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Meeting Abstracts

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Cancer Pathobiology

CAN1

DCLK1 Promotes Cisplatin Resistance in Ovarian Cancer

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Introduction: High grade serous ovarian carcinoma (HGSOC) is the most common and lethal histologic subtype of ovarian cancer (OvCa) that accounts for 80% of OvCa deaths. Poor survival is attributed to disease recurrence and drug resistance to platinum-taxol-based drugs, which are the first-line treatment. Thus, developing effective treatments that overcome resistance to standard-of-care treatments are urgently needed. Doublecortin-like kinase 1 (DCLK1) is a microtubule-associated protein family member known to regulate microtubule turnover and distribution. It is a major regulator of cancer cell "stemness," epithelial-mesenchymal transition (EMT), and promotes tumor progression, and metastasis. The study objective is to assess the functional role of DCLK1 in mediating platinum-resistance in OvCa and associated benefits from antagonizing this pathway as a novel treatment strategy to prevent OvCa recurrence. **Methods:** DCLK1 expression was evaluated under adherent (2D) and suspended spheroid (3D) conditions in a panel of human OvCa cell lines representative of HGSOC, and platinum-sensitive and resistant cell lines using western blot. Pharmacological inhibition (DCLK1-IN-1) and genetic manipulation (DCLK1 CRISPR knockout) were used to assess the role of DCLK1 in mediating cisplatin resistance. Drug sensitivity assays were performed using live-cell imaging analysis (Incucyte) and 3D cell viability assays. Migration and invasion of human OvCa cells was determined *in vitro* in the presence of DCLK1-IN-1. Western blots were performed to evaluate the effect of DCLK1 inhibition on markers for EMT, OvCa cell stemness, and a potential kinase substrate. Differential expression of microRNAs critical for mediating drug resistance and EMT were measured by RT-PCR in DCLK1 deficient cells. **Results:** DCLK1 is differentially expressed in a panel of human OvCa cell lines representative of HGSOC when cultured under both 2D and 3D conditions. We also observed significant DCLK1 upregulation in cisplatin-resistant OvCa cell lines relative to their sensitive controls (OVCAR-8 and IGROV-1). Inhibition of DCLK1 was effective in restoring cisplatin sensitivity and reducing pro-tumorigenic phenotypes (e.g., cell migration & invasion) in platinum-resistant OvCa cells. We observed that DCLK1 knockout differentially affected markers for cancer cell stemness and microRNA expression. **Conclusions:** Our study showed that DCLK1 is important in regulating programs promoting platinum resistance in OvCa. Use of DCLK1 specific inhibitors will provide a rationale and optimal therapeutic strategy to overcome cisplatin resistance in a clinical setting.

CAN2

Targeting the DNA Damage Response Pathway by Combination of ATR and PARP Inhibition Imparts Synthetic Lethality to Cervical Cancer Cells

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Introduction: Cervical cancer (CC) is the fourth most common cancer among women worldwide. Most CC cases are due to high-risk human papillomavirus (HPV) infection, which expresses oncoproteins that inhibit tumor suppressors causing loss of G1-S cell cycle checkpoint control and defective DNA repair. Targeting the DNA damage response (DDR) pathway is being studied as an effective treatment strategy in metastatic CC, with specific focus on increasing dependence upon the Poly (ADP-ribose) Polymerase (PARP) DNA repair pathway. We hypothesized that increasing dependence upon the PARP pathway through abrogation of the G2 cell cycle checkpoint by ATR inhibition would sensitize CC cells to subsequent PARP inhibition. **Methods:** The impact of drug sequencing on cytotoxic cell death was determined in two HPV+ cervical cancer cells lines (Caski and SiHa) by treatment with the ATR inhibitor (ATRi) AZD6738, PARP inhibitor (PARPi) olaparib, or both, given either simultaneously or by pre-treatment with ATRi. The degree of drug interaction was determined by isobologram analysis to determine the combination index for CC cells exposed to simultaneous ATRi/PARPi treatment and ATRi pre-treatment followed by sequential PARPi. Changes in cell viability in the drug combination versus PARPi alone groups was measured using MTS cell viability assays. Expression of proteins involved in the DDR pathway including PARP and ATR, as well as downstream cell cycle checkpoint proteins, were evaluated by western blot. **Results:** A synergistic effect was observed when CC cells were simultaneously treated and when pre-treated with ATRi followed by PARPi, likely due to the observed upregulation of the PARP pathway after ATR inhibition. Cell viability in the drug combination groups, for both 48- and 72-hour incubation periods, showed a reduction in cell survival below 50% compared to the PARPi alone groups. Lower IC50 values were identified when comparing PARPi alone with the drug combination, indicating the potential for PARPi dose de-escalation in treatment with ATRi. Western blotting of Caski cells, with ATRi pre-treatment followed by sequential PARPi, demonstrated both a decrease in total PARP levels and an increase in cleaved PARP indicating increased cell death, while also demonstrating a decrease in Chk-1, P-Chk1 and P-Chk2 affirming therapeutic inhibition of the G2 checkpoint. **Conclusion:** HPV oncoproteins reduce HPV+ CC cells' ability to repair DNA damage via homologous recombination. Treatment with ATRi may increase reliance upon the PARP pathway for DNA repair through the error-prone nonhomologous end joining mechanism. These HPV-induced changes, render this pathway more vulnerable to PARP inhibition. Additionally, they render the combination of ATRi and PARPi more effective at decreasing CC cell viability compared to either inhibitor alone, especially when the cells undergo G2 checkpoint inhibition prior to administration of a PARP inhibitor.

CAN3

Collagen Hydroxyproline Analysis of Colon Cancer Polyps in Patients Within the Appalachian Mountain Region

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Introduction: Emerging evidence suggests that collagen morphology may influence the prognosis and response to treatment of colorectal cancer (CRC). We sought to investigate the overarching differences in collagen posttranslational hydroxyl prolines as a predictive model of tumor stage and to investigate population differences in the American Appalachian population. **Methods:** Tissue microarrays (TMAs) comprising of matched benign and malignant tissue from 45 patients were constructed to evaluate the degree of collagen alpha-1(I) hydroxylation of proline. **Results:** In total 86 samples were analyzed. 5 specific peaks were discovered to differ between benign and malignant polyps. ROC curve analysis indicated high sensitivity and specificity of these peaks to predict polyp malignancy. 17 individual peaks indicated differences between early-stage (Stage I+II) and late-stage (Stage III+IV) polyps with a high predictive power (Area; 0.7355, 95% CI: 0.7014-0.7696, $p=1.000x10^{-15}$). Analysis of late-stage malignant polyps showed the same 17 peaks were significantly increased in patients from the Appalachian region of the United States vs Non-Appalachian residents. This present study highlights the potential for utilizing TMA as a method for detecting with high predictive power the influence of collagen on overall tumor prognosis. Further, we provide evidence that TMA analysis can be utilized to detect differences between specific at-risk populations, a method that might improve diagnostic and prognostic outcomes. **Conclusion:** Taken together we provide evidence for the investigation of collagen proline hydroxylation in CRC as a prognostic and diagnostic marker which can have significant clinical outcomes.

CAN4

CECR2 Drives Breast Cancer Metastasis by Suppressing Macrophage Inflammatory Responses

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Introduction: Epigenetic and transcriptional changes are critical for metastasis, the major cause of cancer-related deaths. Metastatic tumor cells escape immune surveillance more efficiently than tumor cells in the primary sites, but the mechanisms controlling their immune evasion are poorly understood. **Methods:** RNA-seq, ChIP-seq, TMA IHC staining and different mouse models were used for the study. **Results:** We found

that distal metastases are more immune inert with increased M2 macrophages compared to their matched primary tumors. Acetyl-lysine reader CECR2 is an epigenetic regulator upregulated in metastases and positively associated with M2 macrophages. CECR2 specifically promotes breast cancer metastasis in multiple mouse models, with more profound effect in the immunocompetent setting. Mechanistically, NF-κB family member RELA recruits CECR2 to activate CSF1 and CXCL1, which are critical for macrophage-mediated immunosuppression at the metastatic sites. Furthermore, pharmacological inhibition of CECR2 bromodomain impedes NF-κB-mediated immune suppression by macrophages and inhibits breast cancer metastasis. **Conclusions:** These results reveal CECR2 as a key epigenetic regulator in modulating breast cancer metastatic microenvironment and a novel therapeutic target to treat metastatic breast cancer.

Neuropathology

NEURO1

PECAM-1 Blockade Reduces Neutrophil Infiltration into the Subcortex Post-Stroke

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Introduction: Current therapies for ischemic stroke focus on reperfusion but do not address the acute inflammatory response that results in significant reperfusion injury. To advance future therapies, a thorough understanding of the precise spatiotemporal underpinnings of leukocyte extravasation and infiltration is necessary. We describe the evolution of the inflammatory response in a mouse transient middle cerebral artery occlusion (tMCAO) stroke model at several time points after reperfusion and the modulation of this response with PECAM blockade. **Methods:** The transient Middle Cerebral Artery Occlusion model (90 minutes of ischemia followed by reperfusion) was used to simulate large vessel occlusion stroke and recanalization. We used wide field and confocal immunofluorescence microscopy to examine the exact distribution of neutrophils with close examination of the leukocyte position with regard to the brain vasculature and the perivascular space. Flow cytometry of single cell suspensions was used to confirm cell identity at different time points post-stroke. **Results:** At 12 and 24 hours, neutrophil recruitment and extravasation was predominated localized to the cortical surface. This contrasts with other organs where there is considerable migration of neutrophils into the inflamed tissue by 24 hours. Over 48 to 72 hours, neutrophils were increasingly found deeper into the subcortex. Throughout the infarct (determined with triphenyl tetrazolium chloride staining), neutrophil recruitment was not uniform but rather organized in clusters. Disrupting leukocyte diapedesis with PECAM function-blocking antibodies restricted leukocytes to within 500 microns of the surface when compared to control; and this was still evident at 72 hours (n=3 mice per group, $p<0.01$, Control 46% ± 4.0 %; PECAM-1 Ab 62% ± 5.0%). High-power wide-field microscopy confirmed limitation of TEM by PECAM-1 blockade at 24h. Flow cytometry confirmed that a majority of the infiltrating cells were neutrophils both at 24h and 72h. **Conclusions:** Our findings demonstrate that neutrophil infiltration into a stroke evolves over several days following reperfusion. The use of PECAM blockade modulates the natural progression of neutrophils into the infarcted stroke bed. A better understanding of neutrophil spatiotemporal infiltration and its regulators could help inform the next generation of therapeutic interventions.

