned to HF (60% Kcal from fat) for 20 weeks, and randomized to receive vehicle (V, 50% Ora swift in drinking water) or spironolactone (Sp, 100 mg/kg/d in vehicle) treatment for 2 weeks. Plasma was collected to measure cytokines using milliplex assay (n=5 per group). Thoracic aortas were isolated and cleaned for vascular reactivity studies, and aortic and mesenteric PVAT (aPVAT and mPVAT) were collected in DMEM (2% BSA, 2-hour incubation, 37C). Cumulative concentration response curves were performed for acetylcholine (10-5 to 10-9 M) and sodium nitroprusside (1x10-6 to 1x10-14 M) after preconstriction with serotonin (2×10-3 M, 5 ul). Maximal vascular relaxation (VR) was similar in all groups, however, preincubation with mPVAT media impaired VR in MS-V mice (p<0.05), but not MS-Sp. Compared to C, MS mice showed increased plasma IL17 (65.8 ±16.8 MS-V vs 181.9 ± 39.4 C-V, p<0.05). This increase was blunted by Sp treatment (95.5±24.5 MS-V vs 105.1±21.4 MS-C, p<0.05). Levels of IL17 in PVAT media were similar in pattern to plasma, suggesting that infiltrating cells may contribute to impaired vascular function.

**A multiomics approach to deciphering the cellular responses of the brain in the context of aging, inflammation, and Alzheimer's disease.**

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Aging represents the strongest risk factor for the acquisition of many dementia-associated neurodegenerative diseases, especially Alzheimer's disease. In tandem, it is well appreciated that aging alone is sufficient to drive altered neuroinflammatory responses that may underlie neurodegenerative susceptibility. However, despite the convergence of these two critical predictors of brain dysfunction, little is known regarding how aging affects the underlaying epigenetic elements associated with cellular phenotypes. To address this, we utilized an mutliomics approach that allows the simultaneous quantification of gene expression and cis repones elements from single nuclei. In total, we harvested 60,000 high quality nuclei from young (3 months) and aged (18 months) male C57BL/6J wild type or 5xFAD cohorts that were treated with either saline (NaCl) or LPS. Together this provided models recapitulating conditions of advanced aging, systemically induced neuroinflammation and amyloid pathology as well as combination of these factors. Overall, our findings demonstrated that new methods to simultaneously and robustly harvest multiple cell types for use in mutliomic analysis approaches may offer new insights into the underlying cellular mechanisms associated with age-related neuroinflammatory responses.

**APOE Genotype Modulates Lipid Droplet Dynamics**

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Background: Aging microglia accumulate lipid droplets (LDs), secrete pro-inflammatory cytokines, and are defective in phagocytosis. The E4 allele of Apolipoprotein E (APOE) is the strongest genetic risk factor for late-onset Alzheimer's disease, and is associated with increased neuroinflammation and LD accumulation. We hypothesize E4 microglia have increased LD formation under basal conditions and a higher capacity to form LDs under stress, resulting in greater pro-inflammatory cytokine production. Further, we hypothesize that APOE genotype modulates the LD proteome and lipidome at baseline and with inflammation.

Method: Primary microglia from ApoE3 and ApoE4 mice were exposed to 250uM oleic acid (OA), 10ug/mL LPS, OA+LPS, dead N2A cells, or dead N2As+LPS, stained with BODIPY, and analyzed for LDs. For quantitative proteomics and lipidomics, we used liver tissue, which provides enough material for analysis. ApoE3 and ApoE4 mice were injected with saline (control) or LPS (5mg/kg) and perfused at 24h. LD-enriched buoyant fractions were collected after density gradient centrifugation and analyzed for -omics.

Result: Primary microglia from ApoE4 mice accumulated significantly more LDs at baseline, with OA, LPS, and N2As compared to E3 with reliable reproducibility. Western blots on LD fractions confirm LD enrichment via surface protein PLIN2. Proteomics revealed that LD fractions from E4 mice are enriched for proteins involved in innate immunity, while E3 LDs are enriched for proteins involved in lipid b-oxidation. Lipidomics showed an increase in phosphatidylcholine ratio in the LD membrane of E4-control and LPS-treated droplets.

Conclusion: E4 microglia accumulate more LDs compared to E3 microglia under all conditions tested. The proteomic profile of E4 liver LDs support the hypothesis that E4 expression increases inflammation under basal conditions, and upon stimulation, causes a more robust response.

**Characterization of Perivascular TAR DNA-Binding Protein 43 Microvasculopathy in Hippocampal Sclerosis**

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In the healthy brain, TAR DNA binding protein of 43 kd (TDP-43) maintains a strictly nuclear localization where it interacts with a large population of RNA and is therefore involved in transcription, splicing, and transport. However, mislocalization to the cytoplasm and abnormal phosphorylation (pTDP-43) results in the formation of intracellular inclusions. These inclusions are a major facet of several neurodegenerative diseases including, frontal temporal lobar degeneration with TDP (FTLD-TDP), and Limbic-predominant age-related TDP-43 encephalopathy (LATE-NC) both of which may lead to focal degeneration of the hippocampus /mid temporal gyrus, known as Hippocampal Sclerosis (HS).

Recent studies have reported the presence of perivascular pTDP-43-positive microstructures in the brains of patients with FTLD and DLB. Immunohistochemical staining and electron microscopy, suggests that while these microstructures showed variable reactivity for B-crystallin or glial fibrillary acidic protein (GFAP), they appeared in close proximity to GFAP-positive astrocytes, leading to the assumption that they are indeed associated with astrocytic end feet, and therefore may affect the integrity of the blood-brain barrier. However, little else is known about these micro-structures.

Using our method of high-volume multiplex staining and analysis (QUIVER), we began characterizing these TDP-positive structures in the brains of patients with hippocampal sclerosis. Concurrent with previous work, our work shows that these inclusions were rarely immunoreactive for GFAP staining, however, many of these inclusions were immunoreactive for a pan-microglial marker (Iba1), suggesting that microvasculopathy may be associated with macrophages and not astrocytes.

Using our QUIVER method of multiplexed staining, we identified and evaluated the location and density of pTDP-43 inclusions colocalized with Iba1 or GFAP in addition to other known markers associated with neurodegenerative diseases.

**Porcupine inhibition enhances the efficacy of Enzalutamide in drug resistant prostate cancer**

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Androgen receptor (AR) signaling continues to participate as a vital component of castration-resistant prostate cancer (CRPC). Subsequently, this has led to the development of Androgen Signaling Inhibitors (ASI), specifically Enzalutamide (ENZ), which is a direct inhibitor of AR, to clinically manage CRPC. Inevitably, ENZ treatment only provides improvement for approximately two months before advancing to an incurable form, ENZ-resistant CRPC. With PCa ranking as the second leading cause of cancer-related deaths in USA males, there is an urgency and necessity for the discovery and development of novel therapeutic approaches for CRPC. Wnt signaling has been extensively documented in its involvement in PCa and the tumor microenvironment (TME), however the mechanism of how the Wnt signaling cascades contribute to ENZ resistance is still ambiguous. Recently we have published that the activation of the canonical Wnt pathway contributes to the progression of ENZ resistance in CRPC and using a combination of Î²-catenin inhibitor with ENZ resulted in the synergistic inhibition of patient derived xenograft (PDX) tumor growth. Regarding the non-canonical Wnt pathway, we confirmed its contribution to invasion and migration which leads to metastasis in ENZ-resistant CRPC, and when the downstream effector ROCK1/2 is depleted and cells are treated with ENZ, there is a significant hindering of cell migration and invasion. Also, utilizing a combination therapy of ROCK1/2 inhibitor with ENZ synergistically inhibited the growth of PDX tumors. Hence the reasoning that by simultaneously inhibiting both the canonical and non-canonical Wnt signaling cascade will result in the inhibition of cell proliferation, migration, and invasion. The objective of the proposed research is to determine how Porcupine (PORCN) is associated with CRPC progression to ENZ-resistance, and develop a novel approach sensitizing ENZ-resistant CRPC to ENZ therapy, providing terminal patients with clinical options.

