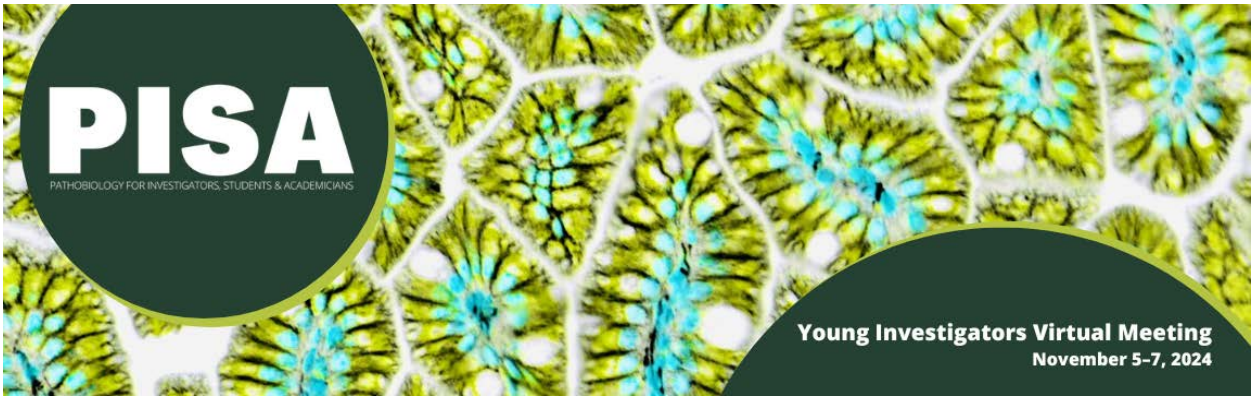

Pathobiology for Investigators, Students, and Academicians

Young Investigators Virtual Meeting

PISA 2024 – Abstracts

Session 1 – Liver Pathobiology

Abstract 1

HNF1B Controls the Cell's Commitment to Hepatic Fate

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Introduction/Background: Gene expression during embryonic development determines cell differentiation and organ morphogenesis. The transcription factor Hepatocyte Nuclear Factor 1 homeobox beta (*HNF1B*) is required for liver development. Heterozygous mutations in *HNF1B* result in a multi-system disorder in humans. In mice, deletion of *Hnf1b* causes embryonic lethality by embryonic day (e)6.5-7.0 due to defective visceral endoderm differentiation. When the defect in the visceral endoderm was rescued, *HNF1B* was found to be required for liver bud formation. Although HNF1B is thought to be critical for the developmental transition of the endoderm to a hepatic fate, the molecular mechanisms through which *HNF1B* mediates hepatic cell fate in humans are poorly defined. **Methods:** Using iPSCS as a model, we are studying the mechanism through which *HNF1B* controls the transition of the endoderm to form hepatic progenitor cells. We generated an *HNF1B* knockout, a dox-inducible *HNF1B* rescue, and dox-inducible HNF4A rescue human iPSC lines using CRISPR-Cas9 gene editing. We confirmed the integrity of the cell lines by DNA sequencing, immunofluorescence staining, and western blot analyses. We characterized the differentiation of our *HNF1B* -/- iPSCs by defining the changes in the expression of characteristic hepatic markers. We also performed RNA sequencing, ATACseq, and ChIPseq to understand the molecular mechanisms by which HNF1B controls the transition from the endoderm to a hepatic fate. **Results:** We established the expression pattern of important transcription factors, including HNF1B, in hepatic differentiation, showing that *HNF1B* precedes the expression of key hepatic genes such as *HNF4A*. We confirmed the knockout and rescue cell lines. Importantly, *HNF4A,* which is a central regulator of hepatocyte fate, was markedly downregulated in the *HNF1B* -/- cells. We observed that the gene expression signature of the *HNF1B* -/- cells was indicative of a complete block in the transition of the endoderm to a hepatic fate. In the absence of *HNF1B*, transcription factors known to be essential for hepatic differentiation such as *HNF4A* and *HNF1A* were significantly downregulated, while genes characteristic of the endoderm such as SOX17 and FOXA2 were significantly upregulated. We determined the direct binding of HNF1B to important TFs during liver development which concided with the accessibility of chromatin. **Conclusion:** We conclude that HNF1B is essential to establish hepatic fate by controlling the expression of the master regulator of hepatic gene expression, HNF4A.

Abstract 2

Role of Autophagy in Hepatic Proteome and its Post-translational Modification

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Introduction: Post translational modification (PTM) of the proteome via acetylation results in the homeostatic maintenance of the hepatic acetylome, the level of which reflects cellular metabolic state. The hepatic acetylome is involved in a variatey of hepatocellular processes including metabolic pathways, epigenetic modification, signal transduction, cell proliferation, and apoptosis. How liver maintains the homeostatic level of acetylome is unknown. Here we show that autophagy function is necessary to maintain the acetylation of hepatic proteome and *hence* maintain the acetylome by a non-degradative transcriptional pathway. **Methods:** In this study various autophagy deficient mouse models were used to dissect the role of autophagy in hepatic acetylome regulation. Mice with hepatic deletion of autophagy-related gene 7 (*Atg7*) or autophagy-related gene 5 (*Atg5*) were bred crossing *Atg7* or *Atg5* floxed mice with *Alb-Cre* mice. Mice of both genders at the age of 9 weeks old were studied. Wild type (WT) mice were injected with chloroquine (60 mg/Kg, *i.p*) for 6 consecutive days to inhibit autophagy. Contrary to this, autophagy activation was done by fasting, rapamycin treatment or by genetic activation of autophagy ($Becn1^{-F121A/F121A}$). Total liver lysate and subcellular fraction autophagy impaired mice were analyzed by immunoblotting for acetylated lysine and multiple PTM. Liver sections stained for Acetyl-Lysine and Hoechst. Hepatic estimation of Acetyl-CoA, CoA and Histone H_3 acetylation, and quantitative PCR of various enzymes involved in acetyl-CoA regulation were analyzed. For rescue experiment, Acetyl-CoA was administered intraperitoneally (10mg/kg body weight) to $Atg5^{FF}$ and $Atg5^{-/-}$ mice for eight consecutive days. Liver sections were subjected to H&E staining and serum ALT was examined as a measure of liver injury. Human liver autopsy samples of chronic liver diseases were immunostained with acetyl lysine to validate the preclinical findings. **Results:** Examination of PTM of hepatic proteome showed that ubiquitination, SUMOylation, methylation, ADP-ribosylation, and phosphorylation of hepatic proteome was significantly upregulated in autophagy-deficient liver. In contrast, the acetylation of hepatic proteome(acetylome) was dramatically downregulated in both autophagy-deficient and autophagy-defective livers. Cellar fractionation studies showed that the overall hepatic acetylome covering nuclear, cytosolic, mitochondrial, membrane fractions were downregulated in autophagy-deficient liver. Contrary to this, autophagy activation by fasting, rapamycin treatment or by genetic activation of autophagy ($Becn1^{-F121A/F121A}$) increased the level of hepatic acetylome suggesting the critical role of autophagy in maintenance of hepatic acetylome. Furthermore, mechanistic studies showed that autophagy maintains levels of acetyl-CoA, an important intermediate metabolite required for protein acetylation. By downregulating key enzymes involved in the acetyl-CoA biosynthesis, such as *Acly, AceCS1, AceCS2, Mlycd*, and *Pdha1*, autophagy impairment significantly reduced hepatic acetyl-CoA production. Notably, in the autophagy-deficient liver, replenishing hepatic acetyl-CoA rescued the lower acetylome and protected against liver injury. Moreover, screening for transcription factors that regulate acetyl-CoA biosynthesis enzymes revealed multiple hits, including nuclear erythroid-derived 2-like 2 (*Nrf2*). The co-deletion of Nrf2 transcription factor rescued the expression of acetyl-CoA biosynthesis enzymes and rescued the lower hepatic acetylome levels. Finally, lower hepatic acetylome, autophagy defect and Nrf2 activation was observed in the human liver autopsy samples of chronic liver diseases validating the preclinical findings and suggesting the clinical relevance of the study. **Conclusion**: Autophagy regulates hepatic acetylome through transcriptional regulation of the enzymes that synthesize acetyl-CoA.