NEURO2

AAV-mediated Gene Replacement Therapy is Beneficial in Unverricht-Lundborg Disease Mouse Model

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Introduction: Unverricht-Lundborg disease (ULD) is an inherited form of progressive myoclonus epilepsy (PME) which is characterized by myoclonus, epilepsy, and progressive neurologic deterioration of varying degrees. It is the most common form of PME. Although its worldwide prevalence is unknown, approximately 4 in 100,000 people are affected in Finland. Patients typically begin showing signs and symptoms between the ages of 6–16. Early symptoms include stimulus-sensitive, action-activated myoclonus, and tonic-clonic seizures. Eventually, people with ULD develop ataxia, lack of coordination, intention tremor, and dysarthria. ULD patients may also develop emotional sensitivity, depression, and mild to moderate cognitive impairment over time. ULD is caused by *CSTB* gene mutation and is inherited autosomal recessively. The most common mutation is a dodecamer repeat expansion in the *CSTB* gene (upstream to the promoter) which leads to a reduction in CSTB protein. Patients usually retain ~10% of Cystatin B activity. *CSTB* encodes Cystatin B, a cysteine protease inhibitor. Despite some progress in understanding the biological function of Cystatin B, the disease mechanism remains unknown. Currently-available treatments aim to control symptoms and increase the quality of life. There is no targeted or disease-modifying therapy available. In this study, we developed and tested a gene-replacement therapy in the ULD mouse model. **Methods:** We used the human, fully-spliced *CSTB* cDNA gene to rescue the mouse model and to reduce future steps in the potential development of a human therapy vector. We hypothesized that replacing Cystatin B would improve the neuropathology and neurobehavioral phenotypes of CSTB-deficient mice. After designing the human codon-optimized *CSTB* plasmid and packaging in Adeno-Associated Virus 9 (AAV9), we administered the AAV-CSTB at two different time-points (p21 and p60) by intrathecal (IT) injections. To study the effect of treatment on early-onset neuropathological phenotypes including neuro-inflammation and granular cell apoptosis in the cerebellum, a cohort of mice at 2-months of age were euthanized. Remaining mice were aged to 9-months in order to study the effect of treatment on the behavioral phenotype (ataxia) and late-onset neuro-inflammation and neuro-degeneration. **Results:** We showed that AAV-CSTB gene replacement therapy reduces early-onset neuro-inflammation and cerebellar granular cell death. AAV-CSTB gene therapy ameliorates behavioral phenotype (ataxia) and improves some of the late-onset neuro-inflammation markers. AAV-CSTB gene therapy does not reduce or prevent the neuro-degeneration-related brain weight loss. **Conclusion:** Replacing *CSTB* may provide therapeutic benefit in the ULD mouse model (*Cstb*$^{-/-}$) by decreasing the severity of neuropathology. Injections at early time-points (neonatal) and/or using different injection routes like intra-cerebroventricular injection (ICV) or intra-cisterna magna (ICM) may provide a greater benefit.

NEURO3

Neuronal Death and Inflammation in Brain of SARS-CoV-2 Infected Non-Human Primates

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Introduction: Neurological manifestations are a significant complication of syndrome coronavirus 2 (SARS-CoV-2) infection and coronavirus infection disease-19 (COVID-19). These likely contribute to post-acute sequalae of COVID-19 (PASC) or "long COVID". Understanding how infection contributes to neuropathogenesis is needed for appropriate treatment of infected patients, as well as in initiating relevant follow-up care after recovery. Brain autopsy series of human subjects who died from COVID-19 have revealed significant pathology, including wide-spread neuroinflammation and microhemorrhages. In this report, we show for the first time, neuropathology in SARS-CoV-2 infected non-human primates (NHPs) that is consistent with that reported among human patients. **Methods:** Eight adult NHPs were inoculated with the 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2 via a multi-route mucosal or aerosol challenge. Immunohistochemistry staining was done on seven brain regions, including frontal, parietal, occipital, and temporal lobes, basal ganglia, cerebellum, and brainstem to elucidate the presence of platelet derived thrombi, neuronal apoptosis, and virus present. General pathology and microhemorrhages were examined on hematoxylin and eosin-stained (H&E) tissues. **Results:** Similar to humans, pathology was variable but included wide-spread neuroinflammation, nodular lesions, neuronal degeneration, and microhemorrhages. Changes in neuronal morphology suggestive of neuronal degeneration were identified with H&E staining and most often seen in the cerebellum and brainstem of infected animals. Neuronal death was confirmed through FluorJade C and cleaved (active) caspase

3 IHC, which showed foci of positivity, particularly among Purkinje cells of the cerebellum. Importantly, this was seen among infected animals that did not develop severe respiratory disease. Sparse virus was detected in brain endothelial cells but did not associate with the severity of CNS injury. **Conclusions:** We anticipate our findings will advance our current understanding of the neuropathogenesis of SARS-CoV-2 infection and demonstrate SARS-CoV-2 infected NHPs are a highly relevant animal model for investigating COVID-19 neuropathogenesis among human subjects.

NEURO4

Interactions Between Autophagy, Herpesvirus, and Neurodegeneration in Alzheimer's Disease

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Introduction: Various microbes and viruses have been proposed to be involved in the pathogenesis of Alzheimer's Diseases (AD). Since microbes and viruses are detected in aging brains, it is not clear if their presence is a byproduct of AD pathology or a direct cause of it. Recently various publications considered the link between several herpesviruses and neurodegeneration in AD. Readhead et al. 2018 utilized multiomics data from post-mortem brain samples with and without neuropathological criteria for AD to construct biological networks and look for associations between viral load, viral activity and AD. This study reported strongest associations between human herpesviruses 6A and 7 with various features of AD. After viral replication and exit from the cell's nucleus, a secondary envelopment process by which HSV acquires its envelope, structurally resembles the process of autophagy. Thus, autophagy might have significant interactions with herpesviruses. Here we sought to determine associations between viral features, AD pathology and autophagy gene allelic variation. **Methods and Results:** We prepared a comprehensive list of over 1000 autophagy-associated genes (ATG) from Tanpaku, PDB databases and genes we found in publications, as well as from an artificial-intelligence-based search for "dark ATG genes". We downloaded the R codes and datasets from Readhead et al. 2018 available on synapse.org. Using custom python codes we mined these digital datasets for ATG. We mined host RNA abundance in pre-AD, non-AD traits, and AD controls for ATG and found virtually all were down regulated in AD compared to controls. We mined quantitative trait loci (QTL) for correlations of host SNPs to viral load in preclinical and clinical AD and found many ATG SNPs associated with viral features. We found QTL of host genes to identify SNPs in ATG that correlated with cognition and Braak stage. We found a dozen genes associated with SNPs in ATG that associated with AD. **Conclusions:** Results from mining different datasets with our comprehensive ATG list supported our initial hypothesis of a correlation between ATG expression and SNPs, viral load and progression of cognitive impairment and AD pathology. The integration of ATG in this project clarifies a potential role microbes and viruses may play in onset and progression of AD pathology. This project is supported by the National Center for Research Resources and the National Center for Advancing Translational Sciences of the National Institutes of Health through Grant Number UL1TR001449.

NEURO5

Early Life Adversity Potentiates Adult Response to Acute Threat: A Brain-wide Neuroimaging and Behavior Study

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Introduction: Adverse childhood experiences increase vulnerability to stress-provoked mental illnesses, such as anxiety/depression and substance use disorders, later in life. How these experiences shape the brain to lead to such disorders remains a mystery. Brain-wide imaging of neural activity in the adult raised with or without early life adversity (ELA) holds promise of finding clues. **Methods:** Longitudinal manganese-enhanced magnetic resonance imaging (MEMRI) provides a methodology to follow brain-wide neural activity dynamics over time in awake-behaving animals. Mn(II) delivered systemically is taken up through voltage-gated calcium channels throughout the brain over 24-26h. Behavior is recorded in our custom arena during Mn(II) uptake. We capture an image of basal behavior (normal exploration) at 22h, and then expose mice to acute threat, predator odor (TMT, 2,3,5-Trimethyl-3-thiazoine), and image again at 26h (Uselman et al, 2020). Here we combine this MEMRI paired with behavior protocol to investigate the effects of ELA on responses to acute threat in normally reared (n=12) and maternally deprived mice (n=12) (ELA-/+). Dams with newborn pups were deprived of adequate bedding from day P2-P9, which induces elevated cortisol in the pups (Rice et al., 2008). At 10 weeks of age, mice +/- ELA were subject our paired behavior-MEMRI longitudinal procedure for both acute and delayed responses. After imaging, mice are euthanized, brains perfusion fixed and serial sections stained by immunohistochemistry. MEMRI images were skull-stripped, spatially co-registered, and intensity normalized. To measure the degree of activity and relationships between brain regions we performed statistical parametric mapping (SPM), segmentation of 90 brain regions, cross-correlation analysis, and Louvain community detection. **Results:** Predator stress increased defensive/avoidance behavior for both groups ($p<0.05$, Tukey-Adjusted), attesting to the expected effect of the TMT. Results suggest basal neural activity of ELA mice resembles that of acute fear in normally reared mice and delayed responses among both groups are similar, with increased activity in the basal forebrain and hindbrain. Additionally, ELA resulted in decreased strength in correlation among brain segments, which fragmented further after acute threat. Staining for the norepinephrine transporter revealed a failure of distal axonal tiling, suggesting that neural activity dynamics could be due in part to ELA's effect on development of the noradrenergic system. **Conclusion:** Together our data find that ELA disrupts the arborization of noradrenergic projections, alters the coordination of basal neural activity in the basal state and in response to acute threat. Supported by NIMH R01MH096093.

Infectious Diseases

IND1

Sofia SARS Antigen FIA and Reverse-Transcriptase Polymerase Chain Reaction: Comparing Diagnostic Methods

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Introduction: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus that causes coronavirus disease 19 (Covid-19), emerged in December 2019 and led to a global pandemic. Timely and accurate diagnostic methods became an absolute necessity and remain a critical tool for detecting Covid-19 cases. Two primary methods for detecting active SARS-CoV-2 infection are currently in use: reverse-transcriptase polymerase chain reaction (RT-PCR) molecular testing and antigen testing. RT-PCR is considered the "gold standard" method for detecting SARS-CoV-2 infection. It is highly sensitive, but requires a molecular testing facility, has a longer turnaround time, and is more expensive than antigen testing. Clinicians and public health authorities must consider these factors when selecting appropriate testing methods for patients and communities. **Methods:** 1285 nasopharyngeal samples were collected from 1282 individuals at Springfield Hospital and Dartmouth-Hitchcock Medical Center between July 2020 and February 2021. Of the 1282 individuals in the study population, two were known to be symptomatic, 16 had a known exposure, and 14 were healthcare workers being tested for surveillance. The remaining samples were collected from out-patients and in-patients for screening. Samples were analyzed using both Sofia SARS antigen FIA test and RT-PCR. Comparing Sofia to RT-PCR with RT-PCR as the gold standard, positive percent agreement and negative percent agreement were calculated. **Results:** Compared to RT-PCR, Sofia rapid antigen test has a positive percent agreement of 72.7% and a negative percent agreement of 98.9%. Eighteen samples tested negative by Sofia, but positive by RT-PCR. **Conclusion:** Compared to RT-PCR, Sofia FIA rapid antigen testing shows high negative percent agreement and reasonable positive percent agreement. Previous studies have compared RT-PCR and Sofia FIA methods in study populations comprised only of symptomatic patients (Beck ET, et al. *J Clin Microbiol*. 2021;59:e02727-20; Brihn A, et al. *MMWR Morb Mortal Wkly Rep*. 2021;70:702-706). In these studies the positive percent agreements values were between 54.5% and

97.6%. Additionally, studies have shown that Sofia FIA testing is significantly less sensitive than RT-PCR in detecting SARS-CoV-2 infection in asymptomatic individuals (Brihn A, et al. *MMWR Morb Mortal Wkly Rep*. 2021;70:702-706). Our results and results from previous studies indicate that Sofia FIA testing should be used with caution for screening and in asymptomatic individuals.

IND2

Estradiol Levels Influence Neutrophil Responses Against HIV in Women

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Introduction: The population is aging, and older women are more susceptible to infections compared to younger women. This fact has been linked to impaired immune responses and changes in hormone levels after menopause. Neutrophils play a crucial role in the innate immune response against viral infections, such as human immunodeficiency virus (HIV), recognizing, trapping, and inactivating the virus using a process known as NETosis. In this response, neutrophils release DNA strands coated with antimicrobial peptides, known as Neutrophil Extracellular Traps (NETs). Interestingly, estradiol (E2), a sex hormone that decreases after menopause, has been shown to decrease susceptibility to HIV infection of T cells and macrophages *in vitro*, but the role of E2 in neutrophil response to HIV is largely unknown. We hypothesize that low levels of E2 due to menopause can impact neutrophil activity and susceptibility to HIV infection in women. **Methods:** Blood neutrophils were isolated from both pre- and postmenopausal women and pre-treated with a concentration of 50 nM E2 for 1 h *in vitro*. Neutrophils were then stimulated with GFP-labelled HIV viral-like particles (HIV-VLPs) or Calcium ionophore, a known NET-inducer, for 30, 60 and 90 min. To quantify NETosis in real-time, time-lapse microscope images were taken every 3 min and analyzed using the Incucyte platform. NETs were separated from supernatants by centrifugation and α-Defensin (αDef) and myeloperoxidase (MPO) were measured in each by ELISA. E2 concentration in plasma was also measured by ELISA. **Results:** Neutrophils from postmenopausal women showed a delayed NETosis response to HIV over 3 h compared to neutrophils from premenopausal women. Whereas increased NETosis with HIV is clear via Incucyte at later time points, after only 30 min of stimulation with HIV, no changes in concentration of αDef were observed in NETs or supernatants. However, neutrophils significantly increased αDef secretion 30 min after Ca^{2+} ionophore stimulation, and significantly more so in premenopausal women compared to postmenopausal. Additionally, the Ca^{2+} ionophore-stimulated neutrophils from premenopausal women had increased levels of MPO present in the NETs compared to postmenopausal women. Ongoing experiments will determine antimicrobial peptide production 60 and 90 min post-stimulation. We wanted to determine if these changes in neutrophil response were due to changes in E2 levels associated with menopausal status and aging. Interestingly, pre-treating neutrophils from postmenopausal women with E2 improved their response to HIV after 30 min, showing increase release of NETs compared to untreated neutrophils. E2-treated neutrophils from young women, however, did not modify their response to HIV. **Conclusion:** Aging delays neutrophil response to HIV stimulation and this was improved when neutrophils were treated with E2, suggesting that circulating E2 levels may play a role in neutrophil function against viral pathogens as women age.