**Serotonin supplementation and response-based optimization of intermittent hypoxia procedure improve expression of respiratory neuroplasticity.**

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Spinal cord injury (SCI) most commonly occurs at the cervical level, rendering individuals unable to breathe. No current clinical treatment can restore independent respiratory function once lost, but intermittent hypoxia (IH) promisingly can induce some increased breathing motor output called long term facilitation (LTF). All IH protocols to date have been defined by periods of alternating exposure to pre-determined durations of hypoxic and non-hypoxic air irrespective of subjects' response. However, some subjects fail to express LTF and the mechanism underlying their unresponsiveness to Std-IH is not fully understood. Explanations are indicated by studies demonstrating that higher levels of subjects' hypoxic respiratory response (HRR) and intraspinal serotonin (5-HT) expression predict greater LTF magnitude. We propose that unresponsiveness to Std-IH can be addressed through targeting subjects' HRR and intraspinal 5-HT during IH treatment. We first examined if diaphragmatic HRR would predict LTF magnitude in uninjured rats as phrenic neurogram data suggest. We then evaluated a novel response-based IH (RIH) protocol in uninjured rats wherein hypoxic duration was defined by the magnitude of each subject's diaphragm HRR. Finally, we supplemented uninjured rats with intrathecal 5-HT during Std-IH, comparing LTF with saline-treated control. Indeed, our linear regression indicated a trend towards positive correlation of HRR and diaphragm LTF ($R2 = 0.2123$, $p = 0.1538$, $n = 11$). Consistent with our hypothesis, RIH treatment successfully induced LTF in 2/2 subjects (~19% above baseline) while Std-IH induced LTF in only 2/5 subjects (~9% above baseline). Finally, 2/2 5-HT-treated subjects exhibited LTF averaging ~5% above baseline whereas only 1/3 saline-treated animals exhibited LTF (~6% above baseline). Altogether, these data introduce interventions able to improve the consistency and magnitude of LTF, thus enhancing efforts to restore breathing function after SCI.

**PTPRF negatively regulates EGFR signaling to inhibit cell migration in colon cancer**

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The spaciotemporal control of cell signaling requires a balancing act of protein kinases and phosphatases. Hyperactivation of signaling downstream of receptor tyrosine kinases (RTKs) is one of the most common mechanisms leading to oncogenic transformation in numerous cancer types. Although the activation process of RTKs has been extensively studied, the inactivation mechanisms mediated by tyrosine phosphatase are less understood. Previously, we have determined the molecular mechanisms by which protein tyrosine phosphatase receptor type F (PTPRF) regulates the Wnt pathway. In this study, we investigated the functional importance of PTPRF in controlling EGFR signaling in colon cancer. Deletion of PTPRF using CRISPR/cas9 in 293T cells led to increased phosphorylation of EGFR and downstream AKT and ERK signaling upon EGF treatment. Similarly, knockdown of PTPRF resulted in an increase in EGFR activation in colon cancer cells. In addition, re-expression of WT, but not phosphatase deficient, PTPRF rescued the phenotype suggesting a phosphatase activity-dependent regulation. Co-Immunoprecipitation experiments indicated that PTPRF interacts with EGFR via its extracellular domain. However, PTPRF-mediated regulation of EGFR phosphorylation had no effect on EGF-induced receptor internalization. Functionally, knockdown of PTPRF promoted cell migration in colon cancer cells. The effect of PTPRF on controlling the specificity of signaling scaffolds downstream of EGFR is being further investigated. Taken together, our study identified PTPRF as an important regulator of EGFR signaling in colon cancer.

**FoxS1 is a Novel Mediator of Hepatic Fibrosis via Kinase Pathways and Transcriptional Control**

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The increasing rates of obesity have led to elevated rates of non-alcoholic fatty liver diseases (NAFLD), which can progress to non-alcoholic steatohepatitis (NASH) and liver fibrosis, liver cirrhosis, and possibly cancer. The hepatic stellate cells (HSCs) have been considered major players in fibrogenesis in liver damage that leads to irreversible scarring. The activation of HSCs occurs as a liver injury signal via transforming growth factor β (TGF-β), which then causes the cells to proliferate and shed their extracellular matrixes. FoxS1, a barely studied transcription factor, was recently found to be increased by tumor growth factor-β (TGF-β) signaling. However, the role of FoxS1 in liver fibrosis has never been demonstrated. Our Real-Time PCR data showed that FoxS1 mRNA was increased in human cirrhotic livers that have significantly higher TGF-β, as well as in a murine model of liver fibrosis. Therefore, we hypothesize that the TGF-β-induction of FoxS1 is an essential process of HSCs activation that leads to hepatic fibrosis and scarring. To test this hypothesis, we created a FoxS1 CRISPR knockout (FoxS1 KO) and scrambled control LX2 cell lines, which are the standard human HSCs used for studying hepatic fibrosis. Then, we performed TGF-β treatments and extracted RNA for RNA sequencing and bioinformatic analysis to determine genes and pathways that are altered by the loss of the FoxS1 transcription factor. Our data demonstrate that FoxS1 regulates genes involved in collagen-containing extracellular matrix pathways, indicating that suppression of FoxS1 might prevent liver scarring. However, more work is needed in animal models to make this determination. In conclusion, these data demonstrate that FoxS1 induces liver fibrosis and plays a critical role in TGF-β-induced human HSC-extracellular matrix production that occurs during hepatic scarring. Based on the findings, FoxS1 may serve as a new therapeutic target for liver fibrosis.

**Astrocyte Activity in the Dorsal Striatum Regulates Cue-Induced Reinstatement of Cocaine Seeking**

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The neuroscience community continues to face significant challenges in understanding cocaine use disorder (CUD). Recent literature indicates that astrocytes play an active role in drug seeking. Several of these studies, highlight that suppression of striatal astrocytic activity results in significant alterations in reinstatement of cocaine seeking, indicating their importance in regulating reinstatement. However, the effects of astrocytic suppression on neuronal signaling in CUD remain unclear. To investigate the roles of astrocytic suppression on behavioral patterns and neuronal activity we performed intracranial viral injections in the dorsal striatum in rats. Animals received injections of neuronal calcium biosensor, GCaMP6f, and “CalEx”, which suppresses astrocyte activity by continually extruding cytosolic Ca2+, or a sham injection of astrocyte specific orange fluorescent protein, tdTomato. Animals also underwent jugular catheterization for cocaine self-administration training. Following recovery, animals underwent cocaine self-administration, extinction, and cue-induced reinstatement. No significant alterations were observed between CalEx and tdTomato groups during self-administration or extinction. However, the suppression of astrocytic activity led to increase in cue-induced reinstatement. Subsequently, brain slices were collected from each animal for ex vivo calcium imaging. There were no significant differences observed in the duration and frequency of Ca2+ events between CalEx and tdTomato. However, the suppression of astrocytic activity increased amplitude of neuronal Ca2+ transients, an indirect measure of cell excitability. Furthermore, the addition of 10 μM cocaine HCl suppressed Ca2+ transients in both CalEx and sham animals. These findings reveal that suppression of astrocytes in the dorsal striatum increases cue-induced reinstatement by magnifying neuronal excitability.

**Role of Mitochondrial Bioenergetics in Platelet Function, Hemostasis, and Thrombosis**

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Platelets are one of the most metabolically active cells in the bloodstream. They show metabolic flexibility, switching between glycolysis and oxidative phosphorylation (OxPhos) depending on oxygen tension and the availability of substrates. Our goal was to investigate platelet energy metabolism under normal physiological conditions to better understand the relative roles of the major ATP-generating processes (glycolysis, OxPhos, and glycogenolysis).

Deletion of platelet glucose transporters (GLUT1 and GLUT3) revealed an essential role of glucose metabolism in hemostasis in vivo. Glucose can power platelets via glycolysis alone or in combination with OxPhos. We sought to probe the relative importance of platelet mitochondrial bioenergetics in hemostasis and thrombosis. Previously used mitochondrial inhibitors (antimycin, oligomycin) are toxic and cannot be used for in vivo studies, thus we developed two novel mouse models with altered mitochondrial function using a platelet-specific deletion of TFAM and QPC. TFAM (Transcription Factor A Mitochondrial), is essential for the maintenance, transcription, and translation of mitochondrial DNA. Its deletion is expected to disrupt platelet mitochondrial DNA, which encodes 13 subunits of OxPhos. QPC is a subunit of ubiquinol-cytochrome c reductase complex III. Its deletion is expected to disrupt Complex III, which is part of the platelet mitochondrial respiratory chain.