Abstract 3

Role of DEK in Liver Fibrosis

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Introduction: In liver fibrosis, excess extracellular matrix proteins, especially collagen, replace normal tissue, resulting in cirrhosis and cancer. In spite of the fact that there are no FDA-approved treatments, understanding the molecular mechanisms responsible for fibrosis is crucial. A multifunctional nuclear protein, DEK, is down-regulated in fibrotic livers, and its deletion worsens fibrosis in preclinical mouse models. Thus, DEK may have anti-fibrotic properties, making it a potential therapeutic target. In this study, we aim to investigate the function of

DEK in liver fibrosis and assess its therapeutic potential. **Methods:** Ccl4 treated DEK WT & DEK KO mice. DEK mice were injected with 0.7ul/gm Ccl4 twice/week, i.p for 2 weeks. On week 3, tissue was harvested and examined for analysis. Western blot to confirm the presence and absence of DEK between the two groups. General IHC staining was done with H&E staining. Specific IHC staining was done for Sirius red, a-SMA, and trichrome. **Results:** DEK is expressed by all types of liver cells. UMAP expression analysis of DEK in hepatocytes. DEK protein is expressed in both the nucleus & cytosol of normal hepatocytes but only in the nucleus of spleen, skeletal muscle, and lung cells. Hepatic hydroxyproline level tested in Ccl4 treated DEK WT and DEK KO mice. Hydroxyproline levels were elevated in Ccl4-treated DEK KO mice. qPCR analysis of liver fibrosis-related genes in Ccl4 treated DEK WT & DEK KO mice showed elevation of the fibrotic gene in DEK-deleted liver. **Conclusions:** DEK deletion exacerbated liver fibrosis suggesting that DEK is a potential anti-fibrotic molecule.

Abstract 4

Neutrophil Infiltration via STING Signaling Promotes Inflammation in Primary Sclerosing Cholangitis

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Introduction: Primary Sclerosing Cholangitis (PSC) is characterized by a pro-inflammatory environment that contributes to liver fibrosis. Human PSC bile ducts are enriched with myeloid cells, indicating a potential role for the innate immune system. The STING (stimulator of interferon genes) signaling pathway is critical in regulating innate immune responses through the release of cytokines. Our goal is to investigate the mechanisms and consequences of peribiliary neutrophil infiltration observed in PSC. **Method:** Primary cholangiocytes isolated from WT and mouse models of PSC (3,5-Diethoxycarbonyl-1,4-Dihydrocollidine (DDC)-fed mice and Mdr2-/- mice) were analyzed by RNA-sequencing. Intrahepatic leukocytes (IHL) isolated from WT and Mdr2-/- were evaluated by flow cytometry and RT-PCR. Mdr2-/- mice injected with Ly6G antibody to deplete neutrophils were analyzed by immunofluorescence (IF), histology, and cytometry by time-of-flight (CyTOF). Autoimmune nCounter analysis was performed on human PSC tissues to interrogate immune signatures. Cholangiocytes exposed to TNF (inflammatory phenotype) were analyzed for chemokines upon genetic knockdown and pharmacological inhibition of STING. **Results:** Primary cholangiocytes from PSC mouse models demonstrated enrichment in inflammatory and neutrophil degranulation pathways. Congruently, flow cytometry analysis on $CD45^+$ IHL revealed an increase in the $Ly6G^+$ $Cd11b^+$ neutrophils in Mdr2-/- mice compared to WT (8.7% vs 2.5%, FC=3.48, *p<0.0001*). These neutrophils displayed an activated phenotype with increased expression of Cxcr1 and Cxcr2. Anti-Ly6G-mediated peripheral depletion of neutrophils in Mdr2-/- mice alleviated liver injury (57.6% and 59.2% reduction in ALT and AST, respectively). IF and histology revealed a substantial reduction in peribiliary neutrophil infiltration and reduced bridging fibrosis with Ly6G treatment in Mdr2-/- mice. CyTOF on IHL revealed an attenuation in cytotoxic CD8 T cells upon neutrophil depletion. nCounter analysis showed an upregulation of STING-associated genes in human PSC tissues. Mechanistically, TNF-induced upregulation of neutrophil chemoattractants, CXCL1 and IL8, was abolished by pharmacologic and genetic inhibition of STING. **Conclusion:** Our findings suggest the STING pathway in activated cholangiocytes triggers an immune response resulting in peri-portal neutrophil infiltration. The sustained presence of these activated neutrophils perpetuates the inflammation seen in PSC.

Abstract 5

Targeting CRM1-HMGB1 Nuclear Translocation in Type 2 Diabetes-Driven Metabolic Dysfunction Associated Steatotic Liver Disease

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Introduction: Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD) has a high incidence in Type-2 Diabetes (T2D) as it relates to the evolution of Non-Alcoholic Steatohepatitis (NASH). Acetyl-High Mobility Group Box 1 (HMGB1) is the proinflammatory isoform of HMGB1, a DAMP released in hepatic inflammatory T2D. The nuclear exporter Chromosomal Maintenance 1 (CRM1) maintains the nuclear-cytoplasm cascade of hepatic HMGB1 in T2D. We hypothesize that target inhibition of CRM1/HMGB1 nuclear shuttle in T2D/NASH. Methods: We performed immunohistochemical quantification of Acetyl-HMGB1 and CRM1 in human liver biopsies from control (healthy), T2D, and T2D-NASH (n:4 per group), and H&E evaluated inflammation and disease stratification. We performed targeted inhibition of CRM1 using Leptomycin-B and HMGB1 with Glycyrrhizin in T2D Huh7 human hepatocytes. Results: T2D subjects with NASH exhibit an increase in acetyl-HMGB1 nuclear and cytoplasmic translocation compared to DM and controls. Acetyl-HMGB1 increased 2-fold in the nucleus and 4-fold in the cytoplasm; CRM1 increased 6-fold in the nucleus and 8-fold in the cytoplasm of T2D/NASH subjects compared to controls. Targeted inhibition of CRM1 and HMGB1 prevented acetyl-HMGB1 hepatocyte release with the most prominent effect in T2D-NASH conditions. Conclusions: Targeting hepatic CRM1/HMGB1 inhibition identifies the potential therapeutic targeting of the CRM1/HMGB1 shuttling process in T2D-driven MASLD.

Abstract 6

Hepatocyte-Specific MET Deletion Exacerbates Liver Damage and Impairs Regenerative Response in Acetaminophen-Induced Hepatotoxicity Model

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Background: HGF receptor, MET, is considered one the most critical drivers of liver regeneration after partial hepatectomy (PH). Despite the well-known regenerative function of MET, its role in the clinically relevant acetaminophen (APAP)-induced liver injury (AILI) model remains unexplored, which markedly differs from the PH model due to the presence of massive liver necrosis and inflammation. The current study aims to delineate the role of MET in AILI, for the first time, using hepatocyte-specific deletion strategy. **Methods:** Hepatocyte-specific MET-KO mice were generated by administering AAV8-TBG-CRE in $MET^{fl/fl}$ mice. MET-KO or WT mice were given a toxic dose of APAP (300 mg/kg) and subsequently assessed for liver injury and regeneration parameters at various time intervals. **Results:** Deletion of MET led to a significant exacerbation of liver injury, and impaired liver regeneration culminating in significant mortality, while all the WT mice recovered spontaneously. Notably, the critical mechanisms initiating AILI such as APAP metabolic activation and APAP-protein adduct formation remained unchanged. However, JNK activation and its mitochondrial translocation were enhanced, and replenishment of antioxidant glutathione was impaired in MET-KO mice, resulting in excessive mitochondrial oxidative damage and subsequent release of cell death mediator, AIF, into cytosol. Excess JNK activation was attributed to increased phosphorylation of its kinase, MKK4, possibly resulting from reduced repressive activity of AKT on MKK4 in the absence of MET signaling. Impaired hepatocyte proliferation in MET-KO mice was linked to the suppression of ERK signaling. RNA-sequencing analysis not only showed repression of cell cycle/proliferation and survival (AKT) signalling but also activation pathways associated with cell death signaling and senescence (TGFβ), along with impaired unfolded protein response in MET-KO mice. Remarkably, HGF/MET signaling was strongly activated in APAP-induced acute liver failure (ALF) patients and vast majority (35%) of the genes altered in human ALF were found be regulated by MET in the mouse AILI model, indicating wider significance of MET signaling for human ALF. **Conclusion:** Overall, our study demonstrates that MET is not only crucial for promoting regeneration/repair but also for restraining injury development after APAP overdose via inhibiting mitochondrial cell death signaling pathway.

