

PISA

PATHOBIOLOGY FOR INVESTIGATORS, STUDENTS & ACADEMICIANS

Young Investigators Virtual Meeting Abstracts

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Pathobiology for Investigators, Students, and Academicians PISA2025 Young Investigators Virtual Meeting October 28-30, 2025

Session 1: AI-driven Insights and Novel Approaches in Understanding Human Pathology

Abstract 001

Assessment of ERBB2 Copy Number Variant Detection by Whole Exome Sequencing in Breast Cancers

Taylor Ticer, Parth S. Shah, Shrey S. Sukhadia, Joel A. Lefferts, Liam L. Donnelly, Jeremiah X. Karrs, Jonathan D. Marotti, and Laura J. Tafe

Department of Pathology and Laboratory Medicine, Dartmouth Hitchcock Medical Center, Lebanon, NH

Introduction: ERBB2, which encodes for the human epidermal growth factor receptor-2 (HER2), is a receptor tyrosine kinase that frequently undergoes gain-of-function alterations or amplifications in numerous types of solid tumors, including breast, colorectal, gastroesophageal, and endometrial cancer. Current guidelines recommend testing HER2 status in the initial workup of breast cancers, via validated immunohistochemistry (IHC) staining for HER2, or fluorescence *in situ* hybridization (FISH) detection of ERBB2. Advances in next-generation sequencing, such as whole exome sequencing (WES), allows for the detection of ERBB2 copy number (CN) alterations. Here we performed a quality assessment of breast cancer cases that underwent WES and compared ERBB2 CN calls with IHC and FISH results, when performed. **Methods:** NGS data were obtained for all breast cancer samples that underwent WES from February 2022 to April 2025. CN was adjusted for NGS-predicted tumor cellularity on the AUGMET platform. Following identification, a chart review was performed to obtain IHC and/or FISH results. IHC and FISH results were scored according to ASCO/CAP guidelines. WES results were stratified with a threshold of ≥ 4 copies being considered a CN gain. **Results:** Of the 122 WES samples that were identified, 103 had IHC, 68 had FISH, and 58 had both performed. The concordance between WES $CN \geq 4$ and FISH (ERBB2:CEP17 ratio) was 92.6% (63/68). When applying a $CN \geq 4$ to $CN < 6$ equivocal range requiring additional FISH or IHC testing (with $CN < 4$ negative and $CN \geq 6$ positive), all samples but one were concordant. The one discordant sample was WES negative ($CN=3$)/FISH positive (ratio 2.9). By WES, this sample showed a highly complex genome with homologous repair deficiency. The concordance between WES ERBB2 $CN \geq 4$ gains with HER2 3+ overexpression was 92.3% positive concordance ($n=12/13$). One discordant case was 3+ by IHC while negative by FISH (ERBB2:CEP17 = 1.0) and WES ($CN=3$), suggesting a mechanism of HER2 overexpression other than ERBB2 CN alteration. **Conclusions:** A review of our institution's breast cancer samples that were sequenced showed high concordance between ERBB2 CNV calls by WES as compared to FISH and IHC. When applying negative ($CN < 4$), equivocal ($CN \geq 4$ to $< CN 6$), and positive ($CN \geq 6$) criteria, similar to IHC and FISH strategies, concordance was 98.5%. This provides assurance of WES as a method to identify ERBB2 CN gains.

Abstract 002

Deep Learning Stratifies Hepatocellular Carcinoma Using H&E Whole Slide Images

Tyler M. Yasaka^{1,2,3,4,5}, Po-Yuan Chen⁵, Satdarshan P. Monga^{1,2,4,5,6*}, and Yu-Chiao Chiu^{4,5,6*}

¹Organ Pathobiology and Therapeutics Institute, University of Pittsburgh School of Medicine, Pittsburgh, PA;

²Department of Pharmacology and Chemical Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA;

³Medical Scientist Training Program, University of Pittsburgh School of Medicine, Pittsburgh, PA; ⁴Pittsburgh

Liver Research Center, University of Pittsburgh Medical Center and University of Pittsburgh School of Medicine,

Pittsburgh, PA; ⁵University of Pittsburgh Medical Center Hillman Cancer Center, University of Pittsburgh School

of Medicine, Pittsburgh, PA; ⁶Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh,

PA

Introduction: Hepatocellular carcinoma (HCC) is a major cause of cancer-related mortality. Although several transcriptomics-based classification systems exist, their clinical utility remains limited. With the increasing use of tissue biopsies in targeted therapy trials, there is an opportunity to advance both molecular and histologic approaches for HCC stratification. **Methods:** Publicly available spatial transcriptomics data with paired hematoxylin and eosin (H&E) images from 10 HCC slides were used to train deep learning models to predict Hoshida subtype (S1, S2, S3) signatures within spatial transcriptomics spots from corresponding H&E tiles. Models were evaluated using an 80/20 training/test split and subsequently applied to H&E whole-slide images from The Cancer Genome Atlas (TCGA; n=340). Tile-level predictions were aggregated to generate patient-level histologic scores, which were then reduced via principal component analysis into two dimensions (HCC Morphologic Indexes [HMI1 and HMI2]), capturing 97% of score variability. Optimal thresholds for overall survival (OS) stratified patients into high-, intermediate-, and low-risk groups, which were further assessed for clinical and molecular correlates. **Results:** Models achieved holdout AUROCs of 0.93 (S1), 0.92 (S2), and 0.94 (S3). In TCGA, risk groups predicted OS across all comparisons (high vs low, $p=4.4\times 10^{-11}$; high vs intermediate, $p=0.0034$; intermediate vs low, $p=1.9\times 10^{-5}$) and disease-free survival (DFS) in most comparisons. Each group was associated with distinct etiologies (e.g., alcohol, MASLD, HBV), mutations, and molecular phenotypes identified through gene set enrichment analysis: high-risk with proliferation (e.g., cell cycle), intermediate-risk with inflammation (e.g., complement cascade), and low-risk with metabolism (e.g., mitochondrial β -oxidation). **Conclusions:** Using a deep learning model which predicts spatial subtype signatures from H&E whole slide images, we developed a histology-based HMI with improved prognostic power compared to existing HCC subtypes. The associated clinical and molecular features suggest that these subtypes exhibit distinct phenotypes and pathogenesis, supporting the potential of HMI to guide patient stratification in clinical trials and inform personalized therapeutic strategies.

Abstract 003

Accelerating Microbiology Diagnostics: Culture Optimization and Early Testing Performance

Janiece S. Glover, Bailey Demarest, Pam Foster, and Kendall A. Bryant

Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center, Nashville, TN

Introduction: The rising demand for microbiology testing has led clinical laboratories to adopt automation technologies to enhance efficiency and accuracy. Microbiology laboratory automation (MLA) improves sample throughput, speeds up diagnostic processes, and minimizes contamination risks. This study investigates the optimal timepoints for culture workup using MLA and assesses whether these timepoints impact downstream identification and antimicrobial susceptibility testing (AST). **Methods and Results:** A total of 374 patient specimens from various sources were analyzed to optimize culture workflows. Specimens were manually cultured and assessed at standard incubation intervals, while the WASPLab system captured digital images at multiple timepoints between 10 and 72 hours. Technologists reviewed these images to determine when cultures were ready for further testing. For non-urine specimens, 52–60% were ready for workup at 16 hours, increasing to 92–100% by 24 hours. Urine specimens showed 6% readiness at 12 hours, rising to 90% by 16 hours. No significant growth was observed in any specimen type beyond 36 hours. Selected Gram-negative and Gram-positive bacterial strains were subjected to MALDI-TOF identification and AST at 10 and 16 hours to evaluate early testing performance. All Gram-negative organisms showed consistent identification results at both 10 and 16 hours, except for *Acinetobacter* species at 16 hours and *Eikenella* species at 10 hours. Gram-positive organisms also showed high concordance, with the exception of *Paenibacillus* species. AST results for Gram-negative organisms at 10 and 16 hours were comparable to standard 18-24 hour methods for all antibiotics tested, except nitrofurantoin. Gram-positive organisms showed consistent AST results, with the exception of sulfamethoxazole/trimethoprim when using the *Streptococcus* AST card. **Conclusion:** MLA enables earlier culture readings and faster finalization without compromising the accuracy of identification or AST results. Early incubation growth can be effectively used for downstream testing, supporting the clinical utility of automation in microbiology labs.

Abstract 004

Lipid Alterations and Oligodendrocyte Distribution in White Matter Hyperintensities in Alzheimer's Disease

Dana R. Julian¹, Anna Costa², Li Jinghang³, Jr Jiun Jean Liou³, Thomas Pearce¹, and Julia K. Kofler^{1,4}

¹Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA; ²Department of Psychiatry, University of Pittsburgh School of Medicine, Pittsburgh, PA; ³Department of Bioengineering, University of Pittsburgh Swanson School of Engineering, Pittsburgh, PA; ⁴Clinical and Translational Science Institute, University of Pittsburgh School of Medicine, Pittsburgh, PA

Introduction: Disease (AD) is a relentless, progressive neurodegenerative disease leading to severe cognitive decline and affects approximately 26.6 million people worldwide. White matter hyperintensities (WMH) are radiologically defined regions of myelin rarefaction which precede cognitive symptoms in AD by as early as 20 years and are predictive of disease onset, severity, and progression. Lipids play critical and dynamic roles in homeostatic white matter, yet little is known about lipid alterations in the context of WMH. **Methods and Results:** We performed untargeted lipid chromatography mass spectrometry (LC-MS) on fresh frozen samples (n=39) from either normal appearing white matter (NAWM) or WMH frontal parietal periventricular tissue. WMH status was determined by postmortem 7T MRI of the contralateral hemisphere and assigned a grade of normal, mild, moderate, or severe by local WMH volume. We uncovered distinctive lipidomic signatures in NAWM and mild WMH vs. moderate and severe WMH tissue with altered chain length and saturation levels in myelin-enriched lipids. To investigate myelin and oligodendrocyte alterations in the spatial context of WMH, we performed MRI-whole slide image alignment. We developed a quantitative digital image analysis pipeline to identify alterations in myelin (Luxol Fast Blue H&E staining) and oligodendrocyte (PLP1⁺ *in situ* hybridization) distribution. These data were processed through algorithms identifying periventricular zones, subcortical vs. deep white matter, and WMH lesion depth vs. the WMH transition zone. We also performed semi-supervised machine learning to identify tissue regions most significant to WMH vs. NAWM prediction. **Conclusions:** Bringing this all together, we built a multi-modal machine learning algorithm to elucidate significant tissue regions in WMH using WSI data using a convolutional neural network, cellular distribution using a graph convolutional network, and lipidomics data using a feed-forward network. These parallel methodologies allow us to interrogate regions of myelin rarefaction in Alzheimer's Disease with changes in lipid signatures. Identifying the biologic underpinnings of myelin loss is critical for the understanding of AD disease pathogenesis.