Both the KO animals showed an increased tail-bleeding time, an increased occlusion time in the FeCl3 carotid injury model, and delayed hemostasis in a jugular puncture injury model with significantly higher rebleeding indicating that mitochondrial bioenergetics is important for clot stability. Using two novel mouse models with dysfunctional mitochondrial bioenergetics, we show that OxPhos is dispensable for low-energy demanding platelet functions such as aggregation but is important for secretion, clot contraction, hemostasis, and thrombosis

**Role of Gut Microbial Metabolites in Colorectal Cancer**

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Interactions between intestinal epithelial cells, microbes, and metabolites in the gastrointestinal tract have substantial impacts on human health, including the development and progression of colorectal cancer (CRC). Here, we explore the link between intestinal dysbiosis, altered microbial metabolism, and oncogenic effects of bacterial metabolites. Metabolomic analysis shows characteristic changes at both the phyla and species level in the gut microbiomes of control, IBD, and CRC mouse models. RNAseq after treating cells with bacterial metabolites highlights upregulated genes and pathways involved in oncogenic processes, while microscopic analysis supports these outcomes. The combination of these results suggest that certain bacterial metabolites are capable of triggering cellular changes leading to the development of CRC.

**Targeting lipid metabolism to improve efficacy of BRAF-targeted therapy in colorectal cancer**

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Aberrant lipid metabolism is a hallmark of cancer associated with poor prognosis in colorectal cancer (CRC). Fatty acid synthase (FASN), a key enzyme of lipid synthesis, is overexpressed and potential therapeutic target in CRC. BRAFV600E is the mutation occurring about 10-15% of CRC cases. BRAF-targeted therapy is effective, but quickly developed resistance is an issue. Therefore, our central hypothesis is that inhibition of lipid metabolism will sensitize CRC cells to BRAF inhibitors and overcome acquired resistance. METHODS. We established HT29 cells and primary PT130 and PT2449pt cells resistant to PLX8394, a novel BRAF inhibitor. IC50 curves, PrestoBlue viability, CytoSelect™ 24-Well Cell Invasion, and TG Assays, Seahorse XF analysis, western blot, confocal microscopy, RNA-seq, and lipid analysis were used to evaluate differences between parental and resistant cells. Combination of PLX8394 and TVB3664 (FASN inhibitor) was tested on cell viability in parental and resistant cells. RESULTS. PLX8394 resistant cells have a higher IC50, increased cellular proliferation and invasion than parental cells. RNA-sequencing show a significant increase in FASN expression. Western blot analysis of resistant confirm upregulation of FASN and levels of triglycerides as compared to parental cells. Seahorse XF Cell Mito Stress Test shows that resistant cells forgo the Warburg effect and instead rely more heavily on oxidative phosphorylation. CONCLUSION. Our study demonstrates that resistance to BRAF inhibitors is associated with a significant increase in proliferation, metastasis, and upregulation of lipid metabolism. Our preliminary data suggests that an addition of FASN inhibitor to the standard regiment for BRAFV600E mutation positive patients can improve efficacy of these therapies. Additional screening of lipid metabolism-targeted therapies in combination with standard BRAF regiment is needed to develop more efficacious strategies for CRC patients with BRAF mutations.

**Skeletal Muscle Exosomal miR-1 Delivery to White Adipose Tissue in Response to an Acute Bout of Resistance Exercise**

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Exosomes are small extracellular vesicles that can serve as intercellular delivery vehicles, thus playing an important role in signaling. Our laboratory has previously shown miR-1, a muscle specific microRNA, promotes adrenergic signaling and lipolysis in adipose tissue in response to mechanical overload in a murine model. The aim of this study was to examine the effects of an acute bout of resistance exercise on miR-1 levels in skeletal muscle, white adipose tissue (WAT), and circulating exosomes in humans. Additionally, we interrogated exosomal subpopulations using tetraspanin surface markers (CD81, CD63, CD9). Our results demonstrate increased miR-1 in WAT in response to resistance exercise. qPCR was used to measure the abundance of the miR-1 primary transcripts (pri-miRNA-1) in WAT. This analysis revealed expression of pri-miRNA-1 in WAT was extremely low to undetectable and unchanged in response to resistance exercise. Moreover, subjects with high BMI (>30) had a distinct serum exosome profile characterized by a significantly lower relative abundance of circulating CD81+/CD9+ vesicles. High BMI participants also presented a significantly different response to exercise for circulating CD63+/CD81+ vesicles. Taken together, these findings indicate an increase in miR-1 delivery to WAT in response to exercise, potentially promoting metabolic adaptations, although increased adiposity may affect exosome biogenesis. However, the source of the increased miR-1 in WAT requires further investigation.

**Length Dependent Properties of Human Left Ventricle and Atrial**

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The Frank-Starling relationship describes the how increasing ventricular filling leads to a proportional increase in cardiac output. This allows for the heart to compensate for changes in preload and increase stroke volume when needed. The Frank-Starling mechanism is a fundamental concept; however, the underlying molecular mechanisms are not fully understood. The cell-level correlate to the Frank-Starling mechanism is length dependent activation wherein an increase in sarcomere length results in increased calcium sensitivity and maximum tension. Here we show length dependent activation of human left ventricle but also the effects of sarcomere length on the rate of tension redevelopment. The rate of tension redevelopment describes the rate at which the myosin heads are able generate force and the kinetics of their attachment, cycling, and detachment. We show with experimental and computational data that at short sarcomere lengths the rate of tension redevelopment is faster than at longer sarcomere lengths. While the bulk of our work has focused on the left ventricle, we also have begun to investigate the length dependent properties of the left atria which has received far less attention. The left atria has an increased proportion of alpha-myosin compared to the ventricle making the myosin kinetics nearly twice as fast. We are continuing to investigate how these changes in the kinetics of atrial myosin alter length dependent properties of the muscle.

**Temporal Dynamics of B cell Diapedesis after Traumatic Brain Injury in Mice**

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TBI is a leading cause of mortality and morbidity for young adults. Survivors of moderate to severe TBI often face persistent cognitive and neurobehavioral deficits. Repeated failures of clinical trials targeting neuronal injury mechanisms have led to expanded efforts to understand the role of other cell types in the complex secondary injury cascade initiated by trauma. The roles of astrocytes and microglia in driving neuroinflammation are now well established, as are contributions of systemic innate immune cells such as neutrophils and monocytes. Much less is understood about the adaptive immune response to TBI. Although clinical studies describe engagement of systemic adaptive immunity, a significant gap in knowledge exists regarding the timing and extent of B cell diapedesis into the brain after TBI and the role of B cells in posttraumatic neurodegeneration or neuroplasticity. Existing studies in experimental TBI are limited largely to a single timepoint. We hypothesize that TBI triggers delayed B cell diapedesis into the cortex following a cortical contusion injury. To test this hypothesis, tissues collected from adult mice euthanized 1, 3, 7, 14 or 28 days after receiving controlled cortical impact TBI or sham injury were immunolabeled with the B cell antibody B220. Our data demonstrate a small number of B220+ B cells within the contused cortex at 1 and 3 days, increased numbers at 7 and 14 days, and few cells at 28 days. Future studies will characterize morphological and phenotypic characteristics of B cells within the injured brain to gain insight to their potential function.