Abstract 7

Targeting EGFR as a Novel Strategy for Treating Acetaminophen Hepatotoxicity

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Introduction: Epidermal growth factor receptor (EGFR) is a well-established mediator of liver regeneration, driving hepatocyte proliferation after partial hepatectomy (PH). However, the role of EGFR in the clinically relevant acetaminophen (APAP)-induced liver injury (AILI) model which significantly differs from the PH model due to the presence of massive inflammation and necrosis has not been fully characterized. Our early explorations using a chemical EGFR inhibitor have found that, paradoxically, EGFR exacerbates AILI. Therefore, we aim to validate this unexpected role of EGFR in promoting liver injury using a hepatocyte-specific EGFR knockout (KO) mouse model, and test the therapeutic relevance using clinically-approved EGFR inhibitors. **Methods:** Hepatocyte-specific EGFR-KO mice were generated by administering AAV8-TBG-CRE in $EGFR^{fl/fl}$ mice. EGFR-KO or wild-type (WT) mice were treated with a toxic dose of APAP and sacrificed at different timepoints to investigate liver injury and regeneration. Similarly, afatinib, osimertinib or erlotinib, three clinically-approved EGFR inhibitors, were administered post-APAP in C57BL6/J mice. A delayed dosing of osimertinib was also tested both alone and in combination with N-acetylcysteine (NAC), the primary treatment for AILI. **Results:** Hepatocyte-specific EGFR deletion resulted in significantly decreased peak liver injury after APAP overdose, despite no observed changes in the key injury initiating events such as APAP metabolic activation and APAP-protein adduct formation. Mechanistically, EGFR deletion inhibited JNK activation and its mitochondrial translocation, resulting in reduced mitochondrial damage and release of cell death drivers. Consistently, clinically-approved EGFR inhibitors, osimertinib and afatinib, decreased AILI without impacting APAP metabolism, with osimertinib having a significantly stronger effect. Importantly, these inhibitors or EGFR deletion did not impair hepatocyte proliferation and liver regeneration. Notably, delayed osimertinib treatment with NAC tended to decrease AILI, when NAC alone is typically ineffective. **Conclusion:** Our studies revealed that EGFR holds an unexpected death-promoting function in the AILI model, which has wide implications in liver biology. Further, clinically approved EGFR inhibitors which decrease liver injury without impairing liver regeneration may be able to be retooled for treating AILI patients.

Abstract 8

Effects of hepatocyte-specific EGFR and ERBB3 deletion in murine fast-food diet model of MASLD

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Introduction: Metabolic dysfunction-associated steatotic liver disease (MASLD) has become the most prevalent chronic liver disorder, with no approved treatment. Our previous work demonstrated the efficacy of a pan-ErbB inhibitor, Canertinib, in reducing steatosis and fibrosis in a murine fast-food diet (FFD) model of MASLD. The current study explores the effects of hepatocyte-specific ERBB1 (EGFR) and ERBB3 receptors deletion in the FFD model. **Methods:** EGFR & ERBB3 specifically in hepatocytes, were fed a FFD diet for 2 or 5 months. **Results:** Hepatocyte-specific EGFR deletion reduced serum triglyceride levels but did not prevent steatosis. Transcriptomic analysis revealed significant alteration of lipid metabolism pathways in EGFR -KO mice with changes in several relevant genes, including downregulation of fatty-acid synthase and induction of lipolysis gene, Pnpla2, without impacting overall steatosis. Interestingly, EGFR downstream signaling mediators, including AKT, remain activated in EGFR-KO mice, which correlated with increased activity pattern of other receptor tyrosine kinases, including ERBB3, in transcriptomic analysis. Further, Canertinib treatment in EGFR-KO mice, which inhibits all ERBB3 receptors, successfully reduced steatosis, suggesting the compensatory roles of ERBB3 receptors in supporting MASLD without EGFR. In contrast to EGFR-KO, ERBB3-KO showed strikingly reduction in steatosis and liver to body weight ratio along with improved glucose tolerance and insulin tolerance. Further, the expression of PPARγ and ATP citrate lyase, the key transcription factor and enzyme, respectively, that regulate fatty acid biosynthesis, were remarkably reduced in ERBB3-KO. **Conclusions:** Hepatocyte-specific EGFR-KO did not impact steatosis, but ERBB3-KO reduced steatosis in the FFD model of MASLD, indicating an important role of ERBB3 in regulating liver lipid metabolism.

Session 3 – Cardiovascular and Pulmonary Disease

Abstract 9

Endothelial Senescence Mediates Hypoxia-induced Vascular Remodeling in the Lung

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Introduction: Pulmonary hypertension (PH) is a fatal pulmonary vascular disease characterized by a sustained elevation of pulmonary arterial (PA) pressure. The major characteristic of PH is uncontrolled accumulation of PA smooth muscle cells (SMCs) to normally non-muscularized distal PAs. Disrupted PA endothelial cell (EC) signaling stimulates PASMC proliferation and accumulation to distal PAs. Cellular senescence contributes to aging and lung diseases associated with PH. Although senescent cells are unable to replicate, they secrete senescence-associated secretory phenotype (SASP) factors, allowing the cells to be metabolically active and control behaviors of neighboring cells. The mechanistic role of EC senescence in pathogenesis of PH has not been fully understood. The Hippo pathway signaling transducer, Yes-associated protein (YAP1) stimulates angiogenesis and controls cell proliferation and survival. **Methods:** We utilize PH patient-derived PAECs to examine YAP1, PDGFB, and EC senescence activity. We also use hypoxia-induced PH model to investigate PA remodeling as well as measure right ventricular (RV) systolic pressure and RV hypertrophy to study the effects of EC senescence. **Results:** YAP1 activity is upregulated in idiopathic pulmonary arterial hypertension (IPAH) patient-derived PAECs. The levels of senescence markers are higher in ECs isolated from PH patients compared to those from healthy individuals. The levels of PDGFB upregulated in PH patient-derived ECs are inhibited by knocking down $p16^{INK4A}$ expression. $p16^{INK4A}$ knockdown decreases YAP1 expression, which suppresses PDGFB expression in IPAH patient PAECs. Accumulation of α-smooth muscle actin (αSMA)-positive cells to the PAs in a hypoxia-induced mouse PH model is attenuated in *$p16^{INK4afl/fl}$-Cdh5(PAC)-Cre^{ERT2}* mice, in which $p16^{INK4a}$ expression is knocked down in ECs after tamoxifen induction. Hypoxia-induced PA remodeling is reversed when mice are reoxygenated after 21 days of hypoxia. **Conclusions:** These results suggest that increases in EC senescence mediate vascular remodeling in PH through endothelial YAP1-PDGFB signaling.

Abstract 10

Sex-Dependent Differences in Lung Vascular Regeneration

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Introduction/Background: Chronic lung diseases, including chronic obstructive pulmonary disease (COPD) contribute to global morbidity and mortality. Lung transplantation remains one of the options for patients with end-stage lung diseases, but it is not optimal. The human lungs possess an ability for regeneration and repair following injury or partial resection. Impaired lung regeneration and repair are implicated in the pathogenesis of lung diseases. Stimulating the regenerative ability of the lungs would be a promising strategy for chronic lung diseases. There are sex-related differences in lung diseases. Females with COPD have a higher risk of hospitalizations with greater morbidity compared to males. Development of the sex-dependent treatment may lead to more efficient strategy for lung diseases, and we need to understand the mechanism. Angiogenesis - formation of new capillary blood vessels- plays key roles in organ regeneration. We have reported that endothelial signaling is necessary for regenerative lung growth after unilateral pneumonectomy (PNX). There are sex-dependent differences in transcriptomics of endothelial cells (ECs) and angiogenic activity. **Methods:** We perform left unilateral PNX on male vs. female C57BL6 mice, measure the remaining lung weight, and analyze vascular and alveolar regeneration and endothelial signaling using immunohistochemical and molecular biological analysis. **Results:** Post-PNX increases in the remaining lung weight are lower in the female mice compared to males. Alveolar numbers and blood vessel formation are significantly lower in females compared to males. Post-PNX increases in the expression of angiogenic genes, VEGFR-2, Tie2, and PDGFB are higher in male mice. **Conclusion:** Post-PNX vascular and alveolar regeneration is less stimulated in females compared to males. Understanding the sex-dependent mechanism would lead to the development of more specific strategies for lung diseases.