Abstract 005

Human iPSC-Derived Skin Organoids Recapitulate Cutaneous Responses to Inflammation and Injury *In Vitro*

Anthony R. Sheets, Shannon M. McNamee, and George F. Murphy

Program in Dermatopathology, Department of Pathology, Brigham and Women's Hospital, Boston, MA

Introduction: Few lab models of human skin recreate the intact spatial microenvironment required to study the development, form, and function of its microcirculation. Advanced systems that capture these aspects of native human skin are needed to better understand and treat microvascular dysfunction underlying many skin conditions, including psoriasis, vasculitis, and non-healing wounds. We have recently discovered microvascular networks within the dermis of human iPSC-derived skin organoids (SKOs), self-organizing structures composed of hollow spheres lined by hair-forming epidermis, encircled by a mesenchyme that also forms cartilage, adipose tissue, and neural elements. Given the importance of the dermal microvasculature in skin homeostasis, inflammation, and wound repair, we examined the dynamics of vascular growth and maturation in the SKO system and characterized its responses to inflammation and mechanical injury. **Methods:** We derived SKOs from human iPSCs expressing GFP-tagged CDH5 and used fluorescence microscopy to track endothelial cell growth and microvascular network expansion in this system. We assayed organoid media for angiogenic growth factor production and used multiplex IHC to compare vasculature between early and late SKOs. We exposed SKOs to pro-inflammatory cytokines (TNF α , IL1 β , and IFN- γ) and profiled their downstream responses through IHC and multiplex antibody arrays for cytokine production. Last, we created sharp injuries in SKOs and investigated tissue repair with brightfield and fluorescence microscopy. **Results:** We identified vasculogenic foci in SKOs by 6 days post-differentiation, driven by secretion of growth factors including FGF2, VEGF, and placental growth factor. SKO microcirculation expands to form arborizing networks lasting beyond 4 months in culture, with progressive increases in microvascular mural cell coverage and basement membrane synthesis demonstrated by IHC. Cytokine-treated SKOs robustly express VCAM1, ICAM1 and secrete additional chemokines, cytokines, and acute phase reactants. Finally, SKOs mount epithelial, fibroblastic, and microvascular responses to sharp trauma, the latter of which are augmented by VEGF. **Conclusions:** Our study

demonstrates human SKOs replicate the native human skin integumentary responses to inflammation and injury, establishing the system as a platform to study cutaneous microvascular insult in the hopes of developing new therapeutic strategies to address unmet clinical needs.

Abstract 006

Bovine Placental-Derived Bio-Ink Reprograms Tissue Microenvironment for Enhanced Regeneration and Senescence Reversal

Fabiana Mastantuono¹, Samantha Ali², Pei Zhuang², Zongliang (Carl) Jiang³, and Mei He²

¹*Department of Biomedical Engineering, University of Florida, Gainesville, FL;* ²*Department of Pharmaceuticals, University of Florida, Gainesville, FL;* ³*Department of Animal Sciences, University of Florida, Gainesville, FL*

Introduction: We propose a bovine placental-derived natural bio-ink for 3D printing, composed of droplets encapsulating placental collagen type I (COL 1) and amniotic fluid-derived extracellular vesicles (AF-EVs) for reprogramming the wound microenvironment to be more responsive to repair. **Methods:** Bovine placenta tissue was dissected and digested in acetic acid and pepsin. COL 1 was precipitated using NaCl and characterized by BCA, Hoechst for DNA contamination, and western blot for collagen type I. Collagen morphology was confirmed by SEM and biocompatibility was evaluated using a scratch assay with C2C12 mouse muscle cells. To assess regenerative potential, AF-EVs from the second (AF1) and third (AF2) trimesters were compared. To assess anti-senescence effects, senescent placental trophoblasts were treated with AF-EVs and stained for γ H2AX and DRAQ5 to quantify DNA damage per nucleus. **Results:** The protein concentration of bovine placental collagen was comparable to concentration of Telocol 10 (Biomatrix). DNA contamination was significantly lower than that of commercial collagen. Western blot confirmed COL 1 presence, and collagen structure was verified by SEM. C2C12 cells seeded on 0.01% bovine collagen achieved complete scratch closure at 48 hours and showed the highest proliferation by Presto Blue compared to other concentrations and all commercial collagen groups. AF2-EVs had a higher particle concentration than AF1-EVs and greater RNA yield. Scratch assay revealed a one-third-fold improvement in wound closure with AF2-EVs at 42 hours, compared to PBS and HUC MSC EVs. γ H2AX/DRAQ5 staining showed nearly twice the DNA damage per nucleus in control placental trophoblast cells versus those treated with AF2-EVs at day 2. A bio-ink composed of collagen droplets encapsulating AF-EVs was successfully generated using the microfluidic platform. **Conclusion:** This bio-ink integrates high-purity placental collagen with bioactive AF-EVs to achieve structural support and targeted modulation of the wound microenvironment. By shifting the microenvironment toward a more responsive state, this platform provides accelerated tissue repair which can be beneficial for complex or chronic wounds.

Session 2: Biomarker Discovery and Therapeutic Advances in Women's Cancers

Abstract 007

Breast Cancer Liver Metastasis: Histopathologic Features at Primary and Metastatic Diagnoses

Hatun Duran Cete, Alexandra Bartlett, Michelle Ozaki, and Pepper Schedin

Knight Cancer Institute, Oregon Health and Science University, Portland, OR

Introduction: Breast cancer metastasis to the liver has poor prognosis, partly because predicting patients at risk for liver metastasis remains elusive as does effective targeting of metastases. Risk factors for liver metastasis are young age and primary breast cancer diagnoses with triple negative and HER2+ subtypes, high grade, and late stage, but overall prognostic sensitivity is low. Lack of histopathologic characterization of metastatic tumors limits targeted therapeutics. **Methods:** We enrolled a cohort of 47 breast cancer patients with liver metastatic tumors, who underwent liver biopsy/surgical resection at OHSU, Portland, Oregon in this retrospective study. Patients were eligible for study inclusion regardless of age or breast cancer subtype. We utilized clinicopathological characteristics obtained at the time of primary breast cancer diagnosis to identify attributes associated with liver metastasis. We then used formalin fixed paraffin embedded liver metastases for histological characterization of growth patterns and expression of ER, PR, and Ki67. **Results:** At primary breast cancer diagnosis, 38% of cases were ≤ 45 years of age and 72% of the young cases were diagnosed within 10 years of childbirth. Across all age groups, 85% of cases were diagnosed with ER+, mostly luminal A breast cancers. Of these primary tumors, 57% were low grade, and 73% were early stage. The majority of the liver metastatic lesions were also ER+, with only 10% conversion to ER-. High GATA3 ($\geq 60\%$) was observed in 86 %

of the liver metastases, confirming luminal origin. Breast differentiation phenotypes were retained in 30% of the liver lesions. PR receptor switching from ER+PR+ primary to ER+PR- liver lesion was frequent. Replacement growth pattern was dominant, observed in 83% of metastases. **Conclusion:** Young age and proximity to recent childbirth is associated with breast cancer metastasis to the liver. Regardless of patient age, we find luminal A, hormone receptor positive, low-grade and early-stage primary tumors represent the majority of these cases, suggesting that these classic good prognostic indicators may not apply to breast cancer metastasis to the liver. Finally, improved understanding of the dominant replacement growth pattern and the relation between tumor cells and the liver metastatic niche may lead to new drug targets and precision therapies.

Abstract 008

Discovering AI-Guided Targeted Therapeutic for Metastatic Triple Negative Breast Cancer

Priyam Kumar¹ and Jayshree Mishra²

¹Texas A&M University, College of Pharmacy, Kingsville, TX; ²Department of Pharmaceutical Sciences, Texas A&M University, Kingsville, TX

Introduction: Triple-negative breast cancer (TNBC) represents 15-25% of breast cancers and disproportionately affects younger women, African American patients, and BRCA1 mutation carriers. TNBC is the most aggressive subtype, with rapid progression and a high frequency of brain metastases (BM), affecting up to 36.4% of patients. Currently available treatments, including chemotherapy and whole-brain radiation therapy, are limited by poor blood–brain barrier (BBB) penetration and high neurotoxicity, underscoring the need for novel therapies.

Methods and Results: In this study, we investigated non-receptor tyrosine kinase (NRTK) as a potential therapeutic target in TNBC-BM. Immunofluorescence analyses of brain sections from TNBC-BM patients confirmed NRTK overexpression in metastatic lesions. Using artificial intelligence (AI)–driven structure-based modeling, we screened billions of small molecules to identify candidates capable of selectively binding NRTK and crossing the BBB. Docking simulations, energy minimization, and functional assays demonstrated strong NRTK-JAK binding activity and induction of apoptosis in TNBC-BM preclinical cell culture models. **Conclusions:** Our findings suggest that AI-guided drug discovery can rapidly identify brain-penetrant, target-specific inhibitors with therapeutic potential for TNBC-BM. This strategy holds promise for overcoming the limitations of current treatment regimens and may serve as a foundation for future translational development after the in vivo pre-clinical testing.

Abstract 009

Evaluating Homologous Recombination Deficiency (HRD), Microsatellite Instability (MSI), and Tumor Mutational Burden (TMB) Biomarkers in Ovarian and Endometrial Cancer patients

Debopriya Chakraborty, Laura J. Tafe, Joel A. Lefferts, Donald C. Green, and Parth S. Shah

Department of Pathology and Laboratory Medicine, Dartmouth-Hitchcock Medical Center, Lebanon, NH

Introduction: HRD is caused by pathogenic variations in HR repair pathway genes e.g. *BRCA1*, *BRCA2* etc., and “genomic scars” or structural variations measured by loss of heterozygosity (LOH), large scale transitions (LSTs), and telomeric allelic imbalances (TAI). HRD is a predictive biomarker for ~50% of high-grade serous ovarian cancer patients among others, determining their eligibility for poly (ADP-ribose) polymerase inhibitor (PARPi) treatment. MSI is caused by deficiency in DNA mismatch repair leading to spontaneous gains or losses in repetitive DNA motifs (microsatellites) in the genome. Both TMB, defined by genome-wide occurrences of non-synonymous mutations, and MSI, vary across cancer types and are predictive biomarkers of response to immune-checkpoint inhibitors. Recent clinical trials are investigating HRD in the context of checkpoint blockade along with PARPi for a potential synergistic effect. Here, we evaluate HRD scores using whole exome sequencing (WES), and compare it with MSI and TMB scores in ovarian and endometrial cancer patients.

Methods: Tumor genomic DNA was extracted from formalin-fixed paraffin-embedded (FFPE) tissues (12 ovarian cancer, one endometrial cancer and 3 control samples) using Purigen system, quantified using Qubit HS, and WES performed on Illumina NovaSeq sequencer. All samples had been previously tested for HRD at Myriad Genetics. HRD, MSI and TMB scores are determined using the respective callers in AUGMET, our in-house bioinformatics pipeline: HRD= Score [LOH+ LST + TAI], ≥ 50 : positive, < 50 : negative; TMB- ≤ 10 Mutations/Mb: Low, > 10 Mutations/Mb: High; MSI- $\geq 5\%$ (MSI-High, unstable), $> 1.6\%$ and $< 5\%$ (MSI-Indeterminate), $\leq 1.6\%$ (MSS, stable). **Results:** Our HRD assay was ~80% concordant compared to Myriad MyChoice results. Three

cases were discordant, likely due to low tumor content (20%) in one, and inter-assay variability in two. There was no correlation of the HRD scores with TMB and MSI biomarkers in the 13 patient samples. **Conclusions:** Due to highly complex nature of HRD biology involving multiple genomic events, the variabilities in its classification techniques are inevitable, leading to discordant results. Although HRD, MSI and TMB originate from pathogenic variants in DNA repair pathways, they vary with respect to immune activation of tumor microenvironment, and across cancer types. This underscores the importance of comparing different biomarkers to guide therapeutic decisions and improve patient outcomes.