IND3

SCFAs Impact Neutrophil Response to HIV Infection

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Introduction: Sexual transmission is the main route for human immunodeficiency virus (HIV) acquisition in women. Local innate immune mechanisms in the female reproductive tract (FRT), such us epithelial cell barrier, mucus and secreted immune mediators, contribute to the prevention of HIV infection. Neutrophils participate in mucosal protection against pathogens through phagocytosis, release of granule contents, and NETosis. NETosis is characterized by the release of Neutrophil Extracellular Traps (NETs), consisting of DNA associated with granular proteins with antimicrobial activity. Neutrophils are abundant in the FRT and can recognize, entrap, and inactivate HIV with NETs *in vitro*. Growing evidence indicates that bacterial vaginosis, alterations in vaginal microbiota mainly due to a reduction in relative abundance of *Lactobacillus* species, decreases the concentration of lactic acid and increases short-chain fatty acids (SCFAs) concentration, promoting colonization of harmful bacteria and increasing the risk of viral infections, such as HIV. Importantly, neutrophils highly express GPR43, the main SCFA receptor. In this context, we hypothesize that neutrophil-microbiota interactions impact innate immune protection in the FRT and the risk for HIV infection. **Methods**: Human purified neutrophils from blood were plated and stimulated with 25 mM of sodium acetate, sodium butyrate or sodium propionate, in the presence or absence of HIV-viral like particles (VLPs)-GFP labeled for at least 3h. NETosis was quantified as capture area (overlap of NETs and HIV-VLPs) using time-lapse microscopy Incucyte S3 system. Neutrophil migration was assessed stimulating neutrophils with 25 mM of the correspondent SCFA, using a transwell migration assay. Neutrophil phenotype was determined by flow cytometry using Cytek Aurora analyzer. **Results**: Pathological concentrations of SCFAs (butyrate or propionate) induced in neutrophils a sustained and stronger release of NETs in response to HIV-VLPs compared to untreated neutrophils. Although SCFAs can be chemoattractants for neutrophils, they migrated at the same ratio when stimulated with acetate and propionate, compared to controls; or significantly less in the presence of butyrate. Interestingly, only propionate-treated neutrophils significantly upregulated high levels of ICAM-1 after migration, a key molecule for transmigration into tissues. In addition, SCFAs significantly increased the proportion of $CD66b^+$ $CD16^+$ and $CD62L^+$ neutrophils. Finally, stimulation with SCFAs altered cytokine and chemokine secretion. **Conclusions**: Our results demonstrate that increased concentrations of SCFAs trigger neutrophil activation and alter their response to HIV infection. Ongoing experiments will shed light about the role of SCFAs on modifying the function of tissue-derived neutrophils in protecting the FRT.

IND4

Elucidating the Role of Host Purinergic Signaling During Rotavirus Infection

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Introduction: Diarrheal diseases are among the top morbidity in children worldwide, with an estimated 1.7 billion cases of diarrhea and resulting in ~525,000 deaths each year. The enteric virus, rotavirus, accounts for ~27% of diarrheal disease in children, however the current pediatric suitable treatment is oral rehydration solution which does not alleviate diarrhea volume or symptoms. This highlights the need to better understand the pathophysiology of viral diarrhea to develop new therapeutics. Rotavirus infects a limited number of cells at the tips of the villi in the small intestine. Yet, rotavirus influences cell types far away from the site of infection. We recently identified, using monkey and human rotavirus, that rotavirus exploits host purinergic signaling via the

P2Y1 receptor to increase uninfected epithelial cell responses during infection. To assess if this dysregulation during rotavirus infection is conserved among all rotavirus strains and relies upon purinergic signaling, we used the recently developed cell culturable murine-like D6/2 rotavirus to assess the role of purinergic signaling *in vitro* and *in vivo*. **Methods:** Using MA104 cell lines that stably express cytosolic genetically-encoded calcium indicators, we characterized calcium signaling throughout D6/2 rotavirus infection by time-lapse imaging and determined if purinergic signaling is required using various small molecule signaling inhibitors. **Results:** *In vitro*, we found that D6/2 infected cells have increased intracellular calcium signals which results in intercellular calcium waves that originate from the infected cells to neighboring cells. Preliminary data shows removal of ADP by Apyrase or blocking the P2Y1 receptor by BPTU results in decreased intercellular calcium waves, suggesting D6/2 infection relies upon purinergic signaling similar to other mammalian rotaviruses. Furthermore, treatment of D6/2 infected neonatal mice with a P2Y1 blocker (MRS2500) results in decreased incidence of diarrhea by day 4 post infection, confirming that P2Y1 receptor is also involved in rotavirus pathogenesis of a homologous virus strain. **Conclusion:** Collectively, these findings point to the conserved role of purinergic signaling and calcium waves in the pathophysiology of rotavirus infection.

IND5

***Klebsiella pneumoniae* in the Colonic Mucus Layer Influences *Clostridioides difficile* Pathogenesis**

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Introduction: *Clostridoides difficile* is an intestinal pathogen responsible for 500,000 infections and 30,000 deaths annually in the US. Intestinal microbiota disruption allows *C. difficile* to colonize the colonic mucus layer; however, the mechanisms by which other microbes influence *C. difficile* remains unclear. Using stool-seeded bioreactors with mucus-coated inserts, we identified *Klebsiella pneumoniae* in the mucus-associated microbiota with *C. difficile*. **Hypothesis:** We therefore hypothesized that *K. pneumoniae* promotes *C. difficile* intestinal colonization. **Methods & Results:** Analysis of 77 *C. difficile* genomes revealed that no *C. difficile* strains harbored the glycosyl hydrolase (GH) families required for mucin degradation. In contrast, 274 *K. pneumoniae* genomes harbored at least 3 mucin-degrading GH families. To confirm the ability of *K. pneumoniae* to degrade mucus, we grew 11 strains of *K. pneumoniae* in a fully defined bacteria media lacking glucose, supplemented with porcine intestinal MUC2 mucus. All strains exhibited robust growth, indicating that *K. pneumoniae* can enzymatically degrade mucus and use it as a primary carbon source. Under the same growth conditions, *C. difficile* was unable to grow. However, *C. difficile* was able to use freely available mucin glycan oligosaccharides (sialic acid, fucose, galactose, GluNAc, and GalNAc) when supplemented into glucose free media. Growth curve revealed that cell-free supernatant from *K. pneumoniae* strains enhanced the growth of *C. difficile* strains, suggesting that *K. pneumoniae* could enhance *C. difficile* growth. Moreover, time-lapse microscopy of LifeAct expressing Vero cell rounding revealed that *K. pneumoniae* cross-fed *C. difficile* and suppressed toxin production. Select *K. pneumoniae* strains produce substantial biofilm. To examine biofilm production, we grew *C. difficile* with *K. pneumoniae* for three days and stained with crystal violet. Interestingly, we observed that some, but not all combinations of *C. difficile* with *K. pneumoniae* increased biofilm production when compared to *C. difficile* alone. Finally, we examined how *K. pneumoniae* impacts *C. difficile* infection *in vitro* using colonic organoids by RNA sequencing. **Conclusions:** These results suggest that *C. difficile* and *K. pneumoniae* interact with one another, which impacts the pathogenicity of *C. difficile.*

Cardiovascular Pathobiology

VASC1

Neuropilin-1 Regulates Vascular Smooth Muscle Cell Contractility and Blood Pressure

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Introduction: Neuropilin-1 (NRP1) is a transmembrane receptor present in vascular smooth muscle cells (VSMC) that binds to the Class 3 Semaphorin (SEMA) ligand SEMA3A. NRP1 mediates the inhibition of RhoA signaling, however, its role in VSMCs and blood pressure regulation is unknown. **Hypothesis:** We hypothesize that loss of NRP1 in VSMC mitigates SEMA3A-induced RhoA inhibition, thereby increasing vascular tone and blood pressure *in vivo*. **Methods:** To study the role of NRP1 in SMCs, we generated mice with inducible, smooth muscle cell-specific deletion of NRP1 (SM22a-Cre^{ERT2} X Nrp1$^{flox/flox}$). Following recombination using 4-hydroxy tamoxifen (SM-NRP1 KO) in male and female adult mice (8-12 weeks), systolic blood pressure (SBP) was measured using a tail cuff and compared to age- and sex-matched mice that did not receive tamoxifen (control). Aortic vascular reactivity to contractile agonists and expression of key proteins in the RhoA signaling cascade were measured using ex vivo tension myography and western blotting, respectively. **Results:** SBP was significantly increased in SM-NRP1 KO mice following recombination compared to control mice (SBP: 136.5 ±10.9 vs 112.9 ±5.6 mmHg; p=0.0006). Aortas of SM-NRP1 KO mice displayed significantly enhanced contractile response to phenylephrine, KCl, and the thromboxane agonist U44619. In support of the increased contractility, expression of total myosin light chain and LIMK-2 proteins were increased in SM-NRP1 KO compared to control aortas. *In vitro*, treatment of murine primary VSMC expressing NRP1 with SEMA3A decreased angiotensin II-induced Rho-GTP activation. Additionally, control and SM-NRP1 KO mice (starting at 2 weeks post-recombination) were administered angiotensin II (490 ng/kg/day) for 4 weeks. While there was no significant difference in SBP at weeks 1 and 2, SM-NRP1 KO mice had significantly lower SBP at weeks 3 and 4 following angiotensin II infusion compared to controls (Week 4 SBP: 150 ±1.4 vs 130.5 ±2.5 mmHg; p=0.02), suggesting a low ejection fraction and cardiac dysfunction in these mice. In support of this observation, gene expression of atrial natriuretic peptide was increased (p=0.06) in hearts of angiotensin II-infused SM-NRP1 KO mice. **Conclusion:** Together, our data point to the role of NRP1 as a novel regulator of basal vascular tone and blood pressure, and the loss of NRP1 promotes the onset of hypertension and exacerbates cardiac dysfunction.

VASC2

Loss of the Smooth-muscle-cell-Angiotensin II-sensitive (SAS) lncRNA Triggers VSMC Hypertrophy and Cell Cycle Arrest: Application to Hypertension and Aortic Stiffness.