Abstract 11

Endothelial Cell STING Contributes to Capillary Rarefaction and Systolic Dysfunction Induced by Cardiac Pressure Overload

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Introduction: Capillary rarefaction and cardiomyocyte (CM) hypertrophy are hallmarks of heart failure (HF). Endothelial cells (EC) and CM communicate in cardiac remodeling and new signals continue to emerge. Stimulator of Interferon Genes (STING) is highly expressed in EC and contributes to vascular inflammation, yet its role in HF is unknown. We hypothesize that EC STING to CM crosstalk modulates CM hypertrophy and systolic dysfunction in HF. **Methods**: Inducible EC STING$^{-/-}$ or EC STING$^{+/+}$ littermate controls (*Cad5*$^{ERTCre2+/-}$ *Sting*$^{fl/fl}$ treated with Tamoxifen or oil, respectively), wildtype (WT), and global deficient (STING$^{-/-}$) mice were subject to transverse aortic constriction (TAC) or Sham surgery. Cardiac function was analyzed by echocardiography after 2-8 weeks and left ventricular (LV) sections were stained with wheat germ agglutinin and isolectin to analyze CM hypertrophy and capillary density. Gene expression was analyzed by qPCR. Bulk RNA Sequencing was performed on CD45- CD31+ heart EC (MHEC) from EC STING$^{+/+}$ and EC STING$^{-/-}$ mice 4 weeks after TAC. **Results**: In contrast to WT mice, global and EC STING$^{-/-}$ mice were protected from declined fractional shortening, capillary rarefaction and LV hypertrophy in response to TAC. RNASeq revealed reduced levels of pro-hypertrophic IL6 and increased anti-hypertrophic and pro-angiogenic Neuregulin1 (Nrg1) in TAC STING$^{-/-}$ MHEC compared to WT. Similarly, IL6 gene expression was reduced in EC STING$^{-/-}$ TAC hearts compared to EC STING$^{+/+}$, as Nrg1 was increased. **Conclusions:** Our data demonstrate that EC STING modulates both EC and cardiac expression of IL6 and Nrg1, suggesting a mechanism of EC-CM communication that contributes to CM hypertrophy, capillary rarefaction and systolic dysfunction in response to pressure overload.

Abstract 12

Differential Contributions of Cell Specific STING to the T-cell Immune Response in Doxorubicin Cardiotoxicity

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Introduction: Doxorubicin (DR), the most commonly used chemotherapy, results in dose-dependent cardiotoxicity, however current therapies are not widely effective. DR causes oxidative stress, cardiomyocyte death, and DNA damage which activates the stimulator of interferon genes (STING) across broad cell types. STING activation induces type I interferon (IFN-I) secretion and downstream IFN-I stimulating genes (ISGs) including the chemokines CXCL9/10. We found that DR induces cardiac and systemic CXCL9/10 and a cytotoxic CD8$^+$ T-cell response, but whether this response is dependent on cell specific activation of STING by DR remains unknown. We **hypothesized** that DR activation of vascular, stromal and immune cell STING differentially mediates adverse cardiac remodeling and CD8$^+$ T-cell cardiotropism in DR cardiomyopathy. **Results:** We treated WT and STING$^{-/-}$ mice with 5.0 mg/kg DR for 4 weeks and found that STING$^{-/-}$ mice were protected from systolic dysfunction and cardiac fibrosis. Further, DR increased total cardiac CD8$^+$ T-cell numbers, CXCR3$^+$CD8$^+$ T-cells, and cardiac *Cxcl9/10* in WT but not STING$^{-/-}$ mice. *In vivo* using flow cytometry, DR enhanced IRF3 phosphorylation, immediately downstream of STING, in endothelial cells (ECs), fibroblasts (CFB), and macrophages, in WT but not STING$^{-/-}$ mice. *In vitro,* treatment of all cell types with 1.0 µg/mL DR increased IFN-I transcription, corroborating STING activation. Cell-specific ablation of STING in ECs (STING$^{fl/fl}$Vecad^{Cre+}), CFBs (STING$^{fl/fl}$Tcf21^{Cre+}), or myeloid cells (STING$^{fl/fl}$LysM^{Cre+}) resulted in improved systolic dysfunction and cardiac fibrosis after DR compared to STING$^{fl/fl}$Cre$^-$ controls. However, we observed distinct mechanisms of protection, as only EC-STING and Myeloid-STING contributed to DR-induced CD8$^+$ T-cell cardiotropism. Mechanistically, we found that direct treatment of primary ECs and macrophages *in vitro* increased *Cxcl9/10* expression and induced subsequent CD8$^+$ chemotaxis and transendothelial migration across primary cardiac ECs in a STING dependent manner. Our data outline a significant role for endothelial and macrophage STING in mediating CD8$^+$ cardiotropism in response to DR.

Session 5 – Inflammation and Ophthalmology

Abstract 13

Exploring the Effects of Epigallocatechin-3-Gallate on the Disaggregation of Serum Amyloid A1 Fibrils

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Introduction: Serum Amyloid A1 (SAA1) is a major acute-phase protein involved in lipid metabolism, cytokine production, and immune responses. It is synthesized in the liver as a 122-amino acid protein, maturing into a 104-amino acid structure that forms a hexamer with a four-helix bundle. Importantly, SAA1 is a key precursor to amyloid A (AA), an insoluble protein that can build up in tissues. This accumulation leads to AA amyloidosis, a condition characterized by tissue damage and organ dysfunction resulting from chronic inflammation. Given these risks, our study explores potential strategies to disaggregate SAA1 fibrils isolated from domestic animals, with particular attention to the effects of epigallocatechin-3-gallate (EGCG), a green tea compound known for its anti-inflammatory and antioxidant properties. **Methods:** The SAA1 fibrils were isolated from the cat spleen, chicken liver, and cow liver and incubated for 5 days in PBS (pH 7.4). To confirm the amyloidogenic properties of these fibrils, we conducted dynamic light scattering (DLS) and transmission electron microscopy (TEM) analyses of these fibrils (at ~0.3 mg/ml) with 0.25% DMSO and 200 to 400 µM of EGCG. **Results:** Our findings revealed that EGCG effectively disaggregates SAA1 amyloid fibrils isolated from domestic animals into non-toxic aggregates. Notably, EGCG caused a significant reduction in particle area in cow liver samples, decreasing from approximately 4.8 µm² to 0.5 µm². A reduction was also observed in chicken liver samples, with particle area decreasing from approximately 1.8 µm² to 1.4 µm². The soluble particle sizes of EGCG treated cow and chicken SAA1 fibrils were reduced from 1000 nm to 100 nm of diameter according to DLS. Significant ultrastructural morphological changes were observed with the cow SAA1 fibrils treated with EGCG. **Conclusions:** Identifying compounds like EGCG that can disaggregate amyloid fibrils emphasizes their potential as therapeutic agents for treating inflammatory conditions and neurodegenerative diseases.

Abstract 14

Persisting Neuroinflammation after 18-weeks in SARS-CoV-2 Infected African Green Monkeys

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Introduction: Many individuals report ongoing neurological symptoms, including impaired concentration and cognition, months to years after infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). We hypothesize that these neurological complaints are due to persistent neuropathological changes/injury after acute infection. **Methods**: To begin to explore our hypothesis, brain and cerebrospinal fluid (CSF) were acquired from ten African green monkeys (AGMs) after 18-weeks post-SARS-CoV-2 infection with the 2019-nCoV/USA-WA1/2020 strain. Formalin-fixed, paraffin-embedded brain tissues underwent a histopathological investigation for the presence of virus/viral proteins and overall pathology, including microhemorrhages, neuronal apoptosis, and neuroinflammation. Our findings from 18-week infected AGMs were compared to the findings from our previously published 4-week infection AGM study, using the same viral strain. Metabolomic analysis of CSF acquired at baseline and necropsy was performed by ultra-HPLC/MS. **Results**: Consistent with most post-mortem human studies, we did not detect viral spike protein in brain tissue. Although we previously reported an increased frequency of microhemorrhages and neuronal cell death, confirmed by active caspase 3 IHC, in 4-week infected AGMs, this appeared to be largely resolved at 18-weeks. Notably, microhemorrhages seen at 18-weeks post-infection (p.i.) had a similar appearance to those seen at 4-weeks p.i., with densely packed red blood cells on the parenchymal side of the blood vessel. Most striking was the presence of significant wide-spread neuroinflammation and nodular lesion formation persisting at 18-weeks p.i., which appeared worse than that seen at 4-weeks p.i. Microglial lesions, a pathological consequence of chronic neuroinflammation, were found at a higher frequency in the brainstem and cerebellum of 18-week p.i., as compared to 4-weeks p.i. and control animals. To gain further insight into the CNS environment, we performed untargeted metabolomics on baseline and necropsy CSF collections. This revealed significant changes in 22 metabolites, including decreased cortisol and malate and increased citrate and hippurate, which suggest inflammation and alterations of the citric acid cycle within the CNS compartment. **Conclusions**: Our findings suggest significant neuroinflammation and possible altered and/or impaired energy metabolism underly the neurological symptoms that continue well after infection.