Abstract 010

Investigating the Role of WT1-CD200 Axis and BRCA Status in Stromal Modulation of TLS Formation in High-Grade Serous Ovarian Cancer

Swathi Suresh¹, Erika Lampert², Grace Gorecki³, Ian P. MacFawn⁴, Huda Atiya⁵, Tullia C. Bruno⁶, and Lan Coffman^{6,7}

¹Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA; ²Department of Obstetrics, Gynecology, and Reproductive Sciences, University of Pittsburgh, Pittsburgh, PA; ³Internal Medicine, Allegheny Health Network, Pittsburgh, PA; ⁴Department of Biology, Grove City College, Grove City, PA; ⁵Division of Hematology/Oncology, Division of Gynecologic Oncology, Department of Obstetrics, Gynecology, and Reproductive Sciences, University of Pittsburgh, Pittsburgh, PA; ⁶Department of Immunology, University of Pittsburgh, Pittsburgh, PA; ⁷Department of Pathology, Magee-Women's Research Institute, University of Pittsburgh, Pittsburgh, PA

Introduction: High-grade serous ovarian cancer (HGSOC) is the most lethal subtype of ovarian cancer, with more than 70% of patients presenting with metastatic disease at the time of diagnosis. While germline BRCA1/2 mutations carry a 30-40-fold higher risk of HGSOC, paradoxically, patients with BRCA mutations have improved responses to treatment and overall survival benefit compared to BRCA-wildtype patients. This study aimed to investigate how BRCA status alters the tumor microenvironment (TME), particularly the stromal compartment, to support anti-tumor immunity. Our lab has previously demonstrated that ovarian cancer cells epigenetically reprogram their resident tissue mesenchymal stromal/stem cells (MSCs) to develop a cancer-supportive phenotype. These cancer-associated mesenchymal stem cells (CA-MSCs) express high levels of WT1, a transcription factor associated with protumorigenic functions and immune evasion. **Methods and Results:** Using our mRNA seq data, we identified a positive correlation between WT1 and CD200, an immune-modulatory protein, in CA-MSCs. With the help of multispectral flow analysis, we found that elevated CD200 expression in CA-MSCs impairs their differentiation into follicular dendritic cells (fDCs), a stromal subtype essential for the development and active function of tertiary lymphoid structures (TLS). These are ectopic lymphoid aggregates that develop in the TME and serve as a hub with multiple immune cells, including B cells and T cells, associated with enhanced anti-tumor immunity and a favorable response to immunotherapy. To investigate whether BRCA status influences this stromal differentiation axis, we isolated CA-MSCs from primary HGSOC tumors with known germline BRCA status and analyzed CD200 expression by qPCR and flow cytometry. We observed that CA-MSCs derived from germline BRCA-mutant tumors express significantly lower levels of CD200 compared to those from BRCA-wildtype tumors. Functionally, MSCs with reduced CD200 expression exhibit an enhanced capacity to differentiate into fDCs in vitro. **Conclusions:** These results suggest that BRCA mutations may downregulate the WT1-CD200 axis in CA-MSCs, promoting stromal reprogramming that supports TLS formation and antitumor immune responses. Overall, our findings reveal a novel mechanism that highlights the WT1-CD200 axis in HGSOC stroma as a potential target for enhancing TLS formation and anti-tumor immune responses.

Session 3: Microbes, Metabolites, and Models of Disease

Abstract 011

Microbial Oxygen Consumption as a Driver of Anaerobic Niche Formation in Burn Wounds

Subhomitra Ghoshal¹, Erin Chard¹, Anna Tingler¹, Selene Shore¹, Alyssa Gutierrez¹, Jessica H. Hartman², Deepak Ozhathil³, and Melinda A. Engevik¹

¹Department of Regenerative Medicine and Cell Biology, Medical University of South Carolina, Charleston, SC; ²Department of Biochemistry, Medical University of South Carolina, Charleston, SC; ³Department of Surgery, Akron Children's Hospital, Akron OH

Introduction: Oxygen availability is a critical factor influencing microbial community dynamics. Facultative anaerobes have been proposed to reduce oxygen in their environment, thereby generating localized anaerobic niches. Although this concept has been suggested, systematic comparisons of oxygen depletion across diverse bacterial species remain limited. We hypothesized that certain bacteria would efficiently reduce local oxygen and create an anaerobic environment that could support strict anaerobes. **Methods:** Burn wound microbial communities were examined by next generation sequencing. Species from genera including *Streptococcus*, *Enterococcus*, *Lactobacillus*, *Klebsiella*, *Pseudomonas*, *Acinetobacter*, *Enterobacter*, *Staphylococcus*, *Proteus*, *Listeria*, *Escherichia*, *Morganella*, *Serratia*, *Citrobacter*, *Salmonella*, and *Lactococcus* were cultured in a chemical defined medium called ZMB1. Growth was monitored using a Synergy H1 plate reader, and oxygen concentrations were tracked continuously with a Resipher system. **Results:** All isolates grew, though with genus-dependent variation in biomass accumulation. Oxygen in uninoculated controls remained stable at ~200 μ M. In contrast, *Morganella morganii*, *Salmonella enterica*, *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, and all *Klebsiella* strains rapidly lowered oxygen below 30 μ M within one hour. Substantial oxygen depletion was also observed for *Pseudomonas putida*, *Serratia marcescens*, *Staphylococcus aureus*, *Citrobacter freundii*, *Enterobacter hormaechei*, *Enterobacter cloacae*, *Escherichia coli*, *Proteus vulgaris*, *Proteus mirabilis*, *Acinetobacter baumannii*, *Acinetobacter calcoaceticus*, and *Enterococcus faecalis*. By contrast, most *Streptococcus* and *Lactobacillus* strains displayed negligible oxygen-reducing activity. Strikingly, we also demonstrated that the strict anaerobe *Fusobacterium nucleatum* was able to persist under aerobic conditions when co-cultured with oxygen-consuming bacteria, such as *Acinetobacter baumannii*. We grew bacterial communities of oxygen-depleting and strict anaerobe together and applied these communities to skin cells and examined cell architecture and pro-inflammatory response. **Conclusion:** These findings indicate that certain facultative anaerobes have high oxygen-depleting potential and can generate anaerobic conditions that support community interactions that ultimately affect the skin.

Abstract 012

Metabolomic Insights into *Lactobacillus* Responses to Breast Milk: Unravelling the Role of Hydroxyphenyllactic Acid in the Gut

Alyssa Gutierrez¹, Katherine Chetta^{2,3}, Thomas D. Horvath^{4,5}, and Melinda A. Engevik^{1,6}

¹Department of Regenerative Medicine and Cell Biology, Medical University of South Carolina, Charleston, SC;

²Department of Pediatrics, C.P. Darby Children's Research Institute, Medical University of South Carolina, Charleston, SC; ³Department of Pediatrics, Division of Neonatal-Perinatal Medicine, Medical University of South Carolina, Shawn Jenkins Children's Hospital, Charleston, SC; ⁴Department of Pathology and Immunology, Baylor College of Medicine, Houston, TX; ⁵Department of Pathology, Texas Children's Hospital, Houston, TX;

⁶Department of Microbiology and Immunology, Medical University of South Carolina, Charleston, SC

Introduction: In early life, *Lactobacillus* species are essential for maintaining intestinal homeostasis. Beyond preventing colonization by pathogens, *Lactobacillus* exerts beneficial effects by producing bioactive metabolites. However, the metabolite profiles of *Lactobacillus* are incompletely defined, particularly in response to neonatal diets such as breast milk and infant formula. The present study identifies a compound, hydroxyphenyllactic acid (HPLA), that is robustly produced across six *Lactobacillus* species in response to human milk but not infant formula. We further investigate the ability of HPLA to modulate reactive oxygen species (ROS), intestinal permeability, and inflammation using *in vitro* models. **Methods:** We mono-cultured six species (*L. acidophilus*, *L. brevis*, *L. johnsonii*, *L. paracasei*, *L. reuteri*, and *L. rhamnosus*) in a chemically defined medium supplemented with human milk or infant formula. After overnight incubation, supernatants were collected for untargeted metabolomics analysis (LC-MS/MS). To assess HPLA's antioxidant activities, HT-29 and RAW 264.7 cells were treated with H₂O₂ and HPLA and cellular ROS was measured via H2DCFDA. HT-29 cells were also treated with H₂O₂ and HPLA for 6h to measure expression of inflammatory cytokines using RT-qPCR. To model gut permeability, we treated HCT-8 cells with HPLA and used lucifer yellow to assess transwell monolayer permeability over 24h. We further used RT-qPCR to assess mRNA levels of tight junctional genes after HPLA treatment. **Results:** Our metabolomics data revealed that HPLA was produced at high levels by all six

Lactobacillus species in the context of breastmilk but not formula. In HT-29 and RAW 264.7 cells, HPLA significantly decreased ROS induced by H₂O₂ treatment. When HT-29 cells were treated with H₂O₂, mRNA levels of *IL-8* and *IL-1a* were significantly increased, but HPLA rescued these effects. We next found that HPLA significantly improved permeability of an HCT-8 monolayer, and we further demonstrated that HPLA treatment led to significant elevations in mRNA levels of *Claudin-1*, *Claudin-3*, and *Occludin*. **Conclusions:** Our results emphasize that *Lactobacillus* metabolite output depends on diet, and we highlight HPLA being most robustly produced in the context of breastmilk. Our *in vitro* findings further reveal that HPLA might play an important role in the neonatal gut as an antioxidant and modulator of the intestinal barrier and inflammation.

Abstract 013

Establishment of a Novel Model of Pediatric Type C Hepatic Encephalopathy in Rodents

Kathryn Rhodes, Julie Venter, Jace Tyson, Mihika Patankar, Yubo Wang, Patrick Mireles, Brandy Routh, Kiersten Bell, Laura Fonken, Kimberly Nixon, and Sharon DeMorrow

University of Texas at Austin, College of Pharmacy, Pharmacology and Toxicology Division, Austin, TX

Introduction: Hepatic encephalopathy (HE) describes the cognitive and neuromuscular deficits arising from liver impairment. Children with chronic liver disease are at a heightened risk for long term consequences of HE if an episode coincides with crucial neurodevelopmental periods. Little is known about the pathology of pediatric HE, nor the long-term consequences, due to a lack of a suitable rodent model of pediatric HE. The aim of the current work was to establish a rodent model of Type C HE in juvenile mice that will allow for the temporal assessment of the impact of an episode of HE on subsequent brain function. **Methods:** C57/Bl6 mice at PND 14 were treated with 0.2 mL/kg (ip) of CCl₄ or corn oil (vehicle) 3 times per week for 8 weeks. Blood ammonia was assessed at 3 weeks, 6 weeks, and 8 weeks of treatment to monitor liver function over time. Behavioral assessments for cognition, neuromuscular ability, balance, and learning and memory were performed during weeks 6 and 7 of treatment. Liver pathology was assessed via serum chemistries, and standard histological assessments. Brain ammonia levels were quantified using Nessler staining. **Results:** Blood ammonia levels were significantly increased in CCl₄-treated mice at all time points and markers of liver pathology was consistent with chronic liver disease. CCl₄-treated mice displayed (i) hyperammonemia in brain tissue; (ii) neuromuscular deficits (decreased grip strength and time and distance on the Rota-rod); (iii) cognition and neuropsychiatric well-being (nest building assay); (iv) Balance and ataxia (balance beam test); and (v) learning and memory (novel object recognition). Neuroinflammation assessed by immunohistochemical staining of microglia and astrocyte markers showed increased microglia presence after treatment, but this was not observed in astrocytes. **Conclusions:** Treatment of juvenile mice with CCl₄ resulted in impaired cognitive and neurological function and hyperammonemia consistent with symptoms observed in children with Type C HE. This model allows for further investigation of brain recovery patterns, impacts of HE on a developing brain, and pathology of pediatric HE.