**Dysregulated Neuroimmune Response in Amylin-Induced Type-2 Diabetic Neurodegeneration**

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Type-2 diabetes is a metabolic disorder that increases the risk for dementia. Studies suggest this risk is associated with elevated blood levels of amylin, an amyloidogenic hormone synthesized and co-secreted with insulin. We have shown that overexpressing human amylin (HA) in the pancreas of rats (HIP rats) leads to cerebral amylin vasculopathy and neurological deficits. Here we tested the hypothesis that neuroinflammation caused by cerebral amylin vasculopathy leads to a dysregulated neuroimmune response. Methods: We conducted RNAseq analysis on the brains of 16-month-old male HIP rats and wild-type (WT) rats (n =10/group). Brain homogenates from WT and HIP (n =7/group) rats were used for western blot to determine leukocyte trafficking protein levels. Immunohistochemistry (IHC) was conducted on the brains of WT and HIP (n=10/group) rats to determine the activation of resident immune cells. We conducted flow cytometry on the blood, spleen, and brains of WT and HIP rats (n = 9/group) to determine immune cell populations. Finally, to determine the acute neuroimmune response to (HA), WT rats were injected with PBS or aggregated HA peptide (n=5/group), then flow cytometry was conducted in the blood, spleen, and brain. Results: In the brains of HIP rats, RNAseq analysis shows significantly altered pathways important for neuroinflammation and immune cell signaling compared to WT. We also saw significant changes in proteins regulating leukocyte trafficking (e.g., VCAM-1, ICAM-1) and activated microglia via IBA-1 and CD68 co-stain. We also see significant changes in $CD45^+$ immune cell populations in the spleen, blood, and brain. Finally, in rats injected with HA, we see significant changes in $CD45^+$ immune cell populations in the spleen and blood with no significant changes in immune cell populations in the brain. These data suggest that increased pancreatic amylin levels in the circulation contribute to diabetes-related neuroimmune dysfunction in the periphery and brain.

**Neuroprogesterone and Chronic Psychosocial Stress**

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Chronic psychosocial stress has deleterious effects on long-term health and exacerbates many age-related conditions. Further, many chronic psychosocial stressors are unavoidable and can negatively affect large swaths of the population, dramatically increasing life-time risk for problems in multiple organs and tissues. Despite years of research, there are no FDA approved pharmacotherapeutic treatments for the brain-related consequences of chronic psychosocial stress exposure. In preliminary data, we have found that exogenously administered progesterone (P4) can reduce glucocorticoid (a key stress hormone in mammals) action in brain tissue. However, the degree to which circulating P4, and neurosterioid P4, may influence the brain's stress response has not been investigated. I hypothesis that augmenting neuroprogesterone synthesis will decrease, while knocking down synthesis will exacerbate, the impact of glucocorticoid/stress exposure in brain tissue.

To test this, we will use adeno-associated virus (AAV) technology to manipulate 3BHSD1 expression (the rate-limiting step in conversion of pregnenolone to progesterone) in astrocytes of animals that have or have not been exposed to behavioral stress. The work will target the entorhinal cortex (EHC), an underexplored brain region involved in coding for the temporal order of memory, and one of the stress-sensitive areas of the brain. To further describe progesterone's contribution to the stress pathway, following microinjections, female F344 rats aged 3 and 18 months will undergo a chronic stress paradigm for 3 months. Cognitive behavioral assessment (MorrisWaterMaze), blood plasma corticosterone, and the estrous cycle will be tracked throughout. Post mortem, EHC transcriptional profiles, ISH (RNAscope) and IHC, and mass spec will be done. Support for the hypothesis would include observations that augmented neuroprogesterone blunts, and knocked down neuroprogesterone strengthens, the brain's response to behavioral stress.

**Evaluating motor and cognitive recovery in aged post-stroke mice via novel reward without food deprivation**

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Background: The measurement of cognitive function in rodents is primarily accomplished using food rewards (e.g. milkshake, sucrose pellets). However, using these rewards requires food deprivation to increase motivation. Food deprivation has been shown to be detrimental to recovery, which impacts post-stroke cognitive functioning and is exacerbated in aged animals. Peanut oil, however, has been used as a reward in reach chambers to test motor function without prior food deprivation.
Aim: We hypothesize that both young and aged animals will continue to learn and perform lever pulls and cognitive touchscreen tasks using a peanut oil reward, allowing the measurement of post-stroke cognitive function without the detrimental effects of food deprivation.

Method: Young (5-12 mos.-old) and aged (16-24 mos.-old) female mice with and without focal ischemic stroke are trained on one of two cognitive touchscreen tasks: paired associate learning (PAL) or autoshaping (AUTO). Following baseline task acquisition, mice undergo a stroke using the SIMPLE model (PMID: 27941784). Task acquisition to evaluate acute cognitive function beginning at 3 days post-stroke. Mice were also trained on a precision forelimb reach task for measurement of fine motor function (PMID: 31889008).

Results: In a cohort of 9 female mice that began training at 13 mos.-old on an operant reach task using peanut oil, 4 mice attempted reach tasks over 75 times within a 1-hr. session. Additionally, mice continued to pull for 5 months, indicating that peanut oil is a desirable reward for aged animals without food deprivation, and can be used for weekly long-term testing over months. Cohorts of aged mice are currently being trained on PAL and AUTO.

Conclusions: With peanut oil successfully being used as a reward for operant tasks, the implementation of this reward for post-stroke cognitive testing is promising, as it allows for long-term evaluation without introducing confounds due to food restriction.

**A comprehensive and optimized protocol for the production of the EcoHIV-1 and essential quality controls**

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Advances in science and HIV1 awareness made HIV1 infection a controllable chronic health issue with increased survival. At the end of 2020, 38 million individuals were living with HIV1, a ~24% increase relative to 2010. To keep HIV1 under control, these patients must adhere to their combined Antiretroviral Therapy (cART) for the rest of their lives as there is no cure. Naive and cART treated HIV1 infected individuals have a higher risk of developing cardiovascular diseases. So, regardless of the cART therapy, HIV1 patients are at risk of developing neurodegenerative and cardiovascular complications at advanced stages of the disease. Therefore, there is an urgent need to identify the main biological players in HIV-1 infection. Currently, there are several models to study HIV-1 and among them is the EcoHIV-1 system. In this system the coding region of gp120 of HIV-1/NL4-3 and HIV-1/NDK was replaced with the gp80 coding sequence of the ectotropic Murine Leukemia Virus (MLV). This modification makes this virus safe to work with as it is not capable any more to infect humans, but capable of infecting rodents only. The EcoHIV-1 model was first described in 2005 and has been widely used to safely investigate HIV-1 vaccines, therapy and HIV-1 related pathogenesis. The current caveat in the EcoHIV-1 model is the lack of standard protocol for viral production and the abscess of quality controls to calculate the viral titer and check the infectivity of the viral preparations. Hence, we aimed to optimize a comprehensive protocol for the production of the EcoHIV-1 and what are essential quality controls that should be used to calculate the virus titer and how infective the virus preparations are. We have successfully demonstrated that we can make infectious EcoHIV-1 in our lab and inject it into different mice strain. In addition, we have successfully detected the virus RNA in the spleen and liver of the infected mice.

**Cancer Reprogramming Via Production of Radiation-derived EVs Containing Mitochondria**

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In spite of extensive efforts, cancer recurrence after radiation therapy(RT) remains one of the significant challenges in the cure of localized prostate cancer(PCa). This study focuses on elucidating a novel adaptive response to RT that could contribute to cancer recurrence. We used PC3, an adenocarcinoma established from a bone metastasis of prostate cancer, and a radio-resistant clone 695, which regrew in vitro after 66Gy radiation (33 x 2Gy) and subsequently regrew in nude mice after exposure to 10Gy radiation (5 x 2Gy). At the single-cell level, confocal microscope images coupled with IMARIS software demonstrate an increase in mitochondrial mass and mitochondrial membrane potential of clone 695. Utilizing a Seahorse XF96 instrument to investigate mitochondrial respiration, clone 695 cells demonstrated a higher basal oxygen consumption rate (OCR), ATP-linked OCR, and proton leak compared to PC3 cells. The elevation of mitochondrial function in clone 695 cells is accompanied by an increase in H2O2 production. These data suggest that prostate cancer cells reprogram their mitochondrial homeostasis, which allows the cancer to survive and regrow after RT. In elucidating how PCa reprograms its mitochondrial homeostasis, we found that RT induces the release of extracellular vesicles (EVs) from PC3 cells (1.2x fold increase). Importantly, these RT-derived EVs carried higher levels of mitochondrial antioxidant proteins, including manganese superoxide dismutase, Peroxiredoxin 3, Glutathione Peroxidase 4, as well as mitochondrial oxidative phosphorylation proteins. Elevated EVs production was also observed in PC3 cells treated with H2O2. In addition to activating EVs release, RT promotes EVs uptake in PC3 cells. Significantly, adding isolated functional mitochondria 24hrs prior to RT showed a two-fold increase in cell survival of PC3 cells. Together, our findings indicate that EVs carrying mitochondrial contents are a novel mediator for cancer reprogramming and recurrence.