Abstract 15

High-Intensity Interval Training Decreases Circulating HMGB1 in Individuals with Insulin Resistance; Plasma Lipidomics Identifies Associated Cardiometabolic Benefits

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Background: Bodyweight high-intensity Interval Training (BW-HIIT) is an effective, time-efficient exercise method that reduces cardiovascular risk factors and improves muscle endurance without needing external equipment. HMGB1 is a proinflammatory protein involved in insulin resistance. Our earlier study revealed that HMGB1 knockout mice show improved insulin sensitivity. This study investigates whether BW-HIIT exercise will reduce proinflammatory markers like HMGB1 in individuals with insulin resistance. **Method:** Fourteen adults (2 male/12 female) aged 18 to 55 were used. Adult male mice (5 per group) were used at 10 weeks of age under 2 groups. Human and mouse pre- and post-exercise serum/plasma samples were analyzed for Lipidomics, metabolic, and Cytokine Multiplex assays. Standard of care, as well as cardiometabolic parameters, were also performed in human subjects. Results: After six weeks, BW-HIIT exercise changes Metabolic hormones like Amylin, Glucagon, and Insulin increased in post-exercise human and mouse models. Also,8 weeks of treadmill exercise of the animal model showed anti-inflammatory cytokines IL-10, IL-12p40, and IL-12p70 increased, and the proinflammatory cytokines Eotaxin, Il-2, and MIP-2 or CXCL2 reduced in the post-exercise mouse model. Post-exercise decreased systolic blood pressure, cholesterol, triglycerides, HDL, and Chol/HDL ratio in individuals with insulin resistance. Reduced circulating HMBG1 levels in insulin-resistant individuals and exercised mice. **Conclusion:** Six weeks of BW-HIIT exercise improves cardiometabolic health, anti-inflammatory markers, metabolic hormones, and insulin sensitivity in the human and exercised mice model. Changes in circulating HMBG1 levels using BW-HIIT exercise make HMGB1 a suitable marker for metabolic disease, potentiating its role beyond an alarmin. Further studies are needed to confirm these effects and to elucidate the underlying physiological mechanisms. **Acknowledgment:** We gratefully acknowledge the assistance and instruction from Dr. Fabiano Amorim, his team for the help with the human samples used for this study. We also acknowledge the assistance of Eve Technologies Corporation in processing the human and mouse samples. We would also like to thank Dr. Changjian Feng and his lab members for their help with the lipidomic studies and analysis.

Abstract 16

Epigenetic Changes and Photoreceptor Neuroprotection in a Mouse Retinitis Pigmentosa Model

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Introduction: DNA methylation is an epigenetic repressor mark for transcription dynamically regulated in neurons without altering the DNA sequence. DNA methylation plays a significant role in the development and differentiation of photoreceptors. To investigate the methylation patterns during the degeneration of photoreceptors and their involvement in photoreceptor plasticity of the degenerating retina, we analyzed the regulation of DNA methylation during the progress of retinitis pigmentosa (RP) in mice. RP is a group of sight-threatening hereditary retinal dystrophies characterized by progressive degeneration of photoreceptor cells, which results in debilitating visual impairment. Genetic defect modulated the expression of factors controlling DNA methylation and exerted similar effects on DNA methylation and the hydroxy-methylation state of the retina. **Results:** Our data found elevated levels of DNMTs and DNA methylation during photoreceptor degeneration in

the classic model of RP, mouse strain with rhodopsin deficiency (*Rho*$^{-/-}$). Blockage of the methyl transferase DNMTs by weekly decitabine treatment (intravitreous injection) led to significant improvement in visual function in *Rho*$^{-/-}$ mice, as evidenced by electroretinogram (ERG), spectral-domain optical coherence tomography (OCT), and optomotor response-based visual behavior assays (OMR). Concurrently, assessment of outer nuclear thickness and immunofluorescence for the cone photoreceptor cell marker PNA demonstrated pronounced increases in the survival of cones and improvement in the morphology of the outer segments. **Conclusion:** DNA methyltransferase (DNMT) inhibition blocked the molecular, morphologic, and visual functional effects of photoreceptor genetic defect, partially reversing the genetic defect-caused photoreceptor degeneration.

Abstract 17

Deciphering Pathogenic Cytokine Interplay in Retinal Inflammation, Angiogenesis and Fibrosis: A Transcriptomic Approach

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Introduction: Dysregulation of cytokine signaling drives endothelial cell (EC) dysfunction, a key feature of retinal neovascular diseases, including neovascular age-related macular degeneration (nAMD). This is characterized by progressive inflammation, angiogenesis, and eventual fibrosis – a transdifferentiation of ECs to a mesenchymal phenotype, impeding tissue function. We explored the transcriptional changes in ECs exposed to six nAMD-associated cytokines (individual and combined effect) in an *in vitro* pathogenic retinal model. **Methods:** Primary human retinal ECs (HRECs) were treated with either no treatment (control), transforming growth factor-beta 1 (TGF-β1), TGF-β2, tumor necrosis factor-alpha (TNF-α), thrombin, interleukin-6 (IL-6), or vascular endothelial growth factor (VEGF), individually (10 ng/mL) or combined at the same concentration (n = 6). After 24h, cells were harvested, and high-throughput, bulk transcriptomic sequencing was performed (Azenta Life Sciences). Differential expression analysis ($|Log_2FC| > 1$, $FDR < 0.05$) was performed for all genes (DEGs) with reference to control. Pathway enrichment analysis (PEA) was employed to characterize gene pathways relative to disease phenotypes. DEGs were compared to nAMD patient eye RNA data (GSE135922) to assess clinical relevance. **Results:** Pro-inflammatory cytokines TNF-α (1823 DEGs) and thrombin (1019) induced a broader DEG profile than IL-6 (6). TGF-β2 (323) enriched mesenchymal pathways more potently than TGF-β1 (17). Angiogenic VEGF (32) induced a narrow, targeted DEG profile. PEA showed pro-fibrotic overlap in TNF-α, thrombin and TGF-β2 and significant enrichment of extracellular matrix and cell migration pathways. The combination group (2559) resulted in novel enhancement to differential expression revealing 884 unique DEGs, including VEGF-encoding *VEGFA*, enriching angiogenic cell-junction pathways. nAMD patient hallmark genes suggested the cytokine combination reflected disease better than individuals. **Conclusions:** Transcriptomic findings in HRECs suggest fibrosis-associated cytokines (TNF-α, thrombin and TGF-β2) to induce a strong transcriptional response in the pathogenic retina. TGF-β2 was confirmed as the dominant TGF-β isoform in HRECs. Combining cytokines presents a more disease-reflective *in vitro* model of nAMD, which may be utilized for pre-clinical drug testing, and extended to model other neovascular retinal diseases such as diabetic retinopathy and retinopathy of prematurity.

Abstract 18

From Brain to Eye: Repurposing Dimethyl Fumarate to Target Vascular Endothelial Growth Factor-Induced Angiogenesis in Retinal Endothelial Cells

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Introduction: Vascular endothelial growth factor (VEGF) plays a role in blood vessel growth during embryogenesis and wound healing but a pathological role in wet age-related macular degeneration (nAMD). Endothelial cells primarily rely on glycolysis for metabolism, but switch to oxidative phosphorylation (OXPHOS)

during angiogenesis. Dimethyl fumarate (DMFu), an FDA-approved oral tablet for multiple sclerosis, has shown anti-angiogenic properties in psoriatic models. Here we explore the ocular context using mouse choroidal explants and human microvascular retinal endothelial cells (HRECs). **Methods:** 1mm^2 choroid/RPE explants from 3-wk-old C57BL/6J mice were embedded in Cultrex and treated with 80µM DMFu. Scratch wound assays were performed on serum starved HRECs treated with 10ng/mL VEGF +/- 80µM DMFu to assess cell migration. Similarly treated HRECs were seeded on Cultrex-coated plates for tube formation and analyzed using the ImageJ Angiogenesis Analyzer. Treated HRECs were RNA sequenced and differentially expressed genes were identified. Pathway enrichment was assessed through over-representation and gene set enrichment analysis (GSEA), and unbiased weighted correlation network analysis (WGCNA). Protein expression of electron transport chain complexes was assessed by western blot and a Seahorse XFe96 Analyzer was used to quantify OXPHOS and glycolysis. **Results:** DMFu reduced choroidal explant sprouting area by 88.65% compared to control (n=4, $p<0.05$); HREC migration and tube formation were also significantly disrupted. GSEA revealed downregulation of VEGF-induced and VEGF-independent proliferation associated pathways with DMFu, including kinesin superfamily protein genes—affecting organelle and chromosome motility during segregation. WGCNA identified a link between tube formation and mitochondrial translation in DMFu groups compared to control. Complex II protein expression decreased by 65.35% (n=3, $p<0.05$) and a concurrent increase in glycolysis was observed with DMFu-treated HRECs through lower maximal OXPHOS and increased glycolytic capacity on Seahorse. **Conclusions:** DMFu exhibited potent anti-angiogenic properties in HRECs and mouse choroidal explants, indicating a promising avenue as an oral therapeutic for nAMD. Mechanistically, DMFu reduced complex II protein and OXPHOS with concomitant increased glycolysis leading to perturbations in microtubule formation and chromosome segregation. Future studies will examine its efficacy in nAMD *in vivo* models.