Abstract 014

Glucocorticoid Receptor Deficiency Impairs Gestational β -cell Compensation and Contributes to Gestational Diabetes

Hsuan Yeh, Taofeek Usman, Chenglin Pan, Wen Quan Zheng, Goma Chhetri, and Henry Dong

Division of Endocrinology, Department of Pediatrics, UPMC Children's Hospital of Pittsburgh, University of Pittsburgh School of Medicine, Pittsburgh, PA

Introduction: During pregnancy, maternal insulin resistance increases to preserve fetal glucose supply. To counteract insulin resistance, gestational β -cells release more insulin into the blood. Such an adaptive response, termed " β -cell compensation", is essential for maintaining normoglycemia during pregnancy. Insufficient β -cell compensation leads to gestational diabetes mellitus (GDM). Our project investigates the role of glucocorticoid receptor (GR) in β -cell compensation during pregnancy. **Methods:** We assessed the activity of GR in β -cells during pregnancy. We generated β -cell GR-knockout (β GR-KO) mice and determined the impact of β GR-KO on maternal insulin secretion, glucose metabolism, islet architecture and β -cell mass. We also determined the effect of β GR-KO on pregnancy outcomes. **Results:** Plasma glucocorticoid (GC) levels surged along with the progression of pregnancy in WT mice. This effect correlated with enhanced β -cell GR activity in maternal islets in late pregnancy. When compared to WT dams, β GR-KO dams developed GDM characterized by glucose intolerance, reduced glucose-stimulated insulin secretion at gestational day 15.5. β GR-KO dams, as opposed to

WT dams, gave birth to significantly larger pups (macrosomia). WT dams underwent 2- to 3-fold β -cell mass expansion during pregnancy. This adaptation was abrogated in β GR-KO dams, as reflected in the reduction of β -cell mass along with aberrant α -cell distribution within islet cores. Consistent with these structural changes, islets from β GR-KO dams displayed significantly reduced insulin content relative to controls. To gain-mechanistic insights into impaired β -cell compensation in β GR-KO dams, we performed RNA-seq assay on islets of β GR-KO and WT dams, revealing that 231 genes were upregulated and 10 genes were downregulated in GR-deficient islets. Loss of β -cell GR activity triggered overexpression of genes in extracellular matrix deposition, contributing to increased collagen accumulation and disrupted islet architecture. β -cell GR deficiency resulted in marked upregulation of “disallowed genes”, a subclass of genes whose islet expressions are kept at a baseline in healthy individuals and whose activities are linked to β -cell failure in patients with diabetes. **Conclusions:** We concluded that (i) β -cell GR functions primarily as a trans-repressor in regulating β -cell adaptation to pregnancy, and (ii) GC-GR signaling is indispensable for gestational β -cell compensation to protect against GDM.

Abstract 015

***Akkermansia muciniphila* Enables Persistent *Clostridioides difficile* Colonization Through Mucin-Derived Cross-Feeding in Antibiotic-Perturbed Mice**

Katherine Psenka and Melinda A. Engevik

Department of Regenerative Medicine and Cell Biology, Medical University of South Carolina, Charleston, SC

Introduction: *Clostridioides difficile* (*C. difficile*) is an anaerobic, gram-positive, spore-forming bacillus that causes over 450,000 healthcare-associated gastrointestinal infections in the United States each year with around 29,000 deaths annually in the United States. *C. difficile* typically flourishes after broad-spectrum antibiotic use, when commensal microbial populations are disrupted. While competitive exclusion has traditionally been viewed as the primary mechanism suppressing *C. difficile*, the role of residual taxa microbial interactions facilitating or hindering its colonization remains unresolved. We focus on *Akkermansia muciniphila*, a mucus-resident Verrucomicrobiota species with a mucin-degrading enzyme repertoire which could free carbohydrates usable by *C. difficile*. We hypothesized that *A. muciniphila* synergises with *C. difficile* to promote gut colonisation. **Methods and Results:** To address this hypothesis, mice were pretreated with an antibiotic cocktail and subsequently gavaged with phosphate-buffered saline (PBS), *C. difficile* R20291 (Cd), or *C. difficile* plus *A. muciniphila* ATCC BAA-835 (Cd + Akk). Body weight and feces were collected at baseline and on days 3, 5 and 7 post-infection. As expected, Cd-infected mice incurred maximal weight loss on day 3 at the peak of infection and began to recover thereafter. 16S rRNA amplicon sequencing revealed profound phylum-level shifts: antibiotic-treated/PBS mice displayed an expansion of Verrucomicrobiota with concomitant reductions in Bacteroidota and Bacillota; Cd infection reproduced this pattern. Strikingly, Cd + Akk mice showed simultaneous expansion of Verrucomicrobiota, Bacillota and Actinomycetota and a near-complete loss of Bacteroidota. Species-level analysis confirmed that *A. muciniphila* abundance rose after antibiotics alone and remained high in both Cd and Cd + Akk groups. *C. difficile* was found in day 3, 5 and became undetectable in Cd-only animals by day 7. However, at day 7 in the Cd + Akk mice *C. difficile* retained $\approx 10\%$ relative abundance. A closer examination of the Cd + Akk fecal communities highlighted enrichment of *Thomasclavelia cocleata*, *Enterococcus dispar*, *Clostridium cocleatum* and *Tissierella* species. In vitro, we grew *C. difficile* and *A. muciniphila* together and when co-cultured in a mucin-supplemented minimal broth (ZMB1) the two showed significant crossfeeding. To further examine this complex model, we setup a tri-culture with *E. dispar* to examine growth of *C. difficile* to underscoring the metabolic complementarity systems at play in vivo. **Conclusion:** This data demonstrates that *A. muciniphila* acts as an ecological ally that extends the window of *C. difficile* colonisation by liberating and sharing mucin glycans and by recruiting additional fermenters that complete the nutrient web. *A. muciniphila* should be avoided as a probiotic option particular for antibiotic microbiota-depleted groups. Targeted depletion or functional inhibition of *A. muciniphila* may therefore represent a novel microbiome-modulating strategy to curtail recurrent *C. difficile* infection after antibiotic exposure.

Session 4: Molecular and Therapeutic Insights into Retinal Degeneration

Abstract 016

Unraveling Transcriptomic Crosstalk of Inflammatory and Angiofibrotic Cytokines in Primary Human Retinal Microvascular Endothelial Cells

Fergus C. McLellan¹, Kelvin Huang¹, Michele C. Madigan^{1,2}, Yichuan G. Liang³, Andrew J.R. White^{1,3}, Pei Qin Ng⁴, Pete A. Williams⁵, and Daisy Y. Shu¹

¹*School of Optometry and Vision Science, University of New South Wales, Sydney, New South Wales, Australia;*

²*Save Sight Institute, University of Sydney, Sydney, New South Wales, Australia;* ³*Centre for Vision Research, Westmead Institute for Medical Research, University of Sydney, Sydney, New South Wales, Australia;*

⁴*Department of Plant Science, University of Cambridge, Downing Street, Cambridge, UK;* ⁵*Division of Eye and Vision, Department of Clinical Neuroscience, St. Erik Eye Hospital, Karolinska Institute, Stockholm, Sweden*

Introduction: Dysregulated cytokine signaling drives endothelial cell (EC) dysfunction in neovascular age-related macular degeneration (nAMD), transitioning between inflammatory, angiogenic, and fibrotic states. We analyzed transcriptional changes in ECs exposed to six nAMD-associated cytokines — individually and combined — using gene interaction network analysis to explore pathological mechanisms. **Methods:** Primary human retinal ECs (Cell Systems) were treated with transforming growth factor-beta 1 (TGF- β 1), TGF- β 2, tumor necrosis factor-alpha (TNF- α), thrombin, interleukin-6 (IL-6), or vascular endothelial growth factor (VEGFA) at 10 ng/mL, either individually or as a combined ‘cocktail’ group (n = 6). Untreated cells served as controls. After 24 hours, total RNA was extracted, and bulk RNA sequencing (RNASeq) was performed (Azenta Life Sciences). Differentially expressed genes (DEGs) ($|\text{Log}_2\text{FC}| > 1$, FDR < 0.05) and pathways were defined relative to control. Pairwise comparison to the cocktail group was applied to identify ubiquitous and unique disease-associated pathways. A machine learning-based gene interaction network used published gene-protein interactions to characterize the synergistic cocktail effect. DEG networks were compared to nAMD patient tissue RNASeq data (GSE135922) to assess potential clinical relevance. **Results:** TNF- α (1823 DEGs) and thrombin (1019 DEGs) induced broader pro-inflammatory transcriptional responses compared to IL-6 (6 DEGs). TGF- β 2 (323 DEGs) enriched more pro-mesenchymal pathways than TGF- β 1 (17 DEGs), while the pro-angiogenic VEGFA (32 DEGs) resulted in low gene enrichment. Pathway analysis revealed a pro-fibrotic overlap among TNF- α , thrombin, and TGF- β 2 treatments. The cocktail induced 2559 DEGs – including 884 unique DEGs – such as pro-angiogenic drivers, *VEGFA* and *FGF2*. Functional clustering described 132 VEGF-associated cocktail DEGs. nAMD-tissue data comparison highlighted potential tissue-specific gene targets, including *INHBA*, *ANGPT1*, and *SERPING1*. **Conclusions:** Fibrosis-associated cytokines (TNF- α , thrombin, TGF- β 2) drive a strong pathogenic response in primary ECs, with overlapping DEGs as potential therapeutic targets. The cocktail amplified transcription changes beyond the sum of individual cytokines, revealing synergistic enrichment of disease pathway genes. A cytokine combination more accurately modeled nAMD endothelial dysfunction, offering a potential platform for preclinical drug testing.