**Rare Coexistence of Spontaneous Coronary Artery Dissection and Takotsubo Syndrome in Two Middle-Aged Females**

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Takotsubo syndrome (TTS) is a transient heart failure syndrome occurring predominantly in females often proceeding a stressful event.1 It represents 2-3% of the emergency department visits for acute coronary syndrome (ACS). Spontaneous coronary artery dissection (SCAD) is a rare and under-diagnosed etiology for ACS that also most commonly affects female gender. Very rarely, these two conditions may co-exist making the diagnosis and management highly complex.2 From our University of Kentucky Takotsubo registry, we present 2 cases of middle-aged women presenting with acute coronary syndrome, with a left ventricular wall motion pattern characteristic for TTS but with subsequent angiographic evidence of SCAD of left anterior descending artery.

**Effect of Sleep Enhancement via Rocking on the Mouse Model of Humanized APPxPS1 Knock-in Induced Alzheimer's Disease**

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Rhythmic passive movement, or "rocking," has been shown to enhance sleep, particularly in infants and young wild type (WT) mice. Rocking can be a potential tool for sleep enhancement because of its added benefits of being non-invasive and easily translatable to humans. Furthermore, sleep enhancement tools will be more valuable to the elderly or patients with sleep disturbance, which is a pervasive feature with age-related dementia, the most well-known of which is Alzheimer's disease (AD). Meanwhile little is known about rocking as a sleep-enhancement tool on AD and the older population in general. Therefore, our study explores the impact of rocking on sleep architecture in both APP/PS1 mice (which mimics the human AD condition) and WT mice.

IACUC approval at the University of Kentucky was obtained for all experimental protocols. C57BL/6J (WT) and APPxPS1 (AD) male mice (Age:10-12 months old) were kept under a 12h:12h light/dark cycle with access to food and water ad libitum. A flat motion platform with reciprocating elliptical movement (HS-260 control, IKA Shakers) was utilized to laterally rock individually housed mice at a frequency of 1Hz for nine hours (9 AM - 7 PM) during the light period. EEG/EMG data was collected on baseline days, rocking days, and post rocking days. We gathered at least six days of baseline, rocking, and post rocking data from both APP/PS1 and WT mice. Vigilance states (Wake, NREM, REM) were scored in 4-second epochs from the EEG/EMG data using a standard criterion. Mean bout duration and time spent in each vigilance state were calculated and compared in both AD and WT mice.

While a trend emerged towards an effect of rocking on some parameters, we did not observe any statistically significant effects. Rocking appeared to increase the % time spent in total Sleep, NREM, and SWS (slow wave sleep) in particular, but a decrease in % REM compared to baseline and post-rocking days.

**Estrogens and olfactomedins induce a feedback loop that increases nicotine consumption in females via the brain reward circuitry**

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Estrogen has been shown to drive nicotine use in women, making them more vulnerable to nicotine use disorders. In ovariectomized (OVX) female rats, estradiol (E2) treatments increased estrogen receptor β (ERβ) and not ERα in the main brain reward region, the nucleus accumbens core (NAcore). Therefore, we hypothesized that ERβ might be important in the reward circuitry for regulating nicotine desire, heightening nicotine use in females. To determine ERβ-specific functions that might regulate nicotine desire, we analyzed large sequencing datasets of estrogen-induced genes and narrowed the list to those expressed in the brain and that have a hormone function. We found one class of genes known as the olfactomedins (OLFMs), which meet these criteria. Therefore, we wanted to determine whether ERα or ERβ regulates the expression of the OLFM isoforms. We used human uterine cells (Ishikawa) that express both ER isoforms and the OLFMs and found that two-hour estradiol (E2) treatments induced the expression of OLFM1 and OLFM3 isoforms. However, nicotine only suppressed the E2-induction of OLFM1 and not the others. We next performed chromatin immunoprecipitation (ChIP) assays with these cells and treatments using antibodies for ERα, ERβ, or GFP control. We found that the OLFM1 promoter was only enriched by ERβ, and no binding of ERα was observed. We next treated OVX female rats with E2, nicotine, E2+nicotine, or vehicle and found that Olfm1 and Olfm2 significantly increased in the NAcore, and all three OLFM isoforms were significantly higher in the ventral tegmental area (VTA). Nicotine suppressed the E2-induced OLFM expression in the NAcore and VTA. These new findings suggest that ERβ-induction of the olfactomedins might serve as a feedback loop for driving desire, which is suppressed when the reward is provided. Hence, we may have uncovered a completely new treatment paradigm to control addiction.

**Neurotoxicity of 1-deoxysphingolipids**

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1-Deoxysphingolipids are an atypical sphingolipid class generated when the first enzyme of the sphingolipid biosynthetic pathway, serine-palmitoyltransferase, utilizes L-alanine as a substrate (Fig 1). L-Serine is the substrate for generating canonical sphingolipids. 1-Deoxysphingolipids lack a functional OH group and as a result, they can neither be converted to complex sphingolipids nor be metabolically degraded as the canonical L-serine-derived sphingolipids. This leads to their accumulation if produced in excess. Increased levels of 1-deoxysphingolipids were implicated in hereditary sensory autonomic neuropathy type 1, diabetic neuropathy, and chemotherapy-induced peripheral neuropathy.

Here, we tested the toxicity of individual 1-deoxysphingolipids species in neuroblastoma cell lines SMS-KCNR and Neuro-2a (N2a), followed by morphological monitoring and analysis. In addition, we studied the affection of 1-deoxysphinganine on the activation state of the actin cytoskeleton regulators RhoA and Rac1.

**Outcomes of the Markey STRONG Scholars Program in Increasing Diversity in Cancer Research and Health Science Careers**

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Participation in research is a widely used tactic for mitigating the challenge of increasing the representation and persistence of minoritized groups in the health sciences. Yet, few undergraduate research training paradigms have programming that collectively address the dramatic inequities in education, opportunity, and proactive mentorship experienced by these students. The Markey Science Training in Research, Oncology, Networking and professional Growth (STRONG) Scholars Program aims to increase the number of individuals who are from underrepresented and/or underserved backgrounds in cancer research through use of inclusive and equitable training practices, as well as boost its participants' scholarly confidence and promote their persistence in their chosen health science careers. Specifically, this summer program provides minoritized undergraduate students with research and clinical experiences, cancer education, personalized mentoring, outreach opportunities, social activities, and personal and professional development. Pre- and post-program surveys and focus groups were developed and employed to assess program outcomes and identify ways to maximize participant interest in cancer and persistence in health science careers. Analysis of mixed methods evaluation data demonstrates that Markey STRONG Scholars Program participation resulted in students' increased understanding of cancer and the scientific process along with pronounced interest in research. Additionally, program participation enhanced students' health science professional identity and sense of belonging to the health science community.

**Development of a Fluorescent Microscope Setup to Measure Myosin Conformations**

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The blood is an essential part of the circulatory system, acting as a medium for molecular exchange. The collective contraction and relaxation of cardiomyocytes change the volume of the ventricles and pump the blood. Myosin heads, bound to actin, go through power stroke to generate force. It was shown that not all available myosin motors contribute to force generation. Myosin heads can be found in a disordered relaxed state (DRX), ready to bind actin, or a super relaxed state (SRX), where actin binding is prohibited. In addition to their functional state, DRX myosin heads turn over ATP 10 times faster than SRX myosin. The dynamic equilibrium of force-generating, DRX, and SRX myosin is disturbed in cardiovascular diseases. Here, we present the development of a fluorescent microscope setup to measure the proportion of myosin conformations and the collected pilot data. The experimental protocol involves a fluorescent ATP analog, mant-ATP. It emits light following excitation with an ultraviolet light source. The mant-ATP is chased by non-fluorescent ATP. Then, the decay in fluorescence is analyzed to find the myosin proportions. The experimental setup includes an inverted microscope, an area scan camera, and an LED light source. A custom-written MATLAB pipeline is used to control the hardware, collect the images from the camera, and analyze the images using image segmentation. The experimental setup will identify the differences in SRX/DRX ratio in diseased human myocardium compared to donors in future experiments.