Session 7 – Cancer Pathobiology and Molecular Diagnostics

Abstract 19

Histamine Receptor 1 Expression is Increase in Head and Neck Cancer Tumors and is Associated with Poor Patient Outcomes

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Introduction: Histamine receptor 1 (HRH1) is a key mediator of histamine signaling in cells and influences several biological processes. While HRH1's role in normal physiology has been well explored, its role in cancer remains unclear. We focused on identifying the levels of HRH1 in head, neck, and throat cancers (HNSCC). In 2020, HNSCC was the 7th most common cancer globally with a 5-year survival rate of 68.5%. **Methods:** We examined transcriptomic data from HNSCC samples in the TCGA database, which harbors samples from a diverse cohort across sexes, ages, and ethnicities (n=520 tumor, n=44 normal). We also examined RNAseq from individuals with tongue cancer for whole tumors (n=26 tumor, n=12 normal) and single-cells. **Results:** We found that HRH1 was significantly upregulated in HNSCC tumors compared to normal tissues, with consistent findings across patients of all sexes, ages, and ethnicities. Interestingly, HRH1 was upregulated at all tumor stages. Methylation analysis revealed decreased levels of methylation in the HRH1 promoter, which correlated with the upregulated expression of the HRH1 gene. In addition, we found that several genes involved in histamine break-down were reduced in expression in tumors while the expression for histamine synthesis and transport was unchanged. We found that HRH1 expression was markedly elevated in tongue tumors specifically. Single-cell RNAseq data indicates that HRH1 was predominantly expressed in epithelial cells and fibroblasts in tongue tumors. Importantly, HRH1 expression levels were strongly correlated with oral cancer patient survival outcomes. Ninety percent of patients with low HRH1 expressing tumors survived, whereas only 50% of patients with high HRH1 levels survived past 72 months. **Conclusions:** These findings suggest that HRH1 upregulation is associated with poor prognosis in head, neck, and throat cancers, particularly in tongue cancer. HRH1 may serve as both a potential prognostic biomarker and a therapeutic target in these malignancies, warranting further investigation into its role in cancer progression and response to treatment.

Abstract 20

Physical Activity Regulates Proteolytic Pathways to Protect Against Cancer-mediated Cardiac Cachexia

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Introduction: Cancer cachexia is a severe and progressive muscle-wasting syndrome characterized by a loss of lean muscle mass, systemic inflammation, and altered muscle metabolism. In addition to skeletal muscle, cardiac muscle often experiences atrophy and cardiac remodeling during cancer-induced cardiac cachexia. Aerobic exercise has been of recent interest as a therapeutic approach for cancer cachexia as it helps preserve muscle mass, however, the exact mechanism is still not fully understood. The purpose of this study is to determine how physical activity concurrent with tumor-bearing affects cardiac atrophy and metabolism in a preclinical lung cancer model. **Methods:** Male mice (n=8-10/group) were randomly assigned to groups: sedentary non-tumor (SED+NT), sedentary tumor (SED+T), wheel running non-tumor (WR+NT), and wheel running tumor (WR+T). Mice were inoculated with Lewis lung carcinoma tumor cells (5x10^5 LLC cells in flank) on day 1 and could (voluntarily) run on a wheel for 4 weeks. Echocardiograms were performed on days 0 and 28 to measure cardiac structure and function. **Results:** Male tumor-bearing mice exhibited the worst cardiac function compared to all other groups, and physical activity concurrent with tumor-bearing protected against tumor-mediated declines in cardiac function (fractional shortening - SED+T: 41% vs WR+T: 53%, $P<0.001$). Additionally, SED+T showed the highest cardiac protein expression of MuRF1, Atrogin-1, and GDF-15, while physically active (WR) mice had lowest cardiac expressions of MuRF1 and Atrogin1. Since MuRF1 and Atrogin1 are known to drive proteolysis resulting in muscle atrophy, this data highlights the critical role of concurrent physical activity in controlling proteolysis to protect against cardiac atrophy and dysfunction. **Conclusion:** Tumor-bearing resulted in severe cardiac dysfunction, atrophy, and proteolysis. Importantly, physical activity initiated during tumor-bearing was capable of downregulating proteolytic signaling to ultimately preserve cardiac structure and function. The study underscores the importance of physical activity for cancer patients experiencing detrimental muscle wasting. More research is needed to understand the exact mechanisms that drive the beneficial effects of exercise and physical activity for clinical populations – especially those suffering pathological muscle wasting. Such information is critical to the safe and effective implementation of rehabilitative exercise programs.

Abstract 21

Selection for a Preferred Threshold Level of PI3K Activation in Myc-driven Mammary Carcinogenesis

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Introduction: Although *Myc* overexpression is a recurring driver event in breast cancer, treatments targeting *Myc* remain elusive. Efforts to identify druggable oncogenic pathways that cooperate with oncogenic *Myc* show recurring selection for activating mutations in *Kras*, but such mutations rarely occur in human breast cancer. Through a transposon-based genetics screen, we discovered that Myc cooperates with a Ras-driven pathway, PI3K (phosphoinositide 3-kinase) signaling. Human breast cancers frequently activate PI3K signaling through mutant *PIK3CA*, a clinically validated target. **Methods and Results:** To verify whether Myc cooperates with oncogenic PI3K signaling, we generated mice that express doxycycline (Dox)-inducible and mammary-specific *Myc* and $PIK3CA^{H1047R}$ transgenes (iMYC/iPIK). Littermates with singular transgene were used as genetic controls. Using Dox-impregnated chow, we discovered that a “high” level of transgene induction (2000 mg/kg) resulted in global mammary overgrowths and lethality in iMYC/iPIK mice within 5-12 days of treatment but not in “single oncogene” controls. Whole-mount and histological analyses on #4 mammary glands were conducted on Carmine alum- and hematoxylin/eosin-stained tissues, respectively. The invasiveness of overgrowths was confirmed by tissue explantation and limiting dilution assays in syngeneic hosts. To generate stochastic $Myc/PIK3CA^{H1047R}$-driven tumors, “moderate” (50 mg/kg) and “low” (10 mg/kg) levels of Dox were administered. Moderate levels of transgene expression consistently generated numerous focal tumors in iMYC/iPIK mice but not in low Dox and Dox-naïve controls. Sanger sequencing analysis of these tumors reveals no cooperating *Kras*, *Hras*, and *Nras* mutations. Rare tumors in low Dox and Dox-naïve iMYC/iPIK mice expressed transgenes through gene-switch mutations. To evaluate whether iPIK transgene expression, in isolation, can impact

Myc/PIK3CA^{H1047R}-driven tumorigenesis, we generated mice that express constitutive *Myc* and inducible *PIK3CA*^{H1047R} transgenes (cMYC/iPIK). The same studies were performed in cMYC/iPIK mice, which showed tumorigenesis and genetic results similar to those observed in iMYC/iPIK. To simulate targeted therapy against mutant *PIK3CA*, Dox-withdrawal studies were conducted on tumor-bearing cMYC/iPIK animals. Notably, nearly all tumors regressed following *PIK3CA*^{H1047R}-deinduction despite continued *Myc* expression. Relapsed tumors evolved to restore *PIK3CA*^{H1047R} expression through gene-switch mutations. **Conclusions and Future Directions:** The expression of *PIK3CA*^{H1047R}-encoded iPIK strongly enhanced *Myc*-driven mammary tumorigenesis and relieved the selective pressure to acquire cooperating *Kras* mutations. A threshold level of PI3K activation is crucial for enhancing *Myc*-driven mammary tumor onset and for tumor maintenance. Ongoing work explores the mechanism underlying *Myc/PIK3CA*^{H1047R} cooperation and the effect of alpelisib (PI3K^{mut} inhibitor) on this interaction.