Abstract 017

Immune Homeostasis Re-establishment by IGFBPL1 Preserves Retinal Function in Experimental Dry AMD

Lu Huang^{1,2}, Anton Lennikov², Farris Elzaridi², Wai Lydia Tai², Kin-Sang Cho², Ajay Ashok², Grace Coyne², Karen Chang², Hio Tong Kam², Qingfeng Li¹, and Dong Feng Chen²

¹*Department of Plastic and Reconstructive Surgery, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China;* ²*Department of Ophthalmology, Schepens Eye Research Institute of Massachusetts Eye and Ear, Harvard Medical School, Boston, MA*

Introduction: Dry age-related macular degeneration (AMD) is a major cause of irreversible blindness worldwide with no effective treatment currently available. Disrupted microglial homeostasis has emerged as a key driver of disease onset and progression. Insulin-like Growth Factor Binding Protein-Like 1 (IGFBPL1) is a secreted regulator that preserves retinal immune balance through the IGF1R axis. Supplementation of IGFBPL1 restores microglial homeostasis and confers neuroprotection in experimental glaucoma models. However, whether resetting immune balance can counteract retinal degeneration in dry AMD remains unknown. Here, we evaluated the therapeutic potential of intravitreal IGFBPL1 in a sodium iodate (NaIO₃)-induced mouse model of dry AMD. **Methods:** Dry AMD was induced in C57BL/6J mice by intraperitoneal injection of NaIO₃, 25 mg/kg. A single intravitreal injection of recombinant IGFBPL1 protein (100 mg/kg) or vehicle was administered immediately after NaIO₃ exposure. Visual function was assessed one-week post-treatment using optomotor response and electroretinography. Retinal structure was evaluated by spectral-domain OCT and cone survival was examined by PNA immunostaining. RPE morphology and junctional integrity were analyzed by wholemount staining for

RPE65 and ZO-1. Retinal transcriptomic changes were profiled by qPCR at day 1 and day 7 after treatment to assess inflammatory and photoreceptor-associated gene expression. **Results:** IGFBPL1 significantly improved dry AMD mice's visual performance one week after treatment, as shown by enhanced optomotor responses and increased photopic and scotopic electroretinogram amplitudes. Structural analysis revealed thickening of the outer nuclear layer on OCT. PNA staining demonstrated increased numbers and elongated outer segments of cone photoreceptors. RPE whole-mounts showed higher RPE65 expression, more regular ZO-1 distribution, and increased junctional nodes. At the molecular level, retinal RNA-seq at day 1 and day 7 post-treatment revealed downregulation of inflammatory genes and upregulation of photoreceptor-associated transcripts. **Conclusion:** Intravitreal IGFBPL1 treatment in NaIO₃-induced dry AMD mice preserved visual function, reinforced photoreceptor and RPE integrity. Our findings suggest immune imbalance as a driver of dry AMD and position IGFBPL1 as a promising therapeutic strategy that acts through immune homeostasis re-establishment to halt retinal degeneration.

Abstract 018

Targeting Angiogenesis and EndMT in Neovascular Age-Related Macular Degeneration: Therapeutic Potential of VAS2870

Yuting Jin¹, Michele C. Madigan¹, Peter R. Wich², Tushar Kumeria³, and Daisy Y. Shu¹

¹*School of Optometry and Vision Science, University of New South Wales, Sydney, New South Wales, Australia;*

²*School of Chemical Engineering, University of New South Wales, Sydney, New South Wales, Australia;* ³*School of Materials Science and Engineering, University of New South Wales, Sydney, New South Wales, Australia*

Introduction: Neovascular age-related macular degeneration (nAMD) remains a leading cause of central vision loss. Although intravitreal anti-VEGF therapy is the current first-line treatment, its efficacy is limited by the frequency of injections and proportion of patients (15-40%) who become refractory and develop subretinal fibrosis. Investigation into alternative therapeutic targets and nano/microparticle-based drug delivery systems is therefore warranted. VAS2870, a NOX inhibitor, reduces oxidative stress and inflammation and may inhibit endothelial-to-mesenchymal transition (EndMT), a driver of fibrosis. Its effect on epithelial-to-mesenchymal transition (EMT) in RPE cells has previously been studied, however its role in endothelial cells is unknown. This study investigates the therapeutic potential of VAS2870 in blocking pathological angiogenesis and fibrosis in vitro. **Methods:** Primary human retinal endothelial cells (HRECs) were cultured in EGM2 and exposed to 0-50µM VAS2870 or DMSO (control). Cytotoxicity was assessed via LDH release. Cell migration was evaluated using a scratch wound assay with VAS2870, DMSO, and 10-20ng/mL of pro-fibrotic and pro-angiogenic cytokines (TGF-β₂, TNF-α, VEGF), with wound closure imaged over 24 hours and analyzed in ImageJ. Tube formation was assessed over 12 hours for untreated and treated HRECs with VAS2870, VEGF, or both, followed by calcein AM staining for cell viability. **Results:** VAS2870 induced a concentration-dependent cytotoxic response in HRECs, with low LDH release comparable to control at 10µM (9.0%±2.2%, mean±SD). 10µM VAS2870 significantly inhibited HRECs migration and wound closure over 24 hours relative to control (48.8%, $p=0.0001$), and under pro-fibrotic (TGF-β₂ co-treatment: 50.4%, $p<0.0001$) and pro-inflammatory (TNF-α co-treatment: 41.7%, $p<0.0001$) conditions, suggesting relevance in modulating EndMT. 10µM VAS2870 further potently suppressed tube formation over 12 hours, significantly reducing the number of nodes, branches, segments and meshes compared to control, VEGF and co-treatment groups. Calcein AM staining confirmed cell viability, indicating reduced tube formation to be from inhibited angiogenesis. **Conclusions:** This study supports the anti-angiogenic and anti-fibrotic potential of VAS2870 in vitro. Its activity in HRECs in combination with earlier studies in RPE cells, makes it a novel alternative to VEGF for ongoing investigation as a therapeutic molecule in nAMD.

Session 5: Therapeutic Modulation in Liver Disease

Abstract 019

The Histone Deacetylase Inhibitor MS-275 Mitigates Hepatobiliary Injury by Maintaining Cell Quiescence and Metabolic Reprogramming in a Murine Model of Cholestatic Liver Disease

Tony (Chun-Cheng) Chiang^{1,3}, Donghun Shin^{2,3}, and Kari Nejak-Bowen^{1,3}

¹*Organ Pathobiology and Therapeutics Institute, Department of Pharmacology and Chemical Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA;* ²*Center for Integrative Organ Systems, Department of Cell*

Introduction: Histone deacetylase (HDAC) inhibitors such as MS-275 were developed as treatments for oncologic diseases given the ability to inhibit cell growth and induce apoptosis, while their value in hepatobiliary disease is unknown. We aimed to evaluate drug responses in mice subject to 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC)-induced biliary injury and understand the drug's underlying mechanism. **Methods:** Mice were given 0.1% DDC diet for 4 weeks. Starting 3 weeks after DDC, mice were given either DMSO or MS-275 along with DDC for 7 days and then sacrificed. Serum and liver were collected at experiment endpoints. Real-time quantitative PCR (qPCR) was used to determine transcript levels. RNA-seq was performed to identify differential gene expression. Immunostainings were applied to validate RNA-seq results. Bile acid composition was analyzed using liquid chromatography-mass spectrometry. Total bile acids were measured with a colorimetric assay. AML12 mouse hepatocytes were used to confirm in vivo results. **Results:** MS-275 significantly reduced DDC-induced hepatobiliary injury, and alleviated liver pathological changes including inflammation and fibrosis. Cluster analysis and differential gene expression indicated that MS-275 normalized DDC-injured livers toward baseline controls. Enrichment analysis disclosed gene expression changes in cell cycle, senescence, bile acid secretion, oxidative phosphorylation, and immune cell activation pathways between DDC alone and MS-275 groups. MS-275 decreased not only G1/S cyclins but also proliferative marker Ki-67 and senescence marker p21. Immunostainings further validated pathway analysis by showing decreased 4-hydroxynonenal and p53 expression in MS-275-treated livers. MS-275 preserves liver functions by increasing expression of metabolic enzymes, transporters, and autophagy proteins compared to injury alone. Though bile acid composition did not differ between DDC alone and MS-275 groups, total bile acid levels were reduced after MS-275 treatment. **Conclusions:** MS-275 exerts hepatoprotective effects against DDC-induced injury in mice by restoring quiescence and reducing oxidative stress. Additionally, MS-275 rescues metabolic enzymes and proteins suppressed by DDC injury. Further epigenetic studies are needed to dissect a more detailed mechanism and correlate with transcriptomic and proteomic findings.

Abstract 020

Dysbiosis in the Gut-Liver Axis is Associated with Low Bone Mass During Murine Cholestasis

Brooke Hutchison¹, Jamie Forsnaglio², Pam Cornuet¹, Fu-Ying Qin³, Xiaochao Ma³, Kari Nejak-Bowen¹, and Matthew D. Carson¹

¹Organ Pathobiology and Therapeutics Institute, Department of Pharmacology and Chemical Biology, Pittsburgh Liver Research Center, University of Pittsburgh and University of Pittsburgh Medical Center, Pittsburgh, PA;

²Department of Biology, Seton Hill University, Greensburg, PA; ³School of Pharmacy, University of Pittsburgh, Pittsburgh, PA

Introduction: Bone disease is a serious complication of cholestatic liver disease, where patients experience accelerated bone loss, fractures, and osteoporosis. However, the mechanisms linking liver injury to skeletal decline remain poorly understood. The gut-liver axis, which regulates bile acid metabolism through interactions between the gut microbiota and liver has a role in the pathogenesis in cholestasis. Notably, prior work by our group has introduced bile acids as mediators of gut-liver signaling actions on bone. Clinically, patients with cholestatic liver disease and low bone mass have higher concentrations of serum bile acids compared to patients with normal bone mass. Therefore, we hypothesize the gut-liver axis and bile acid signaling contributes to bone loss in cholestasis. **Methods:** This study's purpose was to examine the role of the gut-liver axis and bile acid signaling on bone homeostasis during cholestasis. Male C57BL/6J mice were fed a 0.1% 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) diet from 11–15 weeks of age to induce cholestasis. Colonic microbiota composition was assessed by 16S rDNA sequencing. Bone microarchitecture was measured using micro-computed tomography (μ CT), and bone cell activity was quantified by histomorphometry. Bile acid levels in serum and bone marrow were analyzed by mass spectrometry, and hepatic bile acid transporter expression was measured by qRT-PCR. To test direct effects, pre-osteoblast (MC3T3-E1) and pre-osteoclast (RAW 264.7) cells were treated with bile acids at concentrations found in the bone marrow. **Results:** DDC-fed mice developed cholestatic injury characterized by dysbiotic gut microbial shifts, increased hepatic bile acid efflux transporters, and elevated bone marrow bile acids. μ CT analyses revealed significant reductions in trabecular and cortical bone mass. Histomorphometry demonstrated suppressed osteoblast activity and increased osteoclast numbers. *In vitro*, bile

acids suppressed osteoblast mineralization and enhanced osteoclast differentiation, recapitulating *in vivo* findings. **Conclusions:** This study establishes the gut-liver axis as a novel regulator of skeletal homeostasis in cholestasis. These findings provide mechanistic insight into hepatic osteodystrophy and highlight the gut-liver axis and bile acids as potential therapeutic targets for treating bone loss during cholestatic liver disease.