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Introduction: The reactivation of the cell cycle and increase in proliferation rate (hyperplasia) is a common response of vascular smooth muscle cells (VSMC) to modifications of their environment. VSMC can also increase the mass on the remodeled vessel wall by enlarging their size and becoming hypertrophic. That process has been observed in the aorta of hypertensive mice and response to Angiotensin-II (Ang-II). Hypertrophy is usually accompanied by an increased number of binucleated cells, defects in cell division mechanisms, and senescence. However, the molecular mechanisms favoring VSMC hypertrophy vs. hyperplasia in response to Ang-II and their repercussion on SMC phenotype are not fully understood. **Methods:** We performed RNAseq analysis on an irreversible epigenetically dedifferentiated VSMC line. Among more than 40 differentially expressed long-non-coding-RNAs (lncRNA), we characterized the novel lncRNA-SAS (Smooth-muscle-cell-Angiotensin II-sensitive). We tested the expression of SAS by RT-qPCR in VSMC in response to dedifferentiation factors such as PDGFB-B and Ang-II. Using GapmeR

inhibitor technology, we transiently silenced SAS expression in VSMC and tested proliferation, cell cycle, and mitochondrial function changes. **Results:** SAS is preferentially expressed in VSMC-rich tissues, including the aorta, in humans and mice. Yet, the functional relevance of this lncRNA on VSMC function has never been investigated. We observed SAS expression was significantly lost in response to Ang-II treatment in VSMC compared to other dedifferentiation treatments. Gapmer-mediated knockdown of SAS potently reduces proliferation and migration in aortic and renal artery-derived VSMC. Interestingly, SAS knockdown led to VSMC hypertrophy and increased number of binucleated cells, suggesting a defect in cell division and cytokinesis. Decreased SAS expression arrested the VSMC cell cycle and promoted senescence (higher number of β-Gal+ cells), recapitulating the phenotype observed in Ang-II treated VSMC. **Conclusions:** Altogether, our results show that SAS is a potent regulator of VSMC morphology and is required for proper cell division. Ongoing experiments test the role of SAS in the development of systemic hypertension and aortic stiffness-associated vascular remodeling.

VASC3

Specific Role of Endomucin in Vascular Endothelial Growth Factor Receptor 2 (VEGFR2) Internalization and Function

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Introduction: Endomucin (EMCN) is a type I integral membrane glycoprotein selectively expressed by endothelial cells in venous and capillary. We have previously showed that EMCN knockdown significantly inhibits VEGF165-induced VEGFR2 internalization and endothelial cell migration, proliferation, and tube formation. The goal of this study is to further define the specificity of EMCN for the VEGF/VEGFR2 system by determining the role of EMCN in VEGF121-induced VEGFR2 activation and migration, VEGF165-induced VEGFR1 internalization, as well as fibroblast growth factor (FGF)-induced cell migration and receptor internalization. **Methods:** EMCN was knocked down in human retinal endothelial cells (HRECs) using siEMCN, with non-targeting siRNA as a control. siEMCN significantly reduced EMCN protein levels compared to the non-targeting siRNA group by 95% ($P<0.05$). Endothelial cells (EC) migration was assessed in a scratch wound healing assay. **Results:** VEGF165, VEGF121 and FGF stimulation significantly increased HRECs wound closure compared to control (1 ± 0.02 vs. 1.15 ± 0.02, $p=0.004$; 1 ± 0.02 vs. 1.18 ± 0.03, $p<0.0001$. 1 ± 0.03 vs. 1.25 ± 0.04, $p<0.0001$; $n>3$ for all groups). EMCN knockdown prevented HRECs migration induced by VEGF165 (1 ± 0.03 vs. 1.04 ± 0.03, $p=0.9$, $n=3$) and VEGF121 (1 ± 0.03 vs. 1.07 ± 0.02, $p>0.05$, $n=3$), but not FGF induced migration (1 ± 0.03 vs. 1.18 ± 0.05, $p<0.0001$, $n=6$), compared to control. Receptor internalization was examined by cell surface biotinylation assay and quantified by Western blot. EMCN depletion prevented VEGF-165 induced VEGFR2 internalization (0.73 ± 0.32 vs. 0.71 ± 0.29, $p=0.74$, $n=7$) but did not impact VEGFR1 (1.50 ± 0.12 vs. 0.73 ± 0.11, $p<0.001$, $n=6$) or FGF-induced FGFR1 internalization (1.03 ± 0.16 vs. 0.73 ± 0.12, $p<0.05$, $n=7$). **Conclusion:** We conclude that EMCN is essential for VEGF165- and VEGF121-induced EC migration and VEGR2 internalization. However, EMCN does not play a significant role in VEGFR1 internalization or FGF-induced internalization and endothelial cells migration. Our data indicate a specific role for EMCN in the VEGF/VEGFR2 system.

VASC4

Obesity Inhibits Angiogenesis Through TWIST1-SLIT2 Signaling

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Introduction: Angiogenesis is required for functional adipose tissue remodeling and expansion. Physiologically balanced adipogenesis and angiogenesis are inhibited in subcutaneous adipose tissue in obese humans. Transcription factor TWIST1 controls angiogenesis and vascular function. TWIST1 expression is lower in obese adipose tissues. However, the role of endothelial TWIST1 in the impairment of angiogenesis in obese adipose tissue remains unclear. **Methods and Results:** Here we found that the levels of TWIST1 and the guidance molecule SLIT2 that also controls angiogenesis are lower in endothelial cells (ECs) isolated from obese (BMI >30) human subcutaneous adipose tissues compared to that from lean (BMI <30) individuals. Overexpression of TWIST1 restores SLIT2 expression in obese adipose ECs. Angiogenic activities such as EC migration and DNA synthesis are inhibited in obese human subcutaneous adipose ECs compared to lean adipose ECs, while TWIST1 overexpression restores the effects. When we examine the effects of obesity on vascular formation using subcutaneous implantation of fibrin gel supplemented with lean vs. obese human ECs on mice, obese adipose ECs inhibit blood vessel formation in the gel. Overexpression of TWIST1 in obese ECs restores blood vessel formation in the gel, while SLIT2 knockdown inhibits the effects. **Conclusion:** These findings suggest that obesity impairs adipose tissue angiogenesis through TWIST1-SLIT2 signaling. Modulation of TWIST1-SLIT2 signaling in ECs could be a novel therapeutic strategy for obesity and obesity-associated diseases.

VASC5

COVID Coronary Vascular Thrombosis: Correlation with Neutrophil But Not Endothelial Activation

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Introduction: Severe COVID-19 infection increases the risk of myocardial injury that contributes to mortality. Here, we investigated the potential causes of cardiac injury. **Methods:** We used multiparameter immunofluorescence to examine extensively heart autopsy tissue of 7 patients who died of COVID-19 compared to 12 control specimens, some with and some without cardiovascular disease. **Results:** Consistent with prior reports, we found no evidence of viral infection or lymphocytic infiltration indicative of myocarditis but did observe frequent and extensive thrombosis in large and small vessels in the hearts of the COVID cohort, findings that were infrequent in controls. The endothelial lining of thrombosed vessels typically lacked evidence of cytokine-mediated endothelial activation, assessed as nuclear expression of transcription factors p65 (RelA), pSTAT1, or pSTAT3 or evidence of inflammatory activation assessed by expression of intracellular adhesion molecule-1 (ICAM-1), tissue factor, or von Willebrand factor (VWF). Intimal EC lining was also generally preserved with little evidence of cell death or desquamation and these did not co-localize with thrombi. In contrast, there were frequent markers of neutrophil activation within myocardial thrombi of COVID-infected patients including neutrophil-platelet aggregates, neutrophil-rich clusters within macrothrombi, and evidence of neutrophil extracellular trap (NET) formation. **Conclusions:** These findings point to alterations in circulating neutrophils rather than the endothelium as contributors to the increased thrombotic diathesis in the hearts of COVID-19 patients.

VASC6

H3K4 Di-methylation Governs Smooth Muscle Lineage Identity and Promotes Vascular Homeostasis by Restraining Plasticity

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Introduction: Dynamic and reversible phenotypic modulation of vascular smooth muscle cells (SMC) between the contractile state and the dedifferentiated state plays a central role in maintaining vascular

homeostasis. Yet mechanisms of retaining lineage identity and allowing for reacquisition of the quiescent contractile state during reversible dedifferentiation remain unknown. H3K4 di-methylation (H3K4me2) on SMC contractile genes is a lineage-specific epigenetic signature stably retained in both contractile and dedifferentiated SMC, suggesting it could serve as a lineage memory mechanism. Here we aimed to determine the functional relevance of the stable H3K4me2 signature in mature SMC. **Methods:** We employed the loss-of-function strategy by designing a locus-specific H3K4me2 editing system to selectively demethylate H3K4me2 on SMC contractile gene subset *in vitro* and *in vivo*. We then characterized the biological consequences in H3K4me2-edited SMC by performing a combination of epigenome profiling, transcriptome profiling, functional assays *in vitro*, and SMC fate mapping *in vivo*. **Results:** We discovered that selective removal of H3K4me2 from the SMC contractile genes led to a marked loss of contractility and alteration of SMC-mediated vascular remodeling capacities upon injury due to loss of miR-145 expression. We found that H3K4me2 editing was associated with increased DNA methylation levels caused by impaired recruitment of DNA demethylase TET2 on SMC contractile genes. Mechanistically, we revealed TET2 directly and preferentially interacted with H3K4me2, indicating H3K4me2 served as a lineage-specific footprint for the dynamic TET2 recruitment in SMC. Finally, H3K4me2 editing induced a profound alteration of SMC lineage identity by redistributing H3K4me2 towards genes associated with stemness and developmental programs, thus exacerbating plasticity as characterized by the greater ability of H3K4me2-edited SMC to transdifferentiate into other lineages upon stimulation. **Conclusions:** We identified H3K4me2-TET2 as a central epigenetic mechanism controlling SMC lineage identity and specialized functions, whose alteration could contribute to various pathophysiological processes.

VASC7

SNAP29 Restricts Cardiac Arrhythmias by Insulating a Subset of Desmosomal Proteins and Connexin43 from Autophagic Degradation

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Introduction: Desmosomes are strong cell-cell adhesion junctions that connect the cytoskeleton of one cell to another to maintain the mechanical integrity of tissues under constant stress, such as the heart. Classically, human genetic studies and mouse models have linked mutations/deficiencies in components of the desmosome to arrhythmogenic right ventricular cardiomyopathy (ARVC), an inherited cardiac disease of the desmosome that impacts cardiac structure/morphology. However, growing evidence points to a role for loss of desmosomal integrity in cardiac electrical diseases (eg., Brugada syndrome, long QT, sinus node arrhythmias) that have no impact on cardiac structure/morphology; however, how desmosomal protein loss results in these distinct forms of the cardiac disease remains unclear. **Methods:** We exploited an unbiased yeast-two-hybrid screen using the desmosomal protein, desmoplakin (DSP) as a bait, to uncover novel desmosomal protein-protein interactions that may help further explain the pathobiology (structural versus non-structural) encompassed by desmosomal mutations/loss. **Results:** Through this screen, we identified the SNARE protein, synaptosomal-associated protein 29 (SNAP29), as a novel DSP-interacting protein. Traditional functions of SNAP29 are to regulate membrane fusion and play a role in autophagy; however, its role at the desmosome and in the heart is undefined. We hypothesized that SNAP29 is a new subcomponent of the cardiac desmosome that regulates desmosomal protein degradation and thus, may have important roles in desmosomal-based diseases. We show that SNAP29 co-localizes with desmoplakin in the adult mouse heart and that adult cardiac-specific DSP deficient mice harbor loss of cardiac SNAP29, validating SNAP29 as a subcomponent of the cardiac desmosome. By generating novel cardiomyocyte-specific SNAP29 knockout (SNAP29-cKO) mice, we show that SNAP29-cKO mouse hearts displayed baseline and pacing-induced ventricular arrhythmias in an age-dependent manner in the absence of cardiac structural and functional deficits. Molecular analyses further revealed that adult SNAP29-cKO hearts displayed a molecular loss of a subset of desmosomal (DSP, plakophilin2) as well as gap junction (connexin43) proteins when compared to controls. These cell-cell junction defects were accompanied by an accumulation of autophagic markers, as well as machinery specifically at the cell-cell junction in SNAP29 deficient cardiomyocytes, which functionally resulted in cardiac arrhythmias. We show that acute blockade of autophagy was sufficient to rescue levels of desmosomal and gap junction proteins as well as arrhythmias in SNAP29 deficient cardiomyocytes. **Conclusion:** In summary, our data suggest that SNAP29 insulates a subset of desmosomal and gap junction proteins from selective autophagy-mediated degradation to restrict cardiac arrhythmias and that loss of SNAP29-desmosome-gap junction interactome may predispose the heart to desmosomal based diseases of an electrical (non-structural) nature.