**Human HLA-A2 molecule is able to activate protective CD8+ T cells capable of conferring a protection against reactivation of cerebral infection with Toxoplasma gondii**

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Reactivation of chronic infection with T. gondii causes life-threatening toxoplasmic encephalitis (TE) in AIDS patients. We previously identified an importance of IFN-γ production by CD8+ T cells for preventing TE. Notably, once the CD8+ T cells are effectively activated in the presence of CD4+ T cells, the primed CD8+ T cells can prevent TE without further depending on CD4+ T cells. Thus, if we develop a method that efficiently activates the protective CD8+ T cells in individuals co-infected with HIV and T. gondii before their CD4+ T cell counts decrease, those primed CD8+ T cells will be able to prevent TE even when their CD4+ T cell counts decrease later. CD8+ T cells recognize their target antigens presented by the MHC class I molecules (MHC-I), and the HLA-A2.1 is one of the most common MHC-I molecule in humans. Thus, we examined whether HLA-A2.1 can activate CD8+ T cells capable of providing a protection against TE. We found that T. gondii-infected transgenic mice expressing human HLA-A2.1 have significantly lower cerebral T. gondii loads than did wild-type (WT) mice. When their CD8+ T cells were transferred to infected immunodeficient NSG mice expressing HLA-A2.1, the transgenic T cells conferred a significantly greater protection against reactivation of the infection than did WT T cells in association with greater expressions of IFN-γ and effector molecules against tachyzoites in the former than the latter. These results provide a valuable foundation for developing an immunological intervention to prevent or reduce the development of TE in HIV-infected individuals.

**High Mobility Group Box 1 Inhibition by Antisense Oligonucleotide Attenuates Angiotensin II-induced Abdominal Aortic Aneurysm**

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Vascular inflammation is a hallmark of abdominal aortic aneurysm (AAA). However, underlying mechanisms that initiate inflammatory pathways in the aorta are not clearly elucidated. Here, we explore the role of high-mobility group box 1 (HMGB1), a highly conserved nonhistone DNA-binding nuclear inflammatory molecule in AAA.

To determine the molecular signature and biological processes involved in AAA, we analyzed RNA sequencing (RNA-seq) data of patients with AAA (GSE57691) and mice with angiotensin-II (AngII) infusion (GSE17901) downloaded from the GEO database. Gene ontology analysis revealed that the major differences in enriched genes between the two groups involved inflammatory responses and cytokine-cytokine interaction. Both human and murine gene expression profiles indicated a marked upregulation of HMGB1 in the diseased abdominal aorta. AngII infusion also revealed a marked increase of HMGB1 in the abdominal aorta of male LDLr-/- mice after 7 days. mRNA abundance of genes related to HMGB1 secretion was altered in diseased tissue relative to the control, as defined by RNA-seq. To explore the role of HMGB1 in AAA formation, we performed mouse in vivo studies with genetic manipulation in HMGB1, utilizing a novel antisense oligonucleotide (ASO) approach to inhibit HMGB1. Proprotein convertase subtilisin/kexin type 9 (PCSK9)-induced hypercholesterolemic male mice fed a Western diet were infused with AngII (1,000 ng/kg/min) for 4 weeks to induce AAA. Mice (N=15/group) were subcutaneously injected with phosphate-buffered saline (PBS) or HMGB1 ASO (50 mg/kg) at days 0 and 3 in the initial week and then once a week during the remainder of the study. There was no difference in blood pressure between the two groups in response to AngII. However, aortic ex-vivo analysis indicated that the HMGB1 ASO profoundly attenuated AngII-induced AAA. AngII-induced expansion of ascending aorta area was also markedly reduced in the ASO group.

**Platelet secretion in wound healing: the essence of α-granule endocytosis, biogenesis, and release kinetics**

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Wound healing is a multi-step process involving different cell types and growth factors. Platelet-rich plasma has been used to accelerate wound repair, but functional understanding of how platelet functions affect wound healing is limited. I investigate the role of platelet secretion in a full-thickness wound model. Dorsal full-thickness excisions were made on mice defective in platelet α-granule biogenesis, endo- and exocytosis, and C57BL/6J control mice. Wounds were measured daily to assess healing. On days 3 and 7, wound sites were harvested for histology. Bioactive molecules were analysed from extracts prepared from the wound tissue. Nbeal2-/- mice have defective platelet granule biogenesis and cargo packaging, and showed severely impaired wound healing with distinctive wound morphology. The platelet-specific Arf6-/- and VAMP2/3Δ mice, with improper endocytic trafficking and fibrinogen uptake, also showed slower wound healing. Interestingly, Munc13-4Jinx mice, having no dense and delayed α-granule release, presented with faster healing, specifically in the first days. In histological wound sections, Munc13-4Jinx and Serglycin-/- mice resembled wound healing and morphology of C57BL/6J mice. Delayed wound healing progression in Nbeal2-/- mice was reflected by slower scab development and removal. Over the course of wound healing, levels of bioactive molecules changed. Most of the ones examined decreased during wound resolution, except FGF2 and MMP-9, reflecting wound remodelling. These trends were altered in Nbeal2-/- mice, where FGF2 and MMP-9 levels decreased as the wounds healed. The correlation slope between wound resolution and IL-1β, MMP-3, TIMP-1, and VEGF levels was less steep or even flat in Nbeal2-/- mice compared to the other strains. We show that platelet functions have specific roles in wound healing progression and may impact the presence and levels of bioactive molecules in wounded tissue. Supported by the AHA (1020159), NIH/NHLBI (HL150818), and VA.

**Dim light at night blunts the daily rhythms in heart rate and core body temperature in male mice**

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Objective: The 24-hour light-dark cycle entrains the circadian clock in the suprachiasmatic nucleus (SCN). The absence of darkness during the 24-hour cycle, specifically dim light at night (dLAN), has been shown to dampen circadian clock signaling in central and peripheral tissues. We tested the effect that mice housed in dLAN had on the heart rate (HR) core body temperature (Tb), and activity.

Methodology: Wild-type male mice (n=5) were implanted with telemetry devices to continuously record heart rate (HR), core body temperature (Tb), and activity in thermoneutral conditions. The mice were initially housed in a 12 h light: 12 h dark cycle (LD, 200 lux: 0 lux) with ad libitum access to food (ALF) for 7 days. Mice were then subjected to dLAN (200 lux: 5 lux) for two weeks (hour 0: lights on; hour 12: lights off).

Results: Feeding analysis showed that mice housed in dLAN significantly ate food during the light cycle compared to mice housed in LD mice. The HR, Tb, and activity had a significant 24-hour rhythm in mice housed in LD and dLAN. However, the mice housed in dLAN had a smaller amplitude in HR and Tb. Specifically, compared to mice housed in LD, mice housed in DLAN showed a 33% and 49% reduction in the amplitude of heart rate after the first and the second week, respectively. Similarly, there was an 18% and 69% reduction in the Tb after the first and second week of being housed in dLAN. dLAN abolished the differences between day and night in both HR and Tb. Interestingly, mice housed in dLAN showed a 2-3 hour phase advance in the peak amplitude (acrophase) in the HR, Tb, and activity as compared to mice housed in LD. There were no differences in the mesor (mean) for HR, Tb, or activity measure from mice housed in LD or DLAN.

Conclusions: These results showed for the first time that dLAN reduces the daily rhythms in the feeding behavior and subsequently diminishes the day-night differences in the HR and Tb.