Abstract 021

Role of DEK as a Cell Death Switch Molecule in Liver Fibrosis

Ramsey Rohner, Kamal Baral, Leah Spade, Haitao Zhang, and Bilon Khambu

Department of Pathology and Laboratory Medicine, School of Medicine, Tulane University, New Orleans, LA

Introduction: DEK is a nuclear oncoprotein whose role in liver physiology and disease remains largely unexplored. This study investigates the function of DEK in the development of chronic liver disease (CLD), particularly liver fibrosis. **Methods:** We utilized whole-body DEK knockout mice (DEK^{-/-}) to assess the impact of DEK deletion on liver physiology. Chronic liver injury was induced using carbon tetrachloride (CCl₄), administered intraperitoneally at 0.7 μL/g body weight twice weekly for two weeks. Serum and liver tissues were collected post-treatment for analysis. To further delineate DEK's role in hepatocytes, we generated a hepatocyte-specific DEK knockout model (ihpDEK^{-/-}) using the AAV8-TBG-Cre system in DEK floxed (DEK F/F) mice. AAV8-TBG-Cre was administered two weeks prior to CCl₄ treatment (0.5 μL/g body weight, twice weekly for two weeks). Liver injury, inflammation, and fibrosis were evaluated through histology and serum biomarkers. Cell death pathways were analyzed via western blotting, qPCR, and immunofluorescence. **Results:** Under basal conditions, DEK^{-/-} and wild-type mice showed no significant differences in liver physiology or injury. Upon CCl₄ treatment, both groups exhibited liver injury and fibrosis; however, DEK^{-/-} mice demonstrated significantly exacerbated damage compared to wild-type controls. Similar findings were observed in the hepatocyte-specific knockout model, where ihpDEK^{-/-} mice showed more severe liver injury, inflammation, and fibrosis than DEK F/F controls. Importantly, while CCl₄-treated DEK F/F mice showed upregulation of apoptotic markers, DEK^{-/-} mice exhibited activation of both apoptotic and necroptotic pathways. This suggests that DEK deficiency shifts the cell death mechanism from apoptosis to necroptosis in the context of liver fibrosis. **Conclusion:** DEK plays a protective role in liver injury and fibrosis by modulating cell death pathways. It functions as a molecular switch between apoptosis and necroptosis during chronic liver disease progression.

Abstract 022

Beta-catenin Regulates Autophagy in Acute Hepatic Porphyrin

Anu Balogun and Kari Nejak-Bowen

Department of Pharmacology and Chemical Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA

Introduction: Acute hepatic porphyrias are rare metabolic liver disorders characterized by impaired heme biosynthesis, leading to accumulation of toxic porphyrin intermediates, oxidative stress, mitochondrial dysfunction, and liver injury. Current therapies are limited by cost, efficacy, and safety concerns, emphasizing the need for novel treatment strategies. Prior work showed that hepatocyte-specific deletion of β-catenin reduced DDC-induced porphyrin accumulation, protein aggregation, and liver injury; however, the interplay between Wnt signaling, its target glutamine synthetase (GS), and hepatic autophagy remains poorly understood. **Methods and Results:** We investigated how Wnt signaling and GS regulate hepatic porphyrin metabolism, autophagy, and mitochondrial quality control during xenobiotic-induced porphyric liver injury. Using a combination of spatial transcriptomics, high-resolution respirometry, transmission electron microscopy, and traditional molecular biology techniques, we demonstrate that Wnt/GS signaling coordinates transcriptional, metabolic, and organelle-level responses to porphyric injury. Loss of Wnt signaling suppressed DDC-induced activation of porphyrin biosynthesis genes, normalized porphyrin intermediate accumulation, and enhanced autophagic flux. GS deletion similarly suppressed porphyrin biosynthesis, primarily by limiting intracellular glutamine availability, a critical substrate for heme production. Combined Wnt inhibition and GS deletion further amplified autophagy and reduced porphyrin accumulation, establishing distinct but complementary roles for these pathways in hepatic homeostasis. Additionally, Wnt inhibition restored mitophagy during DDC-induced hepatic injury, whereas GS deletion primarily modulated mitochondrial respiration. This work proposes that persistent Wnt activation during injury promotes mitochondrial biogenesis while suppressing mitophagic clearance, exacerbating organelle dysfunction. Loss of Wnt signaling alleviates this constraint, improving mitochondrial quality control and

metabolic resilience, supported by our prior findings that β -catenin deletion enhances autophagy. **Conclusions:** Together, these findings define a Wnt-GS-autophagy axis that regulates hepatic adaptation to porphyric injury through coordinated control of heme biosynthesis, autophagy, mitochondrial turnover, and bioenergetic function. Therapeutic modulation of this axis may represent a novel strategy to mitigate metabolic liver disease.

Abstract 023

TET1 Guards Against the Development of Alcohol-associated Liver Fibrosis

Muhammad Azhar Nisar^{1,2,*}, Hongze Chen^{1,4,*}, Kevin Cao³, Xinjin Li^{1,2}, Shaolei Lu⁵, Zhaoli Sun¹¹, Brandon James Peiffer¹¹, Xiao-Ming Yin¹, Wenke Feng⁹, Tung-Sung Tseng⁶, Hui-Yi Lin⁶, Peng-Sheng Ting⁸, Wei-Ting Lin¹⁰, Tomilola Olaolu¹, Zhijin Wu⁸, Shang Wu¹, Layla Schechner¹, Jenna Copes¹, Sonali Notani¹, Xuewei Bai^{3,4}, and Chiung-Kuei Huang^{1,2}

¹Department of Pathology and Laboratory Medicine, Tulane University School of Medicine, New Orleans, LA;

²Department of Medicine, Division of Gastroenterology and Hepatology, Renaissance School of Medicine at Stony Brook University, Stony Brook, NY; ³Liver Research Center, Division of Gastroenterology and Liver Research Center, Warren Alpert Medical School of Brown University and Rhode Island Hospital, Providence, RI;

⁴Department of Pancreatic and Biliary Surgery, First Affiliated Hospital of Harbin Medical University, Heilongjiang Province, China; ⁵Department of Pathology and Laboratory Medicine, Warren Alpert Medical School of Brown University, Rhode Island Hospital, Providence, RI; ⁶School of Public Health, Louisiana State University Health Sciences Center; ⁷Department of Biostatistics, School of Public Health, Brown University, Providence, RI;

⁸Department of Medicine, Tulane University School of Medicine, New Orleans, LA; ⁹Department Structural Cellular Biology, Tulane University School of Medicine, New Orleans, LA; ¹⁰Tulane University School of Public Health and Tropical Medicine, New Orleans, LA; ¹¹Johns Hopkins University School of Medicine, Baltimore, MD;

*co-first authors

Introduction: Alcohol-associated liver disease (ALD) is a leading cause of liver-related morbidity and mortality worldwide, yet the molecular mechanisms driving its progression remain poorly understood. Emerging evidence suggests that dysregulated epigenetic regulations, particularly DNA methylation, play a pivotal role in ALD pathogenesis. The ten-eleven translocation (TET) family proteins, which catalyze the oxidation of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), are critical regulators of DNA demethylation. However, the role of TET1 in ALD progression remains largely unexplored. We aimed to determine the involvement of TET1 in ALD progression and clarify the underlying mechanisms by which TET1 is involved in ALD progression. **Methods:** To determine the impact of TET1 on ALD, we generated the whole body TET1 knockout (TET1 KO) and liver specific TET1 knockout mice (TET1 LKO) female mice and challenged them with an alcoholic liquid diet containing 5% ethanol (EtOH) for 8 weeks to induce ALD progression. We also used in vitro cell co-culture models for mechanistic evaluation of the role of TET1 in cell-cell interaction. Additionally, wild-type female mice subjected to CCL4 injection combined with alcohol diet for 5 weeks were used to validate the therapeutic potential of TET1 cofactor supplementation, alpha-ketoglutarate (α -KG), in mitigating ALD-related fibrosis. **Results:** We found that TET1 and 5hmC levels are significantly reduced in human and mouse ALD samples, correlating with disease severity. Besides, female TET1 KO and TET1 LKO mice fed an alcohol diet exhibited increased liver to body weight ratio, elevated serum AST levels, and pronounced fibrosis when compared with the control groups. Mechanistically, TET1 deficiency led to upregulation of pro-inflammatory cytokines, including Interleukin-1 alpha (IL1- α), Interleukin-1 beta (IL-1 β), and tumor necrosis factor-alpha (TNF- α) in mouse ALD liver samples. Further analysis using TET1 chromatin immunoprecipitation sequencing, IL-1 β emerged as a central mediator since TET1 binds to the enhancer region of IL1 β . Treatment with 5-aza-2'-deoxycytidine (5AZA), a DNA methylation inhibitor, reversed IL-1 β upregulation in TET1-deficient human hepatocytes, confirming TET1's epigenetic regulation of IL-1 β . TET1 deletion also amplified immune cells infiltration and hepatic stellate cell (HSC) activation, key drivers of fibrosis. These effects were reversed by IL-1 β inhibition using IL-1 receptor antagonist (IL-1Ra) and IL1 β neutralizing antibody, underscoring IL-1 β 's pivotal role in TET1-mediated protection. Importantly, α -KG supplementation in wild-type mice challenged with CCL4 and alcohol diet resulted in reversal of the fibrotic phenotype, providing direct evidence that enhancing TET1 enzymatic activity through cofactor supplementation represents a viable therapeutic approach. **Conclusion:** This study establishes hepatic TET1 plays a protective role in ALD progression through epigenetic suppression of IL-1 β . By maintaining DNA demethylation, TET1 attenuates inflammation, stellate cell activation, and fibrosis,

offering a promising therapeutic target. These findings advance our understanding of ALD pathogenesis and highlight the potential of epigenetic therapies to combat this multifactorial disease.

Session 6: Genetic Models Revealing Mechanistic Pathways in ECM Organization, Hormone Signaling, Stress Responses, and Multi-organ Biology

Abstract 024

A Knock-In Mouse Model of Dermatosparaxis Ehlers–Danlos Syndrome Reveals Impaired Collagen Processing and ECM Disorganization

Taylor Petrucci^{1,2}, Emma Mach¹, Cortney Gensemer^{1,2}, Matthew Huff¹, Cara Virgin¹, Maggie Osterhaus¹, Kathryn Byerly¹, Erika Bistran¹, Sydney Severance¹, Jan Guz¹, Fu Lei Tang¹, and Russell A. Norris^{1,2}

¹*Department of Regenerative Medicine and Cell Biology, Medical University of South Carolina, Charleston, SC;*

²*Department of Neurosurgery, Medical University of South Carolina, Charleston, SC*

Introduction: Dermatosparaxis Ehlers-Danlos Syndrome (dEDS) is a rare connective tissue disorder caused by pathogenic variants in ADAMTS2, yet the molecular mechanisms underlying its pathology remain poorly understood. To address this gap, we generated a knock-in murine model carrying the corresponding genetic mutation (Adamts2^{Q226*}) to investigate the impact of impaired collagen processing on extracellular matrix (ECM) structure and function. **Methods:** Immunohistochemistry was performed to assess Adamts2 expression in skin tissues. ECM organization was evaluated by transmission electron microscopy (TEM). Collagen processing was quantified biochemically, while collagen organization was analyzed using FibroNest digital pathology software. To investigate transcriptional changes, single-nucleus RNA sequencing (snRNA-seq) was conducted on formalin-fixed paraffin-embedded (FFPE) skin, and candidate genes were validated by immunohistochemistry. **Results:** Homozygous Adamts2^{Q226*/Q226*} mice exhibited reduced Adamts2 expression, abnormal ECM ultrastructure, and hieroglyphic collagen fibrils consistent with human dEDS pathology. Collagen processing was decreased in a genotype-dependent manner, with the most severe defects observed in homozygous mice. FibroNest analysis demonstrated significant reductions in composite collagen assembly scores, with increased fine collagen and reduced assembled collagen in Adamts2^{Q226*/Q226*} mice. snRNA-seq identified fibroblasts as the primary population with genotype-dependent transcriptional changes, including downregulation of ECM-associated genes such as Col6a and Fbn1, which were validated by immunohistochemistry. **Conclusions:** This work establishes the first biologically validated Adamts2 knock-in mouse model of dEDS. Our findings demonstrate that impaired collagen processing leads to altered ECM organization and fibroblast-specific transcriptional dysregulation, revealing central mechanisms underlying disease pathology and providing a platform for future therapeutic exploration.