Inflammation and Immunopathology

INFL1

Adipose Tissue Role in Neonatal Sepsis.

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Introduction: The role of adipose tissue (AT) in the course of infectious diseases such as sepsis remains underappreciated. However, AT role has emerged as an important source of endocrine and immunologically active cell populations with multiple effects on homeostasis regulation. Alterations in the systemic and tissue environment can be recognized by adipocytes, leading them to respond to bacterial, fungal, or viral components and promoting altered immune cell responsiveness, metabolic pathways, and inflammatory vigor. Thus, our goal was to investigate the role of the white (WAT) and brown adipose tissue (BAT) depots and physiological outcomes during neonatal sepsis. **Methods:** Postnatal day 5 (PD5) CD1 mice were I.P. treated either with saline (Control), LPS, or Pam3CSK4, weight and temperature were accessed, and pups were euthanized after 3 hours. Subcutaneous WAT and BAT depots were dissected out and snap-frozen in N2. Total RNA was extracted with TRIzol Plus PureLink™ RNA Purification Kit. Clariom™ S Assay mouse and the Affymetrix GeneChip Mouse Gene ST 2.0 arrays were performed for transcriptome analysis. Differential gene expression was analyzed using a cutoff of 2-fold change and P-value of 0.01 and t-test in comparison to the control. **Results:** Systemic inflammation promoted either by Gram-positive or Gram-negative infection resulted in significant weight loss gain. The cutaneous temperature remained without significant changes in the control, whereas LPS induced a major variation of -1.5°C, and -0.5°C followed by Pam3CSK4. Within the decreasing body temperature and mass, we set out the hypothesis the AT is contributing to these alterations. To this end, a total of 103 genes were upregulated by LPS exposure, and 238 genes following Pam3CSK4 in BAT, while 85 and 249 genes were downregulated, respectively. The enriched terms network showed 3 clusters in the LPS group, in which, Collagen-containing extracellular matrix, Hippocampus development, Inflammatory response were the tops GO enriched in BAT. Pam3CSK4 promoted GO enrichment of Blood coagulation, mRNA metabolic process, Condensed chromosome, and Chromocenter showing 5 networks clusters. In WAT, 153 and 204 genes were upregulated by LPS and Pam3CSK4, respectively, while 299 and 402 were downregulated. GSEA followed by LPS revealed Chemokines Receptors, Action Potential, and C1q Complex Response highly enriched. In the network GSEA following Pam3CSK4, solely 2 clusters were observed. Cluster 1 presented a strong network among Lipid Catabolic process, Lipase activity, phenol-containing compound metabolic process and temperature homeostasis. **Conclusions:** Collectively, these findings highlight the heterogeneity of AT depots in response to the systemic inflammatory insult along with dysregulated temperature and decreased bodyweight control. Thus, in the course of neonatal sepsis, AT involvement can lead to important physiological outcomes for the newborn.

INFL2

Differentially Methylated Positions After IL-6 in the Endothelium

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Introduction: Interleukin 6 (IL-6) is a major mediator of the septic cytokine storm, leading to multiorgan dysfunction. While some direct actions of this cytokine in acute inflammation are being elucidated, little is known about long-term effects that may lead to post-intensive care syndrome (PICS). IL-6 activates JAK kinases to phosphorylate the transcription factor STAT3. We hypothesize that STAT3-mediated

endotheliopathy promotes changes in DNA methylation leading to long-lasting effects. Here, we sought to determine if the IL-6/STAT3 signaling axis altered the DNA methylation in the endothelium. **Methods:** DNA methylation was profiled in HUVEC and enrichment endothelial cells from kidneys in mice treated with LPS. To induce IL-6 signaling, cells were treated with a combination of IL-6 and sIL-6Rα (IL-6+R) for 6h and 72h. DNA was isolated and was bisulfite converted and applied to the Infinium Methylation EPIC arrays. Raw methylation data were analyzed using the ChAMP package in R, with the support of several auxiliary scripts. Gene expression was measured by RT-qPCR. **Results:** Our data suggest that an IL-6+R treatment resulted in significant modifications to the human methylome in endothelial cells. Unbiased clustering showed a separation between treated and control cells after 72h, with no significant changes after 6h. 360 CpG sites were significantly modified (FDR-adjusted p=<0.05), where 329 CpG sites were hypomethylated versus 31 CpG sites that were hypermethylated. This result shows that the time after exposure to IL6 is critical for the alteration of DNA methylation. Gene ontology analysis of the differentially methylated genes showed enrichment of pathways directly linked with the platelet and leukocyte interaction, cell proliferation and angiogenesis, and signaling. The annotated genes with a difference with hypomethylation between groups greater than 30% included 46 Differentially methylation positions (DMP). Among the 46 DMPs, 26 mapped to known genes, and 20 were located in intergenic regions. The annotated genes included genes involved in response to IFNα and inflammatory response (LAMP3), TNFα signaling via NFKβ (ABCA1 and RCAN1), regulation of cell division (MACC1) and TGFβ signaling (RAB31). 7 DMR with greater than 15% increase in methylation mapped to annotated genes associated with immune response (MYO10, ADAM19). Consistent with the demethylation of LAMP3 locus, IL-6+R induced 72h post-treatment a dramatic increase in LAMP3 expression. The same treatment induced an inhibition of ADAM19 and MYO10 gene expression, consistent with their hypermethylation status. Mice data showed DMP between LPS and control. **Conclusions:** Persistent activation of IL-6 leads to endothelial DNA methylation changes and corresponding gene expression differences. In mice, we're increasing the sample size. These differences may regulate pathways that can be associated with reduced long-term survival and prolonged organ damage as observed in PICS.

INFL3

Identification and Characterization of a First Series of Small Molecule Inhibitors of Feline Islet Amyloid Polypeptide Aggregation

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Introduction: In a majority of diabetic cats, amyloid deposits have been detected in the islets of Langerhans. These deposits originate from islet amyloid polypeptide (IAPP), a satiety hormone produced and co-secreted with insulin by beta cells. Islet amyloid deposits have been associated with beta cell death, thus contributing to the progression of diabetes. No inhibitors of feline IAPP aggregation are currently available to stop or prevent pancreatic amyloidosis in feline diabetes mellitus. We aim to design and prepare novel small molecules to stop the formation of pancreatic amyloidosis in diabetic cats. **Methods:** We prepared 155 small molecules to inhibit the aggregation of feline IAPP. Herein, we present the screening of these small molecules, focusing on one particular series (family) of about 28 molecules. The inhibition of feline IAPP fibril formation was assessed with thioflavin-T assays and confirmed with transmission electron microscopy. **Results:** Based on the screening of this series of molecules, we discovered three potent inhibitors of feline IAPP aggregation which delayed the lag time: KN-21-48, NBMI-19-24, MO-19-49. **Conclusions:** Identifying inhibitors of feline IAPP aggregation could yield insight into new therapeutic approaches to reduce beta cell death and slow progression of feline diabetes mellitus.

INFL4

Identifying the Targets of Perforin Mediated Killing

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Introduction: Upon activation, CD8+ effector T-cells develop cytotoxic function, suppress antigen presentation, and undertake pathogen clearance via perforin-mediated killing. However, the targets of the perforin-mediated killing remain unknown. In this study we developed a chimeric protein called Sensor of Perforin Ordained Targets (SPOT) to identify the targets of prf mediated killing. **Methods and Results:** The SPOT construct contains a mutant Prf which is expressed at WT levels but is nonfunctional. Excellent antibodies exist for human perforin, making flow cytometric detection of surface bound Prf feasible. However, the amount of perforin secreted onto the target cells in the physiological context would likely be limiting for efficient detection by flow cytometry. Therefore, the mutant prf is attached to a biotyinylating moiety-Turbo ID via a long linker. Adding Turbo-ID molecule, a bacterial biotinylase protein used for probing protein:protein interaction, would help biotinylate the surface proteins on target cells allowing for amplified detection via flow cytometry. This protein would be packaged inside cytotoxic granules of Prf deficient T cells and released appropriately and adhere to the target cells without disturbing the membrane integrity or delivering the Granzyme molecules into the cytoplasm. Using Site Directed Mutagenesis, we were able to demonstrate that a gain of glycosylation mutation in Prf renders the protein non-functional without interfering with its expression. The transgenic T-cells, transduced with the mutant prf demonstrated a remarkable loss of cytotoxic function as observed via 7AAD staining, when cultured with target cells pulsed with cognate antigen. **Conclusion:** We hypothesize that an optimized chimeric SPOT protein transgenically expressed in T cells or NK cells will reveal the normal *in vivo* targets of perforin-mediated killing after infection and elucidate the mechanisms of perforin-mediated immune regulation in unprecedented detail.

INFL5

Cationic Antimicrobial Peptide, E35, Reduces LPS-induced Inflammation in Mice

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Introduction: Cationic antimicrobial peptides (AMPs) are a promising approach against multidrug-resistant bacteria due to their membrane-disruption mechanisms, the lower propensity to invoke selection of resistance compared to conventional antibiotics, and bacterial killing properties that are typically not affected by the metabolic state of the bacterial cells. An engineered cationic AMP, E35, has been shown to be highly effective against multi-drug-resistant bacteria, and also can bind LPS in vitro. We tested whether E35 would reduce LPS-mediated inflammation in a mouse model in vivo. **Methods:** Male C57BL/6 (WT) mice were given no treatment, intravenous (IV via tail vein) E35 peptide alone (5mg/kg and 10mg/kg), intraperitoneal (IP) LPS (5mg/kg), LPS followed 30min later by E35 or E35 followed by LPS immediately. N=3-6/expm.gp. Blood was collected after 4h and TNFα, IL1β and IL-6 were measured by ELISA. Liver was also collected and MAPK activation (JNK, p38MAPK, ERK) measured by Western blot (WB) of whole liver lysates. **Results:** E35 peptide alone showed little or no effect on circulating inflammatory cytokines, but increased JNK activation in the liver at both 5mg/kg and 10mg/kg. LPS, as expected, significantly increased inflammatory cytokines and liver MAPK activation at 4h. Importantly, E35 given before LPS significantly reduced TNFα (p=0.03), and trended towards reduced IL1β (p=0.36) compared to LPS alone. Activation of p38MAPK in liver was also reduced in LPS+E35 mice compared with LPS alone. **Conclusions:** E35 peptide can reduce LPS-mediated inflammation when given before LPS. These data suggest that cationic AMPs may be beneficial to kill bacteria and reduce bacterial-mediated inflammation in sepsis.

INFL6

Perivascular Macrophages Prime Vascular Endothelial Cells to Promote Neutrophil Adhesion and Transendothelial Migration

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Introduction: Neutrophil (PMN) transendothelial migration in post-capillary venules is a key event in inflammatory responses against pathogens and the ensuing tissue damage. Perivascular macrophages (PVMs) have been previously suggested to promote PMN recruitment during bacterial skin infection via chemokine release. However, in the current study we identified a new mechanism where PVMs prime endothelial cell to regulate PMN transendothelial migration. **Methods:** We used intravital microscopy on lipopolysaccharides (LPS)-inflamed intestines to demonstrate that PVMs were critical for PMN migration and accumulation in the intestinal mucosa. **Results:** Anti-CSFR-1 antibody-based macrophage depletion significantly reduced PMN adhesion and transendothelial migration in inflamed intestines *in vivo*. Removal of macrophages also resulted in significantly lowered expression levels of endothelial cell ICAM-1, a major PMN adhesive receptor. Mechanistically, using murine and human endothelial cells and macrophages (bone marrow-derived and human THP-1 macrophages) we determined that TNF□ secreted by activated macrophages led t o a robust endothelial cell ICAM-1 induction and increased PMN retention and migration. Antibody-mediated neutralization of TNF□ in macrophage supernatants inhibited ICAM-1 upregulation and decreased PMN transendothelial migration. **Conclusion:** Our findings identify an important and clinically relevant new mechanism by which macrophages promote PMN recruitment in inflamed mucosa.