Abstract 22

A 5-year Study Involving Clinical Cases to Determine Copy Number Distribution of *SMN1* and *SMN2* Genes in Spinal Muscular Atrophy Testing across Medical Facilities in New Hampshire

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Introduction: Spinal Muscular Atrophy (SMA) is an autosomal recessive inherited neuromuscular disorder characterized by muscle weakness and progressive degeneration of motor neurons leading to atrophy. SMA is typically caused by homozygous deletion of the *SMN1* gene with disease severity related to the number of copies of the *SMN2* gene. Both *SMN1* and *SMN2* are located at the *SMN* locus on chromosome 5q13 and share 99% nucleotide sequence identity making it difficult to differentiate the two genes. Genetic counseling recommends preconception carrier screening or carrier screening of all pregnant women in the population. **Methods:** Our lab at Dartmouth-Hitchcock Medical Center (DHMC) uses Droplet Digital PCR (ddPCR) to determine *SMN1* and *SMN2* copy numbers. In this study, we analyzed the data from SMA testing (almost exclusively carrier screening) across the health system from April 2019 through July 2024 (5718 patients) with a focus on carrier frequency in our population and most common days of the week for specimen collection for possible turnaround time (TAT) improvement. **Results:** The SMA carrier frequency (1 copy of *SMN1*) among all patients tested was 1 in 46 individuals (125/5718; 2.18%), similar to what was observed in previous studies. The average carrier frequency of patients seen locally at DHMC and the immediate vicinity in Lebanon, NH was 2.85%, whereas a carrier frequency of 1.71% was observed among patients seen at other facilities in the network located in the southern part of the state. Results diagnostic for SMA (0 copies of *SMN1*) were found in 4/5718 (1 in 1430) individuals tested. No obvious trends were noted when looking at carrier frequencies or *SMN2* copy number in our population over time (by year or quarter). A review of ordering practices showed specimen collection was most common on Fridays (20.58% of all tests ordered) with only slightly lower numbers of tests ordered on other weekdays. **Conclusions:** Monitoring carrier frequency for SMA in our patient population can be a useful tool for quality assurance purposes since allele frequencies are not expected to fluctuate over time in a given population. Changes in carrier frequency can suggest changes in ordering practices or actual changes make-up of the geographical population.

Abstract 23

Next-Generation Sequencing as a Reliable Method of Quantifying Bone Marrow Engraftment in Bone Marrow Transplant Patients

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Introduction Bone marrow transplantation (BMT) is an effective curative therapy performed on patients with hematological malignancies. Patients undergo conditioning regimens that deplete their existing hematopoietic stem cells, allowing for engraftment of the donor bone marrow. After patients undergo BMT, percent engraftment is periodically measured, determining the success of the therapy. Current molecular testing for bone marrow engraftment (BME) measures short tandem repeats (STR) in the genome to identify differing alleles between the patient and the donor. However, we hypothesize that next generation sequencing (NGS) provides an efficient

method of BME measurement that is more accurate and sensitive than current STR analyses. **Methods** We performed targeted NGS with an existing panel used for unrelated pharmacogenetic testing on DNA from unrelated patients mixed to create a range of varying concentrations of each patient, which was used to assess linearity. We also utilized patient blood samples from BMT samples from the donor, recipient pre-transplant, and recipient post-transplant. STR analysis was performed on samples as well, which was used to provide baseline comparative results. **Results** The targeted NGS panel utilized for these assays targets over 430 single nucleotide variants (SNVs). This was narrowed down to 40 benign SNVs that commonly occur in the human population. The STR panel, however, targets 16 loci. SNVs were considered informative if the genotypes were different between donor and recipient. The most informative loci were those that the pairs were homozygous for different alleles. The number of informative loci obtained via NGS differs from pair to pair, ranging from 15 to around 25 informative SNVs. Linear standard curves generated from NGS showed comparable R^2 values to that generated from STR analysis. Comparison of variant allelic frequencies with NGS provided an easily quantifiable measurement for percent engraftment when comparing donor samples with post-transplant samples with BMT pairs. **Conclusions** NGS offers a method for easily quantifying BME in patients who have undergone a BMT. Compared to STR analysis, NGS is comparable in accuracy of results, while including more targeted loci. The benefit of NGS includes decreased wet bench time, as well as decreased analysis time, when compared to STR analysis. This suggests that NGS could provide a more efficient method of measuring BME in the clinical setting.

Session 9 – Gastrointestinal Pathobiology

Abstract 24

***Akkermansia muciniphila* Alone Increases Small Intestine Tuft Cell Population via Succinate Production**

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Introduction: The gastrointestinal tract harbors millions of different bacteria. These bacteria interact with the intestinal epithelium both directly and via secreted products to stimulate and activate the intestinal mucosa. *Akkermansia muciniphila* has recently become a microbe of interest due to its reported impacts in both health and disease. *A. mucin* is a mucin-degrading microbe which has been extensively studied using *in vivo* models with a complete gut microbiome, but these models are limited in their ability to specifically elucidate the role of *A. muciniphila*. In this study, we utilized a germ-free model to evaluate the impact of *A. muciniphila* alone on gut epithelial cells. **Methods:** We inoculated adult germ-free mice with either Brain Heart Infusion (BHI) bacterial growth media (vehicle control) or 10^9 viable *A. muciniphila* in BHI. The intestines of germ-free control and *A. muciniphila* mono-associated mice were collected after 21 days. Bacterial Fluorescence in Situ Hybridization (FISH) staining was used to confirm the presence of bacteria in mono-associated mice and absence in control mice. Immunofluorescent (IF) staining was performed to examine changes in abundance and function of intestinal cell types. **Results:** In our germ-free model, *A. mucin* alone was not found to affect stem cell or paneth cell populations. Mice mono-associated with *A. mucin* were observed to have decreased numbers of Chromagranin A positive enteroendocrine cells in the small intestine compared to germ-free control mice. While populations of mucus-producing goblet cells were not affected by *A. mucin*, changes in mucus composition were observed. *A. mucin* mono-associated mice exhibited decreased sialic acid and fucose residues compared to germ-free controls. Most notably, we found a significant increase in tuft cell number in *A. mucin* mice compared to controls. Mass spectrometry of conditioned media identified succinate as a byproduct of *A. mucin*. Succinate has been shown to activate small intestinal tuft cells and to expand tuft cell numbers. Analysis of the *A. mucin* genome further confirmed its capability to produce succinate. **Conclusions:** Our data elucidates the impact of *A. mucin* alone on the mammalian small intestine, specifically its ability to increase tuft cell population via succinate production. These findings could help inform human conditions, such as Parkinson's Disease and Multiple Sclerosis, that are associated with an over-abundance of *A. mucin*.

Abstract 25

Distinct Mucin Profiles are Associated with Inflammation in Ulcerative Colitis Patients

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Background: All intestinal epithelial cells have mucins anchored to their apical membrane, while goblet cells additionally synthesize secretory mucins. Both mucin types contribute to a mucosal barrier that is crucial for gut homeostasis. Mucin dysfunction leads to increased risks for infection, inflammation, and may contribute to inflammatory bowel disease (IBD). Prior studies indicate that ulcerative colitis (UC) patients have reduced goblet cell counts, altered mucin glycosylation, and more permeable mucus compared to healthy individuals. The objective of this study was to investigate the relationship between mucin profiles and inflammation in UC. **Methods & Results**: We analyzed RNAseq data from rectal biopsies of 206 control non-IBD individuals and new-onset UC patients (GSE109142). Patients were categorized by inflammation levels determined by calprotectin levels and histological scores from diagnostic colonoscopy. RNAseq revealed that UC patients had increased adherent mucins MUC1, MUC4, and MUC13, and levels were positively correlated with histological severity. Contrarily, MUC3A and MUC20 levels were negatively correlated with inflammation severity. No changes were observed in other adherent mucins. As for secreted mucins, we found that histological severity scores of 2 and 3 were associated with increased MUC2, MUC5B, and MUC5AC. We confirmed differences in MUC5B and MUC5AC protein levels by immunostaining colonic tissue from healthy individuals and UC patients. To determine if host-derived cytokines affect mucin profiles, we examined the mucin profiles of intestinal organoids derived from healthy individuals and UC patients. Interestingly, only MUC4 was significantly elevated in UC organoids, suggesting that other components absent in the cultures likely drive mucin alterations. To identify involved cytokines, we assessed mucin profiles in organoids treated with INF-y, IL-17A, IL-22 or TNF. IL-22, but not the others, increased MUC1, MUC4, MUC13, and MUC5B levels. Finally, we found decreased levels of glycosyltransferases GALNT3, GALNT5, GALNT7, GALNT12, B4GALT4, B4GALT5, B3GNT2, B3GNT3, B3GALT5, B3GNT7, B3GNT8, and ST6GALNAC1, and increased levels of ST3GAL1 and ST3GAL2 in UC patients, suggesting that mucins in UC may also be abnormally glycosylated. **Conclusions**: Inflammatory cytokines may contribute to UC by altering mucin profiles, such as increasing mucin expression and abnormal glycosylation.