Abstract 025

CREBRF Regulates Cardiomyocyte Function and Stress Response

Zana M. Ross, Aneta Kowalski, Divya Gupta, Mahesh Basantani, and Erin E. Kershaw

Division of Endocrinology and Metabolism, Department of Medicine, University of Pittsburgh, Pittsburgh, PA

Introduction: Cardiovascular diseases are among the most common conditions of the modern era, and minority populations are known to be disproportionately affected. A GWAS of Samoan population identified a variant in a sensor of cellular stress/energy, CREBRF (CREBRFR457Q). CREBRF is linked to metabolic phenotypes in humans and functional phenotypes in animal-derived clonal and primary cells. Yet, CREBRF's specific role in cardiomyocytes and heart is unknown. The objective of this study is to evaluate effects and mechanisms by which CREBRF and its variant impact cardiomyocyte development, function, and ultimately, cardiovascular disease. Our hypothesis is CREBRF improves function and stress response in cardiomyocytes. **Methods:** To test this hypothesis, we (i) characterize the regulation of CREBRF in cardiomyocytes and heart across species, (ii) develop and validate preclinical models of altered CREBRF action, and (iii) determine the extent to which CREBRF and risk variant are necessary and/or sufficient to mediate the adaptive response to stress in cardiomyocytes and heart. **Results:** The results demonstrate CREBRF is expressed and regulated by stress in cardiomyocytes and heart across a variety of mammalian species (rat, murine, human). **Conclusion:** Taken together, these data support the hypothesis that CREBRF improves murine cardiomyocyte function and regulates stress response.

Abstract 026

Beyond Bile Ducts: Investigating Kidney Injury and Multi-Organ Effects in *Foxa3-Cre YAP* Knockout Mice

Akshita Piedy^{1,2}, Jia-Jun Liu³, Silvia Liu^{2,3}, Jianhua Luo², Minakshi Poddar³, Sucha Singh³, Pamela Cornuet³, Laura Molina^{4,5}, and Kari Nejak-Bowen^{1,2}

¹Department of Molecular Pharmacology, University of Pittsburgh School of Medicine, Pittsburgh, PA; ²Pittsburgh Liver Research Center, University of Pittsburgh School of Medicine, Pittsburgh, PA; ³High Throughput Genome Center, Department of Pathology, University of Pittsburgh School of Medicine and University of Pittsburgh Medical Center Pittsburgh, PA; ⁴Pathology Residency Training Program, Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA; ⁵Department of Pulmonary, Allergy, and Critical Care Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA

Introduction: Yes-associated protein 1 (YAP) is a key regulator of organ development. A loss of this protein in early liver development (*Foxa3-Cre YAP* KO) leads to a lack of intrahepatic bile duct development and cholestasis. While originally designed to study biliary development, unexpected progressive renal injury was observed. This study is intended to investigate the subsequent renal pathology in this model and evaluate developmental defects in other body systems. Furthermore, the study looks to assess whether kidney injury stems from a genetic cause or as a byproduct of developing cholestasis. **Methods:** *Foxa3-Cre YAP* KO mice, littermate controls, and *Foxa3-Cre* TdTomato healthy mice were examined using Hematoxylin and Eosin (H&E), PicroSirius Red (PSR), immunohistochemistry (IHC), and immunofluorescence (IF). **Results:** *Foxa3-Cre YAP* KO kidneys show inflammation, fibrosis and progressive cyst formation. IHC staining for CD45 as well as PSR stains capture a consistent level of fibrosis and inflammation across the kidneys. To further examine renal cyst formation, IHC with Ki67 and Cleaved Caspase 3 were used to observe cell proliferation and apoptosis. IHC staining with both cellular markers reveal no abnormalities around the cysts indicating that cyst formation is not a product of excessive proliferation or cell death. Additionally, IF was used to assess cilia structure. The IF target for acetylated alpha tubulin confirms consistent cilia structure across all models suggesting it is not a driver of the pathology. Next, *Foxa3-Cre* TdTomato reporter mice were used to observe reporter expression for identification of where *Foxa3-Cre* is active in development. Reporter mice show unexpected *Foxa3* promoter activity in the genitourinary system, specifically in the reproductive organs for both male and female mice. This finding links both renal and reproductive developmental defects to the *Foxa3-Cre YAP* KO model. **Conclusions:** Loss of YAP as shown in the *Foxa3-Cre YAP* KO model appears to disrupt hepatic, renal, and reproductive development highlighting its multi-organ pathology. More work is necessary to identify the impact of cholestasis on kidney injury. Future studies will focus on uncovering other routes to assess mechanisms which drive kidney injury and strengthen the understanding of prolonged cholestasis and its effects on early development.

Abstract 027

Progesterone and Sex Hormone Regulation of LUVA Mast Cells: Impacts on Viability and Degranulation

Sydney Severance¹, Chloe Meyer¹, Roman Fenner¹, Cortney Gensemer^{1,2}, Russell A. Norris^{1,2}

¹Department of Regenerative Medicine and Cell Biology, Medical University of South Carolina, Charleston, SC;

²Department of Neurosurgery, Medical University of South Carolina, Charleston, SC

Introduction: Hypermobile Ehlers–Danlos Syndrome (hEDS) disproportionately affects females and is frequently comorbid with mast cell activation disorders (MCAD). Symptom flares often correspond to hormonal changes, suggesting that sex hormones may influence mast cell activity. We aimed to characterize hormone receptor expression in LUVA cells, an immortalized human mast cell line, and to investigate whether β -estradiol, progesterone, and dihydrotestosterone affect mast cell survival and degranulation responses. **Methods:** Hormone receptor expression was assessed using RT-PCR with gene-specific primers for estrogen, progesterone, and androgen receptors. LUVA cell viability following treatment with β -estradiol, progesterone, or dihydrotestosterone (DHT) was measured at physiologically relevant concentrations. Mast cell degranulation was quantified using β -hexosaminidase release assays. Cells were pretreated with hormones before stimulation with compound 48/80 (C48/80), a known degranulation trigger, to test whether sex hormones stabilize, amplify, or have no effect on mast cell degranulation. **Results:** RT-PCR confirmed expression of multiple hormone receptors, including ESR2 (ER β) and membrane-associated progesterone receptors PAQR5 and PAQR6, while the classical nuclear progesterone receptor (PGR) was absent. Viability assays showed no significant cytotoxicity

at 0.1 μM or 1 μM β -estradiol, 10 μM or 100 μM progesterone, or at 0.1, 1, or 10 nM DHT. However, cell viability dropped significantly (below 60%) following treatment with 10 μM β -estradiol and 1000 μM progesterone. Hormone treatment alone did not induce significant β -hexosaminidase release. Optimization identified 10 $\mu\text{g}/\text{mL}$ C48/80 as the lowest concentration consistently triggering degranulation. Progesterone pretreatment at a dose of 10 μM reduced C48/80-induced degranulation in LUVA cells, suggesting a possible inhibitory role, though results did not reach statistical significance ($p = 0.1014$). **Conclusions:** LUVA cells express functional hormone receptors and remain viable under physiological hormone exposure. While hormones alone do not trigger degranulation, 10 μM progesterone may attenuate C48/80-induced responses, indicating potential hormone-specific modulation of mast cell activity. These findings suggest sex hormones may contribute to symptom variability in hEDS and warrant further investigation into hormone-mediated mast cell regulation.

Session 7: Inflammation and Injury

Abstract 028

***Clostridioides difficile* Toxins Trigger a Macrophage-ILC3 Axis That Drives IL-22 and Mucin Induction**

Erin Chard, Rachel Stuber, Anna Tingler, Selene Shore, Amy C. Engevik, and Melinda A. Engevik

Department of Regenerative Medicine and Cell Biology, Medical University of South Carolina, Charleston, SC

Introduction: *Clostridioides difficile* is a toxin-producing pathogen that causes antibiotic-associated diarrhea. *C. difficile* infection (CDI) is associated with profound epithelial disruption, yet the pathways that link host immune responses to epithelial barrier remodeling are not well understood. We hypothesized that *C. difficile* toxins would stimulate specific cytokines that would promote adherent mucins. **Methods:** Infection was induced in mice by antibiotic pretreatment in drinking water followed by intraperitoneal clindamycin and oral gavage with either PBS (control) or *C. difficile*. Mice were monitored for weight loss and disease activity for 10 days, with peak illness at day 3. Colonic tissue was collected at days 3, 4, 5, 8, and 10 for RNAseq, and day 3 tissue was used for immunostaining. Peritoneal macrophages, splenic T-cells and MNK-3 cells were stimulated with *C. difficile* toxins and IL-22 or IL-1 β production was measured by ELISA. Colonic organoids and CMT93 cells were treated with recombinant IL-22, and mucin gene expression was quantified by qPCR. **Results:** In a murine model, we observed an increase in IL-22 expression at the peak of CDI. Given that both T cells and group 3 innate lymphoid cells (ILC3s) can produce IL-22, we tested their responses to *C. difficile* toxins. T cells exposed to *C. difficile* toxins did not upregulate IL-22 by qPCR or ELISA, whereas MNK-3 cells (ILC3s) generated IL-22 in response to IL-23 and IL-1 β , but not directly to toxins. Instead, we found that peritoneal macrophages secreted IL-1 β upon toxin exposure, and supernatants from these macrophages stimulated IL-22 production in MNK-3 cells. These findings suggest that *C. difficile* toxins indirectly activate ILC3s through macrophage-derived IL-1 β . Importantly, IL-22 is known to regulate the transcription of adherent mucins, including MUC13. Consistent with this, *C. difficile* mice exhibited significantly increased expression of MUC13 at both the mRNA and protein levels compared to controls. IL-22 treatment of colonic CMT93 cells and organoids further confirmed the ability of IL-22 to upregulate MUC13 by qPCR and immunostaining. **Conclusions:** Together, our data reveal a novel immune–epithelial axis in *C. difficile* infection, in which toxin-induced macrophage signaling drives ILC3-derived IL-22, leading to enhanced expression of barrier-promoting mucin MUC13. This pathway highlights a protective host response aimed at reinforcing the mucosal barrier during infection.