**Delineating the effects of Perfluorooctanesulfonic acid in normal intestinal tissues and adenoma organoid models**

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Being contaminant in the drinking water, PFOS can accumulate in the intestine, thus, modulating intestinal homeostasis under physiological and pathological conditions. Although some studies have highlighted the potential mechanisms of PFOS in cancer initiation and progression, there is not much known about the effect of PFOS exposure on normal and pre-malignant intestinal tissues and its contribution to carcinogenesis. Therefore, the goal of this study is to investigate PFOS effects on normal and pre-malignant intestinal epithelium. The effect of PFOS and/or diets supplemented with inulin or pectin on gene expression profiles was assessed by RNA-Seq analysis in intestinal tissue of C57BL/6 mice. Tumor organoids were established from Apc/VillinCre and ApcMin (adenoma models). Colorectal cancer (CRC) cell lines, and human CRC liver metastasis tissue were used to assess the mechanisms involved in PFOS-induced intestinal alterations. Using RNA-Seq analysis, we have identified that PFOS exposure resulted in transcriptome alterations with exacerbated changes in pathways involved in lipid metabolism and immune system regulation. The volcano plot analysis highlighted the decrease in HMGCS2 and the increase in VEGFR expression in intestinal cells exposed to PFOS. Also, PFOS-induced upregulation of FASN and PDL-1 expression levels, in Apc/Cre and ApcMin organoids. Using FASN knockout cells and TVB-3664, a FASN inhibitor, we identified the possible role of FASN in PDL-1 regulation. In summary, our data suggest that PFOS may contribute to upregulation of de novo lipid synthesis and mediate tumor immune escape to promote transition from adenoma stage to colorectal cancer and, thus, contribute to initiation of CRC.

**Invention of a Novel Minimally Invasive Compact Artificial Heart**

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Purpose: Our goal is to develop a compact, single port, pulsatile artificial heart that only requires a minimally invasive single cannulation.

Methods: Our artificial heart consists of a valved single lumen cannula (VSLC) and a valveless single port diaphragm displacement pump (spDDP). Via a small left thoracotomy, the VSLC is inserted from heart apex through LV to aorta. Four one-way inlet valves on VSLC wall allow blood withdrawal from LV, and a tri-leaflet outlet valve at VSLC tip allows blood delivery to aorta. The 28 Fr VSLC was made by polyurethane (PU) dip molding. The spDDP housing was made by polycarbonate vacuum thermoforming with a PU diaphragm in the middle. The spDDP stroke volume was 60 cc. A closed mock loop with 37% glycerin to mimic blood viscosity was used. Priming volume/reservoir height were adjusted to maintain 70 mm Hg diastolic aortic pressure/16 mm Hg preload. In adult sheep (n=5, 44-52 kg) testing, a pressure sensor on the introducer tip allowed the pressure wave form to guide correct placement of VSLC tip into ascending aorta. The VSLC was then connected to spDDP. Artificial heart performance was assessed for 6 hrs.

Results: The VSLC and valveless sppDDP prototypes were made as designed. The artificial heart achieved a pumping flow of up to 3.75 L/min with <3% valve regurgitation in bench test. In the sheep study, the VSLC was easily installed within 1 min. The artificial heart achieved maximal 3.75 L/min pumping flow in a 52 kg sheep. The artificial heart maintained consistent pumping flow through 6 hr with stable hemodynamics (arterial blood pressure, central venous pressure, and cardiac output). Hemoglobin and platelet/white blood cell counts were unchanged through 6 hr. No obvious injury/thrombosis were observed at necropsy.

Conclusions: With the very small, simple installation and great performance, our newly invented artificial heart shows great promise for cardiogenic shock patients.

**A histological and immunochemical comparison of intact and injured human nerve tissues in vitro and in vivo**

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The peripheral nervous system (PNS) is one of the few adult human tissues with a strong capacity for self-repair at least in part due to the presence of Schwann cells (SCs), the PNS-resident glial cells. The repair capability of SCs has been exploited cell-transplantation therapies to promote neuroprotection, regeneration and remyelination in the central nervous system. However, the identity of the predominant cell types within nerve tissues has not yet been addressed in human models of nerve injury. Hence, the goal of this study was to optimize microscopic and immunological methods to study human peripheral nerve-resident cells before and after injury. Intact and injured nerves were compared for the expression of markers typical of glial and non-glial cells. First, we used cultured human nerves (live and fixed) as an in vitro injury model. Second, we used donor-matched nerve samples (fixed) from study participants undergoing experimental axotomy as an in vivo model of nerve injury. We evaluated the expression and localization of markers of mature and immature SCs, and cells within connective tissue layers, to evidence morphological and molecular changes in response to injury in different cell populations. We identified mature (axon-related, intact) and repair (axon-deprived, injured) SCs together with intact or degraded myelin. Interestingly, the typical SC marker p75 (NGFR) not only is expressed in mature and repair SCs, as expected, but also in cells within the perineurium, the epineurium, and blood vessels in both intact and injured nerves. Substantial remodeling of the epineurial and perineurial layers occurred in injured nerves in vivo, but these changes do not occur in vitro. To conclude, we found that in vitro degeneration is a useful model to recapitulate SCs responses to injury such as activation and myelin degradation, but the contribution of non-glial cells is more accurately evidenced in whole nerve tissues collected directly from participants.

**Leucine Zipper-bearing Kinase (LZK) modulates dynamicity of cytoskeleton and migration in astrocytes**

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After Spinal cord injury, reactive astrocytes migrate toward the injury site to form the astrocytic scar. Our lab discovered leucine zipper-bearing kinase (LZK) as a major positive regulator of astrocyte reactivity to injury. This study examines the role of LZK in the regulation of cytoskeleton dynamicity and astrocyte cell migration. Astrocytes were isolated from tamoxifen-inducible, astrocyte-specific LZK knockout (KO) mice and 4-Hydroxytamoxifen was applied to induce gene deletion in vitro. We assessed cell migration by scratch assay, lamellipodia characterization, microtubule acetylation, and filamentous-to-globular actin ratio.

Astrocytes lacking LZK showed significant changes in differentially expressed cytoskeletal-associated genes in primary astrocyte culture and astrocytes isolated from the injury site. LZK deletion decreased cell migration, reduced length of lamellipodia, and lower levels of polymerized actin and acetylated tubulin. Notably, overexpression of LZK (LZK-OE) elevated the level of genes involved in actin polymerization and microtubule stability. The astrocytic LZK-OE increased the ratio of filamentous to globular actin and acetylated tubulin to total tubulin. LZK-OE significantly promoted cell polarity and astrocyte cell migration.

These results suggest that LZK promotes astrocyte migration by regulating tubulin and actin dynamics in cytoskeleton rearrangement. Pathways through which LZK causes these cytoskeletal changes are under investigation.

**The Role of Dennd5b in Dietary Lipid Absorption**

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Background: Dietary lipid absorption is essential for systemic lipid homeostasis. We previously demonstrated a role for Dennd5b in intestinal lipid absorption. Female Dennd5b-/- mice have reduced appearance of dietary triglycerides (TG) in plasma and are less susceptible to diet-induced obesity. In humans, DENND5B gene variants are correlated with BMI in females, but not males.

Objective: Determine if the Dennd5b-/- mouse model recapitulates the sex disparity observed in humans with DENND5B polymorphisms and determine the fate of unsecreted TG in intestinal tissue.

Methods and Results: A non-absorbable fatty acid (FA) tracer was used to quantify the impact of Dennd5b-deficiency on lipid absorption in male and female mice. We observed a relatively modest reduction in lipid absorption efficiency in Dennd5b-/- mice in both sexes, despite a complete absence of plasma TG after oil gavage. We hypothesized that this was due to metabolic utilization of TG by enterocytes. Metabolic cage studies showed that both wildtype and Dennd5b-/- mice shift toward utilization of FAs when fed high-fat diet. Electron microscopy (EM), revealed large electron dense structures that resemble autophagosomes in Dennd5b-/- enterocytes. Western blots revealed an increase in Lc3-II:Lc3-I ratio in Dennd5b-/- intestine, indicating increased autophagy. Levels of the FA sensing transcription factor, Hnf4g, and its target gene involved in beta oxidation, Cpt1a, were increased in Dennd5b-/-. EM also revealed altered mitochondrial morphology in Dennd5b-/- enterocytes.

Conclusions: We conclude that unsecreted TG in the Dennd5b-/- enterocytes are degraded by autophagy, liberating free FAs which are utilized in mitochondrial oxidation. The sexually dimorphic impact of Dennd5b may not be as prominent in mice as it is in humans. Overall, our findings demonstrate that Dennd5b plays a critical role in regulating lipid metabolism in the intestinal tissue that can impact systemic metabolic health.

**Nedd4-2 inactivation enhances SGLT1 stability in diabetic hearts**

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Rationale: Type-2 diabetes (T2D) is a major risk factor for developing heart failure. While the underlying mechanisms are poorly understood, recent evidence suggests that upregulation of the Na+/glucose cotransporter 1 (SGLT1) plays an important role, as it causes myocyte Na+ overload.