Abstract 26

High Fat Diet Alters Gastric Homeostasis Leading to Metaplasia

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Background & Aims: High-fat diets (HFDs) are increasingly prevalent worldwide. The consumption of HFDs are known to disrupt gastrointestinal function, leading to metabolic imbalances and contributing to digestive diseases. While much research has focused on the intestinal alterations that result from HFD, the impact of HFD on the stomach remains underexplored. Given the stomach's key role in nutrient sensing and hormone secretion, cellular changes in response to HFDs may contribute to gastric disorders, including inflammation and cancer. This study investigates the effects of HFD on gastric cell composition and the resulting cellular changes. **Methods:** Male C57BL/6 mice were fed either a standard chow diet (control) or an HFD for 25 weeks. Gastric tissue was collected from mice following euthanasia and was fixed in 10% normal buffered formalin overnight. Gastric tissue was then analyzed by histology and immunostaining for markers that identify individual cell types within the gastric mucosa: tuft cells (DCLK1), enteroendocrine cells (ChgA), goblet cells (MUC2), and proliferative cells (Ki67). Quantification of cell types was performed using Image J software. **Results:** HFD led to notable changes in stomach cell composition. Analysis of H&E-stained tissue showed thickening of the gastric mucosa, loss of parietal cells and an infiltration of immune cells in mice fed a HFD compared to chow fed mice. Tuft cells, which are involved in immune responses and nutrient sensing, significantly increased in HFD fed mice. Enteroendocrine cells, crucial for hormone regulation, were significantly decreased in mice fed a HFD compared to control chow fed mice. Additionally, proliferating cells showed an upward trend, indicating increased turnover which could lead to an increased risk of cancer development. MUC2+ goblet cells were detected in 60% of the HFD mice and were absent in control mice, indicating that intestinal metaplasia was present in some of the HFD

mice. **Conclusion:** Prolonged consumption of a HFD induces significant cellular changes in the stomach, including increased tuft cells, reduced hormone-secreting cells, and heightened cell proliferation, potentially contributing to inflammation and cancer risk. These data highlight the importance of understanding HFD's role in gastric disease progression.

Abstract 27

Antibiotic-Induced Alterations in Gut Microbiota and Their Effect on Mucus Production in the Cystic Fibrosis Colon

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Introduction: Cystic fibrosis (CF) is a genetic disorder caused by mutations in CFTR, leading to thick mucus accumulation in the lungs and frequent respiratory infections requiring antibiotic treatment. We hypothesized that antibiotics could have off-target effects on the gut microbiome and intestinal architecture in CF patients. **Methods:** We conducted 16S sequencing and non-targeted metabolomics via LC-MS/MS on fecal samples from pediatric CF patients and healthy children. Samples were collected from children without antibiotic exposure for 30 days and CF patients currently on antibiotic regimens. We measured the mucin proteins MUC1 and MUC2 in intestinal biopsy specimens through immunostaining and assessed MUC2 levels in mucus-producing cells after antibiotic exposure via qPCR and immunostaining. To evaluate bacterial community functions, we cultivated bioreactors from stool samples of both groups and treated them with various antibiotics, applying sterile supernatants to mucus-producing cells to examine MUC2 levels. **Results:** 16S RNA sequencing revealed that CF children had significantly altered gut microbiota compared to non-CF children. Stool samples from CF children on antibiotics showed decreased Firmicutes (e.g., Anaerostipes, Ruminococcus, Blautia) and Acintobacteria (e.g., Bifidobacterium), while Bacteroidetes (e.g., Bacteroides, Parabacteroides) were elevated. Non-targeted metabolomics indicated a significant reduction in amino acids and metabolites promoting colonic mucus due to antibiotic use. Immunostaining of colonic biopsies showed decreased mucus-filled goblet cells, lower MUC2 levels, and reduced sialic acid in CF patients on antibiotics compared to non-CF individuals and CF patients without recent antibiotic exposure. This mucus depletion was particularly notable with certain antibiotic classes. In vitro, antibiotics did not affect mucus expression or protein in mucus-producing cells, but metabolites from untreated bioreactors upregulated MUC2 production, while those from antibiotic-treated bioreactors did not. **Conclusions:** Our findings indicate that antibiotics significantly alter gut microbiota and deplete metabolites essential for mucus production. This underscores the importance of considering antibiotic type in CF management, as certain therapies may worsen gastrointestinal complications by disrupting microbiota balance and reducing critical mucus-promoting metabolites.

Abstract 28

Claudin-23 Expression in Intestinal Epithelial Cells Surrounding Mucosal Wounds Enhances Wound Repair *In Vivo*

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Background: Epithelial cells play a crucial role in controlling mucosal barrier function. Mucosal injury resulting in wounds is seen in chronic in inflammatory diseases. In response to damage, epithelial cells migrate as a collective sheet to restore critical barrier function. Claudins in epithelial tight junctions are key regulators of barrier function, and recent studies suggest they contribute to epithelial homeostasis, migration, and proliferation. We

observed increased claudin-23 (CLDN23) expression in intestinal epithelial cells (IECs) within crypts surrounding mucosal wounds. Therefore, our studies were aimed at investigating the role of CLDN23 in regulation of intestinal epithelial wound repair. **Methods:** *Cldn23*$^{ER\Delta IEC}$ mice were used to silence CLDN23 expression in IECs, with *Cldn23*$^{f/f}$ controls. Human and murine IECs (colonoids) were used to analyze CLDN23 expression post-injury. Human model IEC lines with overexpression (OE) and knockdown (KD) of CLDN23 were generated as previously described (Raya-Sandino, A. *et al.*, 2023, *Nature Commun*. **14**:6214). CLDN23 expression in colonic wounds was detected using scRNAseq, RNAScope, and immunofluorescence labeling. *In vitro*, scratch-wounded CLDN23 OE or KD IEC monolayers were monitored for 24h via time-lapse imaging. *In vivo*, punch-biopsy wounding was used to assess repair over 72h using colonoscope videos. To assess the role of CLDN23 in cell migration, colonoid movement was tracked for 48h. Cell directionality and speed were calculated in the DiPer software (Gorelik R, Gautreau A., 2014, *Nature Protoc*. 9:1931-1943). **Results:** Upon injury, CLDN23 mRNA and protein expression increased in crypts adjacent to the wound at 6 and 12h, suggesting a role for CLDN23 in regulating mucosal wound healing. Mice lacking epithelial CLDN23 expression showed significantly delayed mucosal wound repair (p<0.0001) following biopsy-induced injury in the colon. In keeping with *in vivo* findings, scratch-wounded colonoids from CLDN23 KD mice exhibited delayed wound repair compared with colonoids from floxed mice. Furthermore, in vitro studies using IECs with either CLDN23 OE or KD confirmed functional effect of CLDN23 modulation on epithelial wound repair. Mechanistically, perturbed wound repair observed in the absence of CLDN23 was attributable to a reduced speed of cell migration without affecting cell directionality. **Conclusion:** CLDN23 plays a novel role in mucosal wound repair by regulating collective epithelial cell migration. Understanding this mechanism could lead to new therapies for promoting wound repair in chronic inflammatory diseases.

Abstract 29

Enhancing the Expression of Barrier Structure Proteins in Human Colon Organoids: An In Vitro Study of Combined Mesalamine and Aquamin Treatment

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Introduction: Inflammation and gut barrier dysfunction are salient features of Ulcerative Colitis (UC). This study aimed to explore the interactions between Aquamin and Mesalamine, assessing their combined potential as a treatment for UC. **Methods:** Aquamin, a multi-mineral supplement derived from red marine algae, contains calcium, magnesium, and 72 additional trace elements. It has been shown to improve barrier structure and function. Mesalamine, an approved drug for treating ulcerative colitis, was studied alongside Aquamin in this in-vitro trial. Human colon organoids, maintained in either control culture medium or exposed to a proinflammatory stimulus (lipopolysaccharide with a combination of three pro-inflammatory cytokines [tumor necrosis factor-α, interleukin-1β and interferon-γ] - LPS-cytokines), were treated with Aquamin, Mesalamine, or their combination for 14 days. Proteomic analysis was conducted on the organoids to evaluate protein changes induced by the treatments, both individually and combined, under normal and inflammatory conditions. **Results:** Upon proteomic assessment, the barrier proteomic expression with Aquamin was consistent with previous studies. The combination of Aquamin and Mesalamine increased the expression of numerous proteins involved in barrier structure. These proteins include mucins-related proteins (Mucin-3A, Mucin-3B, and Zymogen granule membrane protein 16), Junctional adhesion molecule A, cadherins (Cadherin-17, Cadherin-13, Cadherin-3, and Protocadherin-1), desmosomal proteins (Desmoglein-2 and Desmocollin-2), and proteins involved in the basement membrane (Nidogen-1, Nidogen-2, Laminin subunit alpha-1, beta-1, gamma-1, beta-2, alpha-4, and alpha-2 and heparan sulfate proteoglycan core protein). Stimulation with LPS/cytokines caused an upregulation of multiple proinflammatory moieties, which were decreased with the combination of Aquamin and Mesalamine. **Conclusion:** These findings suggest that Aquamin, a multi-mineral supplement, may offer benefits as an adjuvant therapy with Mesalamine for UC. It achieves this by upregulating proteins involved in gut barrier structure and suppressing the expression of specific pro-inflammatory proteins.