INFL7

Human L2 Regulates Leukocyte Transmigration at a Unique Step between those Regulated by PECAM and CD99

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Introduction: Inflammation is the innate response to tissue damage and infection. Transendothelial migration (TEM), arguably the most important step of inflammation, is the process by which leukocytes leave the bloodstream to enter inflamed tissues. PECAM and CD99 are adhesion molecules that are essential for the initiation and completion of TEM, respectively. Identification of the proteins involved and their mechanisms are imperative for resolving uncontrolled TEM, which is the cause of chronic inflammatory diseases such as cancer, arthritis, and multiple sclerosis. CD99L2 (L2) is a type-1 transmembrane protein required for TEM *in vivo*. Mice deficient in L2 have reduced leukocyte recruitment to sites of inflammation. **Methods and Results:** Intravital microscopy and the acute dermatitis model show that L2 is required for leukocyte extravasation across the endothelium specifically and not leukocyte adhesion or rolling. L2 function in inflammation has been extensively studied in mice. The relevance of L2 in human inflammation remains unknown. To characterize the role of L2 in human inflammation, we used *in vitro* TEM assays with primary human endothelial cells and leukocytes. Inhibition of L2 function using shRNAs or antibodies against L2 reduces TEM of monocytes and neutrophils to ~20-30%. TEM is restored upon crosslinking or re-expression of L2. Blockade of either leukocytes or endothelial cells blocks equivalently to blocking both. TEM is significantly reduced in the presence of soluble L2-Fc. **Conclusions:** These data are consistent with a homophilic mechanism of interaction. Our data also show that L2 functions at a step in TEM between steps regulated by PECAM and CD99. Similar to PECAM and CD99, L2 promotes transmigration by recruiting the lateral border recycling compartment to the site of TEM, specifically downstream of PECAM initiation. Ongoing studies are focused on identifying signaling pathways used by L2 to promote TEM and validating them *in vivo* using intravital confocal microscopy to study TEM in real time.

INFL8

Interplay Between TNFα-induced Inflammation and Metabolic Dysfunction in Retinal Pigment Epithelial Cells

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Introduction: Dysfunction of the retinal pigment epithelial cells (RPE) is a key feature of age-related macular degeneration (AMD), the leading cause of blindness in developed countries. Inflammation is a known pathogenic mechanism in AMD through complement system activation, upregulation of inflammatory cytokines and macrophage recruitment. A critical cytokine mediating inflammatory responses is tumor necrosis factor-alpha (TNFα), which has been implicated in the pathogenesis of AMD. Growing evidence supports metabolic dysfunction as another key mechanism driving AMD. To date, little is known about the metabolic effects of TNFα on RPE. Here we bridge the gap between the interplay of inflammation and metabolic dysfunction in RPE showing that the pro-inflammatory effects of TNFα are associated with a dramatic change in mitochondrial morphology, function, and a rewiring of the metabolic pathways. **Methods:** Matured ARPE-19 and primary human RPE cells (H-RPE, Lonza) were treated with TNFα (10 ng/ml). Glycolysis and oxidative phosphorylation (OXPHOS) were examined by high-resolution respirometry. Gene expression of EMT and metabolic markers were assessed using qPCR. The pro-inflammatory effect of TNFα was assessed by measuring IL-6 secretion using ELISA. ATP content was measured using a bioluminescent assay. Cells were stained with MitoTracker Orange and imaged using confocal microscopy to visualize changes in mitochondrial morphology. Ultrastructural features of mitochondria were assessed using transmission electron microscopy (TEM). **Results:** TNFα significantly upregulated the expression of pro-inflammatory cytokines and IL-6 secretion and induced RPE to elongate into spindle-shaped cells, reminiscent of epithelial-mesenchymal transition (EMT). However, gene expression analysis showed that TNFα repressed numerous EMT genes (Col1A1, α-SMA) indicating that the elongated cells were not mesenchymal in nature. Metabolic profiling by high-resolution respirometry revealed an increase in basal OXPHOS levels and increased glycolytic capacity, associated with increased ATP content. TNFα significantly upregulated expression levels of the mitochondrial antioxidant SOD2 and induced defects in both the mitochondrial network morphology and ultrastructure with loss of cristae integrity. **Conclusions:** Taken together, we show that TNFα-induced pro-inflammatory activation of RPE is associated with robust disruption of mitochondrial function, mitochondrial ultrastructure, and bioenergetic rewiring towards higher mitochondrial respiration and ATP production. Understanding the interplay between TNFα-induced inflammation and metabolic dysfunction opens new therapeutic avenues for druggable targets in treating AMD.

Epithelial and Mucosal Pathobiology

MUC1

***Giardia duodenalis* Alters Biochemical Properties of Intestinal Mucus During Infection**

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Introduction: The intestinal secretory mucin MUC2 is a highly glycosylated protein that is critical for intestinal barrier function and homeostasis. Mucin glycans are synthesized by glycosyltransferase enzymes via progressive addition of various sugar residues to form a polysaccharide chain. The composition of glycan chains and their terminal modifications dramatically influences physical and chemical properties of a mucin and overall mucus barrier function (Arike et al, 2017). Disruptions to mucin glycosylation patterns have been demonstrated during intestinal diseases including gastrointestinal cancers, inflammatory bowel disease, and enteric infections (Bergstrom et al, 2013). During *Giardia duodenalis* infections, intestinal barrier function is disrupted, resulting in an increase in intestinal permeability that may facilitate translocation of microbes and microbial antigens to trigger disease (Allain et al, 2020). To date, interactions between *Giardia* and mucin glycans have not been studied, and it is unknown if disruptions to mucosal glycosylation patterns may contribute to *Giardia* pathogenesis. **Methods:** 3-4 week-old C57BL/6 mice were infected with *Giardia duodenalis* strain GS/M for 7 days. Tissue sections from the jejunum and colon were collected and stained with various fluorescein-

coupled lectins (CONA, DBA, PNA, WGA, SNA, UEA-1) and fluorescence was quantified and normalized to tissue area. Quantitative PCR (qPCR) was performed for glycosyltransferase genes in the jejunum and colon. **Results:** The abundance of mucin-associated glycans was altered in the small and large intestines of *Giardia*-infected mice in comparison to controls at day 7 PI. In the jejunum, N-acetylglucosamine abundance was increased upon infection, while sialic acid and fucose abundance decreased. Conversely, mannose and sialic acid abundance increased in the distal colon. Although N-acetylglucosamine abundance was similar between infected and control mice, upon infection the distribution was altered, and abundance decreased in the epithelium but increased in the lumen. Expression of mucin-associated glycosyltransferase genes was also altered in the small and large intestines of *Giardia* infected mice. Gene expression of the Core 2 synthase C2GnT2 increased in both the jejunum and distal colon upon infection, expression of the sulfotransferase Chst4 decreased, and expression of both the fucosyltransferase Fut2 and the sialyltransferase St6GalNAc1 increased. **Conclusions:** Glycosylation patterns and the expression of glycosyltransferase genes are altered in the small and large intestines of *Giardia*-infected mice. These alterations represent a novel mechanism of pathogenesis and may contribute to *Giardia*-induced intestinal barrier dysfunction and dysbiosis. Additionally, disruption of intestinal mucus glycosylation may contribute to the pathogenesis of many intestinal parasites.

MUC2

Characterizing the Mucin-degrading Capacity of the Human Gut Microbiota

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Introduction: Mucus provides a critical barrier for the colonic epithelium by excluding microbes and luminal antigens from direct interaction with epithelial cells, providing a lubricant for stool, and limiting the diffusion of harmful compounds. This barrier function is due in part to the extensive glycan structures found in mucus. Mucin protein is heavily O-glycosylated with core structures containing α- and β-linked N-acetyl-galactosamine, N-acetyl-glucosamine, and galactose. These core structures are elongated and commonly modified by α-linked fucose, sialic acid and sulfate residues. The sialic acid and sulfate residues protect the underlying mucin glycans from degradation. However, some gut microbes harbor glycosyl hydrolases (GHs) that enzymatically degrade mucin glycans. The released oligosaccharides can then be used as a primary carbohydrate source for the mucus-associated microbiota. Despite the growing number of bacterial genome sequences available, our knowledge of the mucin-degrading capacity of human gut commensal microbes remains fragmented. The aim of the present study was to systematically examine the CAZyme mucin-degrading profiles of the human gut microbiota. **Methods and Results**: Using the CAZy database, we identified 13,156 genomes harboring at least one gene copy of GH families involved in mucin degradation. Commensal microbes were identified using the Human Microbiome Project consortium in the Joint Genomes Institute Integrated Microbial Genomes database, resulting in 4,385 genomes for downstream analysis. Within the Verrucomicrobia phlyum, all *Akkermansia glycaniphila* and *muciniphila* genomes harbored gene copies of sialidases (GH33), fucosidases (GH29, GH95), endo-β-1,4-galactosidases (GH2, 35), mucin core GHs (GH84 and GH89), endo-acting O-glycanases (GH16), and sulfatases (GH20, GH2, GH42); consistent with their known ability to degrade mucus. Interestingly, the only representative of the Lentisphaerae phylum, *Victivallales*, harbored a GH profile that closely mirrored *Akkermanisa*. In the Actinobacteria phlyum, we found that several *Actinomadura, Actinomyces, Bifidobacteria, Streptacidiphilus* and *Streptomyces* species contained gene copies of mucin-degrading GHs. Within the Bacteroidetes phylum, we identified mucin degrading GHs in *Alistipes, Alloprevotella, Bacteroides, Fermenitomonas Parabacteroides, Prevotella* and *Phocaeicola* species. Firmicutes contained *Abiotrophia, Blautia, Enterococcus, Paenibacillus, Ruminococcus, Streptococcus,* and *Viridibacillus* species with mucin-degrading GHs. Far fewer mucin-degrading GHs were observed in the Proteobacteria phylum, with only 3-4 GHs families found in *Klebsiella, Mixta* and *Enterobacter*. We confirmed the mucin-degrading capability of 26 representative gut microbes by growing these bacteria in a chemically defined media lacking glucose supplemented with porcine intestinal mucus. **Conclusions**: These data greatly expand our knowledge of mucin degradation within the human gut microbiota.

MUC3

***Annickia polycarpa* Extract Attenuates DSS-induced Colitis by Balancing Neutrophil Recruitment**

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Introduction: Inflammatory bowel diseases (IBD) are chronic inflammatory diseases of the gastrointestinal tract with an increasing incidence worldwide. IBD consists of Crohn's disease (CD) and ulcerative colitis (UC) and is characterized by intestinal epithelial barrier dysfunction, excessive neutrophil recruitment, and oxidative stress. Current treatment strategies involve anti-inflammatory drugs that often show adverse side effects thus warranting the need for safer alternatives. The chloroform fraction of *Annickia polycarpa* (APE) possesses potent antioxidant and anti-inflammatory activity. Thus, we hypothesized that APE could improve the outcome of murine colitis by ameliorating the chronic inflammatory response. **Methods:** We induced colitis using dextran sulfate sodium (DSS) and gavage-fed mice with the chloroform fraction of *Annickia polycarpa* daily for 7 days. Survival and disease activity index (DAI) consisting of symptoms such as weight loss, diarrhea, and perianal bleeding was determined daily. Subsequently, colon tissues were recovered for histological analysis using hematoxylin-eosin. mRNA levels of inflammatory cytokines in the colon were determined by quantitative real-time PCR. Neutrophil levels in the colon tissue were determined after immunofluorescence staining with anti-mouse Gr-1 antibodies; and oxidative stress was analyzed using the dihydroethidium assay. Finally, using the Evans-blue-based permeability assay, we determined epithelial permeability in the colon. **Results:** APE-treated mice had improved survival and significantly reduced DAI. DSS-induced colon tissue damage was ameliorated upon APE administration as manifested by reduced colon length shortening and edema formation, less leukocyte infiltration and preservation of colon crypts. Colon tissue expression of the proinflammatory cytokines TNF-α, IL-1β, and IL-6 were attenuated upon APE administration. Also, we observed that fewer neutrophils infiltrated the lamina propria of the colon tissue in APE-treated mice resulting in reduced oxidative stress. Consequently, we observed reduced intestinal permeability in the colon of APE-treated mice. **Conclusion:** In summary, daily administration of *Annickia polycarpa* reduces disease severity and improves survival during murine colitis by limiting neutrophil influx, inflammation, and oxidative stress in the colon. Thus, APE may serve as a therapeutic alternative to improve the management of IBD.