Objective: To identify the mechanisms involved in promoting an increase in SGLT1 protein level in diabetic hearts.

Methods/Results: We compared hearts from patients with and without T2D and hearts from rats with late-onset T2D caused by overexpression of human amylin in pancreatic Î²-cells versus hearts from wild-type littermates. SGLT1 protein level was significantly higher in hearts from both humans and rats with T2D. In contrast, the SGLT1 mRNA levels were comparable in hearts from T2D and non-diabetic patients and rats. This result suggests that theT2D-induced increase in cardiac SGLT1 protein occurs at a post-transcriptional level. Co-immunoprecipitation of SGLT1 with ubiquitin was reduced in diabetic human and rat hearts, which indicates that SGLT1 degradation is impaired. The E3 ubiquitin ligase Nedd4-2 is responsible for ubiquitination of several cardiac membrane transporters. Pharmacological inhibition of Nedd4-2 with Heclin in HL-1 cells resulted in significantly higher SGLT1 protein levels, suggesting that Nedd4-2 also mediates SGLT1 degradation. Nedd4-2 is inactivated upon phosphorylation by several kinases, including the serum and glucocorticoid-regulated kinase-1 (SGK1). Using western blotting, we found increased Nedd4-2 phosphorylation in hearts from humans and rats with T2D compared to their non-diabetic counterparts. Moreover, hearts from T2D patients exhibited higher levels of SGK1 and its activated phosphorylated form compared to their non-diabetic counterparts.

Conclusion/New Hypothesis: The increase in SGLT1 protein in the diabetic heart is regulated post-transcriptionally by an impairment in Nedd4-2 dependent ubiquitination and degradation of SGLT1.

**Tobacco use, secondhand smoke exposure and infant feeding practices among mothers living in rural Kentucky**

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The purpose is to examine tobacco use, secondhand smoke (SHS) exposure and infant feeding practices including breastfeeding (BF) duration in mothers living in rural Kentucky. Smoking rates in Kentucky are higher than the general US population and conversely, BF initiation and continuation rates are lower in the state than the national average. Women in rural communities have higher rates of tobacco use and SHS exposure as well as lower rates of BF initiation and duration compared to their urban counterparts. Policy outcomes research shows pregnant women living communities with strong smoke-free laws have lower rates of preterm birth. Research is lacking on the association of tobacco use, SHS exposure and infant feeding status in rural communities. This study uses a cross-sectional retrospective design and purposive cluster sampling with stratification by strength of municipal smoke-free laws and tobacco use and/or exposure status. Women between 18-45 years of age currently residing in one of the six identified rural Kentucky counties: Knott, Owsley and Perry (strong smoke-free laws) and Bath, Menifee, Morgan (no smoke-free laws), who have given birth to a live infant within the past two years and speak English are eligible. Recruitment methods include ResearchMatch, Craigslist and Facebook. The projected sample size is 280 mothers with 40% of participants from each county cluster with self-reported tobacco use and/or secondhand smoke exposure. Measures include demographics; infant feeding practices (prior BF history and infant feeding status); tobacco use (previous and current; cigarettes and e-cigarettes); SHS exposure (home, workplace and vehicle); lung cancer (prior screening, personal and family history and worry); depression; anxiety and alcohol and substance abuse (prenatal and current). Participants completing the survey receive a $15 Amazon gift card. Data analysis includes descriptive statistics, bivariate associations and multi-level modelling.

**High Density Lipoprotein Targeting Protease Inhibitor Preserves Lung Function in Alpha-1 Antitrypsin Deficient Mice**

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Background: Alpha-1-antitrypsin deficiency (AATD) is caused by genetic mutations in the alpha-1-antitrypsin (AAT) gene. The AAT protein acts as an endogenous regulator of the protease enzyme elastase. When an infection or injury occurs in lung tissue, neutrophils and macrophages move into the lungs and release elastase. However, patients with AATD are unable to regulate this inflammatory response, and elastase-mediated damage to lung tissue causes emphysema and COPD in many of these patients. Previous studies have demonstrated high-density lipoprotein (HDL) mediated transport of AAT. We have designed an HDL-targeting protease inhibitor peptide (HTPI) peptide to allow for the synthetic enrichment of elastase inhibitor activity on plasma HDL particles. Hypothesis: We hypothesized that the novel HTPI can protect against lung injury in alpha-1-antitrypsin deficient mice.

Methods and Results: The HTPI peptide contains both an HDL targeting domain and an elastase inhibitor domain. We demonstrate preferential targeting of HTPI to HDL in human plasma ex vivo and to mouse HDL in vivo after intravenous administration. To examine HTPI efficacy in a mouse model of lung injury, AAT- deficient mice were given retroorbital (r.o.) injections of either saline or HTPI (7 mg/kg) prior to oro-tracheal (o.t.) installation of either saline or lipopolysaccharide (LPS). The next day, lung fluid was collected by bronchoalveolar lavage (BAL) and the BAL fluid was analyzed for total cell counts and differential counts of neutrophils and macrophages. HTPI-treated mice had lower total cell counts compared to saline controls. The lower BAL cell content in HTPI-treated mice was due primarily to reduced neutrophil infiltration.

Conclusions: Venous administration of HTPI prior to LPS-induced lung injury prevents the neutrophilic inflammatory response in mice. These findings support the potential utility of the HTPI peptide in the preservation of lung function in the context of AAT-def

**A differential role for YAP signaling in regeneration and fibrosis**

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A fundamental question in regeneration is how cell proliferation is activated and regulated to appropriately restore tissue architecture. The mechanosensitive transcriptional coactivator, Yes associated protein (YAP), is a potent inducer of cell proliferation. Activation of YAP has been shown to induce or enhance regeneration in several tissues. However, high YAP activity is also associated with fibrosis and oncogenesis. The African spiny mouse (Acomys cahirinus) is a mammal with the remarkable ability to regenerate complex tissues such as ear pinna and skin. Previous studies show Acomys primary fibroblasts have increased cycling of active YAP compared to lab mouse (Mus musculus). In addition, inhibition of YAP delays ear pinna regeneration in the spiny mouse, suggesting that YAP plays an important role in spiny mouse regenerative ability. Using spiny mouse ear pinna regeneration and lab mouse ear pinna fibrosis, we investigated the role of YAP signaling at different stages of regeneration and fibrosis. We found that YAP activation accelerates Acomys ear pinna regeneration and increases the amount of tissue generated during Mus ear pinna fibrosis. During the injury response period YAP activation in both species increases macrophage recruitment and accelerates re-epithelialization. Interestingly, after inflammatory resolution, YAP activation increases proliferation in the Mus fibrosing ear pinna, but not the Acomys regenerating ear pinna. In addition, while YAP activation increases the proportion of tissue differentiating into cartilage in Mus, it had no effect on tissue architecture in Acomys. Together, these data show YAP modulates the intensity and duration of the injury response in both fibrosis and regeneration but only mediates proliferation after inflammatory resolution during fibrosis.

**Energy Balance and Pathology Core Services Provided by COCVD at University of Kentucky**

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The NIH P30 COBRE Center of Research in Obesity and Cardiovascular Disease (COCVD) at the University of Kentucky provides services to investigators across UK. The Energy Balance Core offers metabolic phenotyping in mice and the Pathology Core prepares for histologic characterization tissue samples from many species. Metabolic phenotyping is performed in the Sable Promethion system and EchoMRI. Sable Promethion offers chambers equipped to quantify food intake, water intake, physical activity, monitoring of body weight and to perform indirect calorimetry. The system includes optional running wheel, timed or yoked control of access to food, and incubation at thermoneutral or other custom temperatures. Core personnel have performed experiments to validate and optimize the protocols and data analysis used with the Sable Promethion system. The EchoMRI allows quantification of body composition in awake mice and in tissue samples. The Pathology Research Core provides equipment and technical expertise to embed, section and stain tissue specimens and to train investigators in staining, imaging and data analysis. Staining services provided include H&E, Masson Trichrome, PAS, Picrosirius Red, Alcian Blue and Verhoeff Van Gieson. We currently are phasing in rate systems for the cores using the PPMS Stratocore system. Supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences (P30 GM127211) from the National Institutes of Health.