MUC4

***Giardia Muris* Infection Leads to Tuft Cell Hyperplasia at Later Stages of Infection**

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Introduction: Chemosensory enteric tuft cells (ETCs) can detect and respond to certain enteric parasitic infections. ETCs play important roles in modulating Th2 immune responses and in promoting parasite clearance (Nevo et al. *Immunol Lett*. 2019;210:1-9; Schneider et al. *Nat Rev Immunol*. 2019;19:584-593). They can utilize various luminal surface receptors to detect their surroundings including ligands which can be supplied directly by the parasite or indirectly *via* excretory/secretory products (Nevo et al. *Immunol Lett*. 2019;210:1-9; Schneider et al. *Nat Rev Immunol*. 2019;19:584-593). ETCs are characterized by the secretion of IL-25, which subsequently activates type 2 innate lymphoid cells (ILC2s), leading to tuft and goblet cell hyperplasia. To date, interactions between *Giardia*, a protozoan that causes intestinal barrier dysfunction (Allain et al. *Tissue Barriers*. 2017;5:e1274354), and ETCs has not been previously studied. In this study, we aim to characterise the tuft cell response to *Giardia muris* both during the acute and post-infectious phases of infection. **Methods:** C57BL/6 mice and tuft cell-deficient mice (*Pou2f3*$^{-/-}$) were orally gavaged with 5x10^4 *Giardia muris* trophozoites. Parasite burden was measured in the duodenum at days 4, 11, and 21 post-infection. Paraffin

embedded jejunum tissue sections were stained using antibodies for doublecortin-like kinase 1 (DCLK1), a tuft cell marker, at days 4, 11, and 21 post-infection. DCLK1$^+$ cells were quantified to assess tuft cell hyperplasia. The expression of genes involved in tuft cell sensing and activity were assessed using quantitative PCR (qPCR). **Results:** *G. muris* infected C57BL/6 mice displayed increased levels of tuft (DCLK1$^+$) cells in the jejunum at 21 days post-infection, but not at days 4 and 11 post-infection. At 21 days post-infection, infected mice showed an increase in *Dclk1* mRNA expression. Tuft knock-out mice (*Pou2f3*$^{-/-}$) showed decreased trophozoite burden during the acute phase of infection. Interestingly, *Pou2f3*$^{-/-}$ mice did not clear the infection at day 21 (post-infectious phase), compared to WT mice. **Conclusions:** Upon *G. muris* infection, tuft cell levels were increased during the later stages of infection, thus ETCs may play a role in mechanisms pertaining to the clearance or repair of infection. Tuft cells may contribute to the establishment of and in the timely clearance of *Giardia* infection, however the mechanism through which tuft cells sense and respond to this environment remains to be characterized.

MUC5

Transcorneal Electric Stimulation Therapy Induce Dry Eye by [Ca2+]i Depletion of Goblet Cells and Reduce Mucin Production

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Background: Electrical stimulation (ES) is a therapeutic approach for numerous diseases including glaucoma and diseases of the retina. It delivers a low-intensity electric current to target tissue non-invasively through the application of electrodes to the cornea's surface (transcorneal ES [tES]) or the skin of the eyelid (transpalpebral ES [tpES]). In a recent clinical study, while experiencing an increase in visual acuity, 37% of the study subjects complained symptoms of dry eye after undergoing tES. Dry eye symptom is a multi-factoral condition that involves tearfilm stability, chronic ocular surface inflammation, corneal nerve structure and many more. Unfortunately, the clinical studies done on human subjects did not measure any objective parameters of dry eye, making it hard to distinguish the cause of the dryness sensation. The propose of this study is to understand the mechanism of dry eye symptoms induced by ES, and investigate the effect of electric on the ocular surface. **Methods:** Female mice of C57BL6j, 12-week-old underwent tES in one eye for 14 days. At day 14, sodium fluorescence staining test was preformed and observed under slit lamp for measurement of dry eye level. Phenol red thread test was used for tear production. The animals were then be scarified and immunofluorescence staining of Mucin 4 (Muc4) was performed on fixed cornea tissue. Primary human conjunctival goblet cells underwent ES for 1h at 300μA stimulated with carbacholine (10^{-4}M) and change of intracellular [Ca^{2+}] ([Ca^{2+}]$_i$) was measured. **Results:** 14 days of tES, 4 min per day significantly increased the cornea florescence staining comparing to the adjacent eye. The sodium fluorescence staining was scored in superior, inferior, nasal, temporal and central regions of cornea, with no staining scored as 0, and a massive staining of florescence scored as 3. The scores of each region of the cornea are averaged to represent the score of dryness of the cornea. The score of sodium florescence stain in tES eye is 2.25 ± 0.19 while the adjacent eye scored 0.6 ± 0.24 (p=0.001), indicating that tES treatment could induce dry eye. The phenol red thread test for tear production showed no difference between tES eye and the adjacent eye (p=0.40), indicating that the dry eye induced by tES was not due to a lack of tear production. The immunofluorescence staining of the cornea whole mount showed a significant decreased signal of Muc 4, which indicated a decreased mucin production. 1h of ES in cultured goblet cells significantly down regulated the [Ca^{2+}]$_i$ increase induced by carbacholine (p=0.03). **Conclusion:** tES 4 min per day for 14 days induced dry eye in female C57BL6j mice. The dry eye induced by tES is possibly a result of reduced mucin production from depleting [Ca^{2+}]$_i$ signals. Transpalpebral may be a better delivery of ES in future clinical applications.

Liver Pathobiology

LIVER1

TLR4 Deficiency Alters Platelet-Derived Exosome Release and Increases Mortality in a Model of Liver Regeneration

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Introduction: Platelets have a strong role in liver regeneration; however, the exact mechanisms are still unknown. A novel mechanism proposes platelet-derived exosomes (PDE), small particles with high biological activity, to be activators of inflammatory and regenerative molecules via activation of the HMGB1/TLR4 pathway. We hypothesize that mice deficient of TLR4 will have decreased PDE release after 24hr polytrauma, resulting in decreased liver regeneration compared to WT mice. **Methods**: Following 24hr polytrauma to activate platelet HMGB1, PDEs were isolated from adult male WT and TLR4-KO mice using a kit-based preparation. Nanosight was used to assess exosome concentration and size, and PDE characterization was performed by ImageStream system. Adoptive transfer of 2x10^6 PDEs was carried out subsequent to 70% hepatectomy in WT and TLR4-KO mice. Hepatocyte proliferation was evaluated using Ki67 MUSE. Data is represented as mean±SEM, and statistical analysis was performed using one- and two-way ANOVA with Tukey's correction. **Results**: All exosomes were effectively CD41+CD63+, confirming their platelet origin. Nanosight analysis showed that following polytrauma, TLR4-KO mice have decreased PDE concentration with size remaining unaffected. Following hepatectomy and PDE injection, WT mice showed increased hepatocyte proliferation 48 hours after, while TLR4-KO did not show any proliferation and exhibited a >80% mortality rate. **Conclusions**: These data suggest that PDE release is dependent on HMGB1 signaling, and TLR4 deficiency severely increases mortality and halts hepatocyte proliferation following liver resection. Future studies will be aimed at understanding the high mortality in this model and to comprehend the exact mechanism behind the results. **Financial Support:** Department of Surgery Funding for RIMA, 1R35GM119526-01 from NIGMS to MDN

LIVER2

β-catenin Conditional Knockout Elicits Protection Against Lithocholic Acid-induced Cholestatic Injury in a Murine Model

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Introduction: Lithocholic acid (LCA) is a secondary hydrophobic bile acid (BA) with the potential to cause cholestatic liver disease. Our lab recently reported that liver-specific conditional loss or inhibition of β-catenin elicits protection and prevents the development of cholestatic liver injury after bile duct ligation (BDL). BA accumulation is the causal factor for both BDL and LCA-induced cholestatic injury. Therefore, we hypothesized that β-catenin conditional knockout would provide protection from LCA-induced injury as well. **Methods:** Age-matched wild-type control (Con) and β-catenin liver-specific knockout (KO) mice were fed 0.6% LCA diet or normal diet for 7 days and then euthanized. Liver histology and serum biochemistry were analyzed for parameters of cholestatic injury. Immunostaining was performed to analyze ductular reaction and immune response. Relative gene expression was assessed for regulatory bile detoxifying enzymes and transporters. **Results:** The KO mice had fewer and smaller necrotic areas as compared to the Con mice after LCA diet administration. Serum biochemical levels showed a significant decrease in biliary injury in the KO mice. Analyzing the BA homeostasis and transport genes revealed that KO mice had decreased BA uptake transporters, increased apical and basolateral efflux transporters, and increased expression of detoxifying cytochrome P450 enzymes. Surprisingly, the total BA levels in liver and serum were comparable between KO and Con mice. Interestingly, immunostaining for pan-cytokeratin showed increased ductular response in KO mice, which could be a defense mechanism for facilitating enhanced BA clearance. We also found a significant increase in the number of CD45-positive cells in the KO mice after LCA diet, indicative of an increased immune response. **Conclusions:** β-catenin conditional knockout improves the overall outcome and confers

protection from LCA-induced cholestatic injury despite insignificant alterations in BA accumulation. Instead, this protection may be accounted to increased number of ducts in the KO mice that expand the bile flow and help alleviate the load of bile accumulation. Alternatively, the increased number of lymphocyte population in KO mice may help clear the necrotic areas.

LIVER3

Mitigation of High-Fat Diet-Induced Liver Injury in MS-NASH Mice: Modifications in Proteomic Signature

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Introduction: Non-alcoholic fatty liver disease (NAFLD) is rapidly becoming the most important cause of liver damage in Western society. Non-alcoholic steatohepatitis (NASH) represents an advanced stage of NAFLD. Our previous long-term murine studies have shown the beneficial role of a red marine algae-derived multi-mineral (calcium, magnesium, multiple trace element-rich) supplement (Aquamin) in reducing liver injury and decreasing liver tumor incidence. The purpose of the present study was to determine how manifestations and effects of NASH can be mitigated by use of Aquamin. **Methods:** Two cohorts of MS-NASH mice were placed on a high-fat Western diet (HFWD) with and without mineral supplementation for a period of 16 weeks. A group of mice (C57BL/6) on regular chow was included as a control. During the in-life phase of the study, weight changes were assessed weekly. At the time of euthanasia, livers were assessed histologically for steatosis and fibrosis by using Sirius red staining for collagen deposition. Liver tissue samples were also evaluated using a tandem mass tag (TMT) mass-spectroscopic proteomic approach for protein expression levels in individual animals. **Results:** Mice on the HFWD gained more weight than animals on the control diet. However, there was no overall change in weight for mice fed a high-fat diet, irrespective of mineral intervention. On histological assessment, there was no difference in the steatosis between two high-fat groups but mice on Aquamin have shown reduced Sirius red staining as compared to placebo mice. Regarding the proteomic profile of liver samples, there was a clear distinction among control and high-fat groups. Mice fed a high-fat diet with Aquamin upregulated 75 moieties at 2-fold-change when compared to Placebo (high-fat diet alone) group. These altered proteins by Aquamin intervention led to upregulate 39 unique pathways with a p-value less than 0.05 as assessed by the Reactome database. Pathways significantly impacted by Aquamin intervention include but are not limited to the following: formation of cornified envelope; keratinization; gap junction assembly; type I hemidesmosome assembly; apoptosis-related pathways; plasma lipoprotein assembly, remodeling, and clearance; and hedgehog 'off' state pathways. **Conclusion:** Addition of these specific dietary minerals may have a protective role in interfering downstream advancement from steatosis to NASH, a serious manifestation of liver injury. This preliminary work warrants additional studies to pursue the role of these minerals in the context of high-fat diet-induced liver injury.

LIVER4

PDGF Increases Hepatocyte-HMGB1 Synergistically with Stimulation of Heparan Sulfate *in vitro* and Alters Hepatocyte Exosome Release In TLR4 Deficient Mice

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Introduction: Exosome release has unique effects depending on the tissue of origin. PDGF is a strong stimulant of multiple tissue processes like exosome release. Increase in HMGB1 via TLR4 receptor activation has been proposed to induce extracellular vesicle release. Our hypothesis is that Heparan Sulfate (a known TLR4 ligand) will be able to evidence PDGF dependence on TLR4 to promote exosome release in hepatocytes. **Methods**: Isolated primary WT and TLR4-KO hepatocytes were stimulated for 24hrs with PDGF, supernatant was evaluated with a Nanosight for exosome release quantification. Primary hepatocytes were also stimulated 24hrs with the following conditions: Heparan Sulfate, PDGF, and Heparan Sulfate+PDGF. Hepatocytes were lysed for protein quantification by western Blot (n=3). Data is represented as mean ± SEM. Statistical analysis was performed using one-way ANOVA with Tukey's correction. **Results**: Hepatocyte exosome concentration is increased *in vitro* in the presence of PDGF after 24hrs in WT hepatocytes. However, in TLR4-KO mice, exosome particle concentration did not differ from levels observed in unstimulated WT hepatocyte exosomes. As for exosome size, 24hr-PDGF stimulation increased WT hepatocyte exosome size, but not in TLR4-KO hepatocytes. Western blot analysis showed that the highest increase in HMGB1 protein levels is observed only after 24hr combined stimulation of both Heparan Sulfate and PDGF compared to individual stimulation. **Conclusions**: PDGF has a direct effect in hepatocyte exosome release whenever TLR4 signaling is intact and appears to be associated with the HMGB1 activation *in vitro*. Future studies will be directed to evidence the downstream HMGB1 pathway associated with PDGF-independent platelet stimulation. **Financial Support:** Department of Surgery Funding for RIMA, 1R35GM119526-01 from NIGMS to MDN.

LIVER5

Hepatic Autophagy Deficiency Leads to Exosome Overproduction and Pro-inflammatory Response

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Introduction: Autophagy is an evolutionarily conserved metabolic process and plays a homeostatic role to maintain a normal liver function. Autophagy deficiency induces a plethora of pathological changes including inflammation, fibrosis, metabolic disturbance, and tumorigenesis. We are interested in determining how the inflammation is induced under this condition. We hypothesized that exosomes could contribute to the process. Exosomes are defined as membrane-surrounded, nanometer-sized vesicles with an average size of 40 to 160 nm in diameter. Exosomes contain a serious of cargos including nucleic acids, proteins, and lipids, which can play critical role in cell-cell communications. **Methods and Results:** In the present study, nanoparticle tracking analysis (NTA) showed an increased production of exosomes in circulation under the condition of hepatic autophagy deficiency. To understand the functional role of these exosomes, they were incubated with macrophages derived from the bone marrow (BMDM), which can be recruited to the liver and contribute to the hepatic inflammation. We found that BMDM could effectively internalize the exosomes. Notably, the expression of a number of pro-inflammatory genes were increased in BMDM co-cultured with exosomes isolated from autophagy deficient mice, but not in BMDM co-cultured with exosomes isolated from wild type mice. Systemic sequencing of miRNA carried by these exosomes identified miR-3072-3p that can target to the inflammatory pathways. Indeed, BMDM transfected with miR-3072-3p mimics expressed a higher level of pro-inflammatory genes, suggesting that these cells can be activated by the exosomes via miRNA mediated signaling. **Conclusion:** Increased production of exosome with miRNA unique to the condition of autophagy deficiency can lead to the activation of inflammatory cells.

LIVER6

Histamine increases Hepatic and Intestinal Mast Cell Activation and Regulates Bile Acid Signaling During PSC

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Introduction: Primary sclerosing cholangitis (PSC) is characterized by ductular reaction (DR), impaired bile flow, altered bile acid (BA) composition and increased histamine (HA) levels/mast cell (MC) activation. Master BA regulator, Farnesoid X receptor (FXR), is dysregulated in PSC patients altering intestinal BA transport via apical sodium BA transport (ASBT) leading to irritable bowel disease (IBD). MCs secrete HA; catalyzed by l-histidine decarboxylase (HDC). $Hdc^{-/-}$ /$Mdr2^{-/-}$ (DKO) mice exhibit reduced DR, hepatic fibrosis, and total BA (TBA) compared to $Mdr2^{-/-}$ mice. Biliary BA toxicity is reduced via expression of fatty acid binding protein (FABP6). MCs and cholangiocytes both express FXR and ASBT and secrete fibroblast growth factor (FGFs), which are elevated in cholestatic liver disease and involved in BA enterohepatic circulation. **Aim:** To investigate HA regulation and MC activation of BA circulation and signaling during PSC. **Methods:** 16 wk old wild-type (WT), $Mdr2^{-/-}$ (PSC model), treated with NaCl or cromolyn sodium (MC stabilizer) via osmotic minipump for 1 week, and DKO mice, treated with NaCl or exogenous HA via osmotic minipump for 1 month, were used in this study. Serum, liver, small intestine, isolated cholangiocytes and cholangiocyte supers were collected from all groups. Serum and hepatic TBA, chenodeoxycholic

acid (CDCA) and cholic acid (CA) were measured in all groups. Hepatic BA signaling (FXR, SHP, Cyp7a1 and Cyp27a1) and biliary BA transporter (ASBT) expression were measured by *q*PCR in total liver or by immunofluorescence (IF), co-stained with CK-19 to mark bile ducts. Intracellular biliary BA binding protein was assessed by IF for FABP6, co-stained with CK-19. Serum and biliary FGF15 was measured by EIA and IF, co-stained with CK-19. Intestinal MC activation (chymase, tryptase and Fcεr1α) was measured in liver and small intestine via *q*PCR, IHC, and EIA. **Results:** Mdr2$^{-/-}$ mice had elevated (i) serum and hepatic TBA, CDCA and CA, (ii) serum and biliary FGF15, and (iii) biliary FABP6 and ASBT compared to WT, which were reduced in DKO and Mdr2$^{-/-}$ + cromolyn mice. Exogenous HA increased all these parameters in DKO mice compared to WT and DKO. BA signaling was dysregulated in Mdr2$^{-/-}$ and DKO + HA mice compared to WT, DKO, and Mdr2$^{-/-}$ + cromolyn. There was increased MC presence and activation in liver and small intestine of Mdr2$^{-/-}$ compared to WT, DKO, and Mdr2$^{-/-}$ + cromolyn. Exogenous HA increased intestinal BA transport and MC activation markers in DKO mice. **Conclusion:** MCs infiltrate the intestine and liver during cholestatic liver disease and lead to increased hydrophobic BA and TBA pool, FGF15 secretion, increased enterohepatic BA circulation and biliary BA cholehepatic shunting. Inhibition of MC activation may provide therapeutic intervention for patients with PSC and IBD co-morbidities.

LIVER7

Durable Persistence of the Fetal Hepatocyte Phenotype After Liver Cell Transplantation

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Introduction: Chronic Liver Disease (CLD) is a leading cause of death in the US and is increasing in incidence for adult and pediatric populations. The only current clinical treatment is orthotopic liver transplantation, which is hindered by the availability of donor livers suitable for transplant. Hepatic cell transplantation offers a potential alternative by using isolated cells, thereby relaxing reliance on whole livers. While adult hepatocytes have shown a limited window of efficacy, fetal hepatocytes have demonstrated in animal models and human clinical trials to durably persist and proliferate and correct hepatic deficiencies. Due to ethical and practical limitations sourcing fetal human hepatocytes, we are striving to understand the mechanisms by which fetal cells repopulate an injured liver, with the goal of enabling this phenotype in adult hepatocytes or induced cells. Our study examines histone post-translational modification (hPTM) and gene expression profiles of post-transplantation colonies, as well as those of primary fetal and adult liver cells. **Methods:** We used the Dipeptidyl Peptidase IV rat model to transplant fetal hepatocytes into adult hosts. After 10 months, livers were flash-frozen and cryosectioned. Using an enzyme activity stain and laser capture microdissection, we isolated sections of fetal-derived colonies and surrounding adult host hepatocytes. Separately, primary adult and fetal rat hepatocytes were isolated or immunopurified using a hepatic lineage marker. Histones and RNA were extracted from these tissue and cell samples for hPTM abundance quantification and RNA-Seq. Data was analyzed using R. **Results:** We have identified 13 distinct marks on Histone H3 that have significant differences in relative abundance in fetal-derived colonies vs. host tissue, as well as in fetal vs. adult hepatocytes. Our RNA-Seq study clustered post-transplant samples by their type (colony or host) rather than by biological replicate and resulted in 1,046 differentially expressed genes (DEGs) between colony and host. Ontological analysis of the gene set expressed higher in host tissue showed enriched metabolic processes, while the genes expressed higher in colonies showed enriched ion transmembrane transport. **Conclusions:** In both hPTM and mRNA expression, a distinct profile common to pre-transplant fetal cells is retained for at least 10 months following transplantation into an adult liver microenvironment. The 13 significantly different histone marks were all on Histone H3, which has known associations with gene transcription. The 186 DEGs in the host tissue population correspond to the incomplete metabolic maturity of the transplanted cells, while the 860 DEGs in the colonies reflect activities associated with their continued growth and proliferation activity. Our future work will explore the link between specific differentially expressed genes and the differentially abundant histone marks that may be regulating them.

LIVER8

Transcriptional Correlation Analysis of Livers Undergoing Normothermic Machine Perfusion Links Gene Expression Patterns with Graft Functional Metrics

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Introduction: Normothermic machine perfusion (NMP) allows dynamic organ preservation under physiological conditions and facilitates real-time assessment of graft viability for transplant. The aim of this study was to delineate transcriptional patterns that correlate with liver functional metrics during NMP. **Methods:** Ten discarded human livers, rejected by all regional transplant centers, were obtained from donation after circulatory death (DCD). The livers underwent 12 hours of NMP with oxygenated red blood cells. Serial tissue and plasma samples were collected. Perfusate lactate clearance <2.5 mmol/L was used to denote livers with adequate hepatocellular function. Transcriptome sequencing was performed on serial biopsies taken at 0 (pre-perfusion), 3, and 6hr of NMP. Weighted gene co-expression network analysis (WGCNA) was performed to correlate each gene set module with functional metrics measured during NMP. Functional enrichment analysis was performed on modules of interest. **Results:** Expression data at 0, 3 and 6 hours of NMP was used to construct the weighted co-expression network. The WGCNA package was then used to correlate each gene set module with various liver demographics and functional data collected during NMP. Genes from the module with the highest correlation value with lactate clearance were further analyzed. Functional enrichment of this module revealed biological processes related to cellular metabolism, biosynthesis, cell death, and autophagy. These genes were found to be more highly expressed in livers with adequate hepatocellular function compared to those with inadequate function. In addition, this gene co-expression module was significantly correlated with other traits including steatosis, age, sex and cold ischemic time indicating a functional relationship with these factors. **Conclusion:** WGCNA leverages the wide variability in human liver tissues to facilitate identification of gene expression patterns with specific liver characteristics (such as donor age and cold ischemic time) and measurable functional metrics during NMP (lactate clearance, vascular resistance). Understanding these correlations will be critical to designing targeted therapeutics for rehabilitation of discarded or high-risk livers during NMP.