Abstract 029

***Clostridium perfringens* Exhibits Different Pathogenesis when Exposed to Different Types of Formula**

Margaret Largent, Alyssa Gutierrez, and Melinda A. Engevik

Department of Regenerative Medicine and Cell Biology, Medical University of South Carolina, Charleston, SC

Introduction: Necrotizing Enterocolitis (NEC) is a GI emergency most common in pre-term, low-birth-weight infants, who are supplementally fed with formula. NEC is associated with gut dysbiosis, specifically the presence of *Clostridium perfringens* – a pathobiont which is often present prior to NEC onset, exacerbating the severity. Infant formula use is also associated with NEC. The carbohydrate source in formula, lactose or maltodextrin (MDX), may influence *C. perfringens* colonization and NEC risk. We hypothesized that maltodextrin and maltodextrin-based formulas increase the pathogenesis of *C. perfringens*. **Methods and Results:** We evaluated the growth of four *C. perfringens* strains in ZMBI media containing lactose or MDX using OD600 readings over

24 hours. Both carbohydrates supported bacterial growth, but MDX sustained more prolonged growth across all strains. To quantify bacterial growth with formula, we used qPCR with a *C. perfringens* specific primer (ftsZ) to create a standard curve to back calculate CFU counts. This was done because of the opacity of the formula. MDX-based formula significantly increased bacterial growth compared to lactose-based formula and control. We also optimized a resazurin assay to identify the most sensitive cell line to *C. perfringens* toxins, finding T84 cells most responsive. In our last experiment, *C. perfringens* strains grown in ZMBI with lactose or MDX were used to treat T84 cells. The resazurin assay showed that statistically more cells survived that were exposed to lactose-fed *C. perfringens* than the MDX or CSS fed bacteria. **Conclusions:** The data indicates that *C. perfringens* both grows better in a MDX-rich environment and has an increased ability to harm cells when exposed to MDX. *C. perfringens* has been associated with NEC and a worse outcome of the disease. This suggests that feeding pre-term infants with MDX based formula is more harmful than using lactose-based formula.

Abstract 030

TNFAIP6 Drives Glioblastoma Stem Cell Growth via EGF Signaling and Macrophage Reprogramming

Zhe Zhu¹, Kailing Yang², Junxia Zhang³, Xiuxing Wang³, and Jeremy N. Rich⁴

¹Department of Pathology and Cell Biology, Columbia University Irving Medical Center, NY; ²Department of Radiation Oncology, Carver College of Medicine, University of Iowa, Iowa City, IA; ³Institute for Brain Tumors, Jiangsu Key Lab of Cancer Biomarkers, Prevention and Treatment, Collaborative Innovation Center for Cancer Personalized Medicine, Nanjing Medical University, Nanjing, China; ⁴University of North Carolina School of Medicine, Department of Neurology and UNC Lineberger Comprehensive Cancer Center, Chapel Hill, NC

Introduction: Glioblastoma (GBM) is an aggressive primary brain tumor driven by therapy-resistant glioblastoma stem cells (GSCs). Pro-inflammatory tumor-associated macrophages (pTAMs) in the GBM microenvironment produce cytokines like TNF- α that can stress GSCs, but how GSCs adapt to this inflammatory pressure is unknown. We investigated the mechanisms that enable GSCs to withstand pro-inflammatory stress, focusing on the role of tumor necrosis factor α -induced protein 6 (TNFAIP6). **Methods:** Patient-derived GSC cultures were exposed to pro-inflammatory conditions using macrophage-derived conditioned medium or recombinant TNF- α . Gene expression profiling identified *TNFAIP6* as a TNF- α -responsive gene in GSCs. Loss- and gain-of-function studies (shRNA knockdown and TNFAIP6 overexpression) were performed to assess its role in GSC proliferation, self-renewal, and signaling. Macrophage polarization was evaluated by treating monocyte-derived macrophages with GSC-conditioned media and analyzing surface markers. An orthotopic xenograft mouse model was used to test the effect of TNFAIP6 on tumor growth in vivo. **Results:** Co-culture with pTAMs or TNF- α exposure significantly increased GSC proliferation and self-renewal, accompanied by induction of *TNFAIP6*. TNFAIP6 knockdown abrogated the pro-proliferative effects of TNF- α in vitro and reduced tumor formation in mouse xenografts, indicating that TNFAIP6 is required for the maintenance of GSCs under inflammatory stress. Mechanistically, the TNFAIP6 protein bound to epidermal growth factor (EGF), thereby prolonging the activation of the EGFR–PI3K–AKT signaling pathway in GSCs. In addition, TNFAIP6 secreted by stressed GSCs acted on macrophages, shifting them from a pro-inflammatory phenotype (pTAMs) to a suppressive phenotype (sTAMs), thereby dampening inflammatory signaling. Disrupting this TNFAIP6-mediated autocrine and paracrine communication significantly impaired GSC proliferation, self-renewal, and tumor growth. **Conclusions:** TNFAIP6 serves as a critical autocrine and paracrine mediator that enables GSC survival and growth under pro-inflammatory stress. By prolonging growth factor signaling and reprogramming macrophages into a suppressive state, TNFAIP6 helps GSCs to overcome inflammatory challenges. These mechanistic insights reveal a novel tumor–macrophage crosstalk that drives GBM progression.

Abstract 031

Elucidating the Impact of Rotavirus Infection on Intestinal Cell Responses

Kayla McGary, Ana G. Pettijohn, and Kristen A. Engevik

Department of Regenerative Medicine and Cell Biology, Medical University of South Carolina, Charleston, SC

Introduction: Rotavirus is the leading cause of severe gastroenteritis in children under 5 years old, causing life threatening diarrhea and resulting in ~200,000 deaths per year. Rotavirus primarily infects epithelial cells on the tips of villi in the distal small intestine. Despite this specific localization, rotavirus has far-reaching effects on uninfected cells' response, including increased fluid secretion from enterocytes and enteroendocrine cells which

contribute to diarrhea. Epithelial cell types are known to alter during parasitic and bacterial infection; however, few studies have investigated epithelial cell alterations and responses during rotavirus infection. We hypothesize that rotavirus infection alters epithelial cell function and affects specific cell populations, including goblet cells and mucus secretion, tuft cell populations, and proliferation. **Methods:** Using a pediatric mouse model of infection, we gavaged pups with rotavirus and collected tissue samples at 1 day and 3 days post infection for immunostaining. Age matched pups were gavaged with lysate as a control. We immunostained for goblet cells (MUC2), glycans (WGA, SNA, and UEA1), tuft cells (DCLK1, PEGFR), and proliferative cells (Ki67) in pups at 1 day and 3 days post infection. **Results:** Rotavirus infection resulted in peak diarrhea at 3 days post infection, with rotavirus detected in stool starting at 1 day post infection. Uninfected pups showed no signs of diarrhea and had no rotavirus detected in stool. Immunostaining for goblet cells using MUC2 and glycans staining showed a decrease in mucus retention in infected pups at 1 day post infection, suggesting increased mucus secretion early during infection. We observed no changes in the number of tuft cells between rotavirus infection and uninfected pups. In rotavirus infection, we did see significant increases in proliferation at 3 days post infection. **Conclusion:** Collectively, these findings indicate that rotavirus infection increases mucus secretion from goblet cells and proliferation at specific times during infection.

Abstract 032

Histamine-HRH1 Signaling Regulates Mucus Secretion and Is Disrupted by *Clostridioides difficile* Toxins

Selene Shore, Anna Tingler, Ana Pettijohn, Rachel Edens-Valentine, Amy C. Engevik, Kristen A. Engevik, and Melinda A. Engevik

Department of Regenerative Medicine and Cell Biology, Medical University of South Carolina, Charleston, SC

Introduction: Histamine is a biogenic amine with diverse physiological functions. Although classically considered a host-derived molecule, histamine can also be produced by microbes and act directly on the intestinal epithelium. Histamine signals through four G-protein coupled receptors (HRH1–HRH4), each activating distinct downstream pathways. We sought to identify which histamine receptors are present in the human intestine and define their function. **Methods:** Single-cell RNA sequencing data from the human small and large intestine were analyzed to identify histamine receptor expression. Calcium signaling, a downstream effector of histamine receptors, was assessed by live-cell imaging of human colonic cell lines and organoids expressing the genetically encoded calcium sensor GCAMP6S. To further characterize receptor function, we performed explant studies using murine and porcine colon tissue and treated human colonic models with histamine while monitoring mucus secretion. MAPK signaling and cytokine production were evaluated using Luminex Magpix multiplexing. **Results:** Single-cell RNA sequencing revealed HRH1 as the only histamine receptor expressed in intestinal epithelial cells under homeostatic conditions. This was confirmed in human intestinal organoids by qPCR and RNAseq, which demonstrated exclusive HRH1 expression. Functionally, histamine stimulation of colonic organoids enhanced calcium signaling and promoted mucus secretion by goblet cells, indicating that histamine contributes to epithelial barrier integrity through HRH1-mediated pathways. Unexpectedly, histamine did not activate MAPK signaling or induce pro-inflammatory cytokine production in intestinal organoids. To determine whether this protective axis is altered during *Clostridioides difficile* infection (CDI), we examined a mouse model of CDI and observed no change in HRH1 expression. However, *in vitro* treatment of human colonic cells with *C. difficile* toxins markedly reduced histamine-induced calcium flux and suppressed mucus secretion. **Conclusions:** HRH1 is the dominant histamine receptor in the intestinal epithelium and promotes mucus release without stimulating inflammatory signaling. *C. difficile* toxins disrupt this protective pathway by dampening HRH1-mediated calcium signaling and mucus secretion. This work identifies a novel mechanism by which pathogens subvert host defenses and highlights HRH1 signaling as a critical regulator of mucosal homeostasis.

Session 8: From Genetic Models to Therapeutic Strategies in Vascular Biology

Abstract 033

Lymphatic YAP1 in hypoxia-induced vascular remodeling

Priscilla Kyj^{1,2}, Mikaela Scheer¹, Tadanori Mammoto^{1,3}, and Akiko Mammoto^{1,2}

¹Department of Pediatrics, ²Department of Cell Biology, Neurobiology and Anatomy, and ³Department of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI

Introduction: Pulmonary hypertension (PH) is a life-threatening pulmonary vascular disease characterized by sustained elevation of pulmonary arterial (PA) pressure. Remodeling of distal pulmonary arterioles (PAs) is a key feature of PH and involves marked accumulation of PA smooth muscle cells (PASMCs) to PAs. Lymphatic endothelial cells (LECs) contribute to cardiovascular and lung diseases associated with PH. The role of lymphatics in PH pathology has not been examined before. **Methods:** We utilize a mouse hypoxia-induced PH model to examine lymphatic structure and PA remodeling as well as measure right ventricular (RV) systolic pressure and RV hypertrophy. **Results:** Depletion of lymphatics accelerates hypoxia-induced PA remodeling and right ventricular (RV) hypertrophy. Expression of Hippo signaling transducer, Yes associated protein (YAP1) increases in hypoxia-treated mouse lung LECs. Hypoxia induced PA remodeling and RV hypertrophy are enhanced in the *Prox1-Cre^{ERT2}-Yap1^{fl/fl}* mouse lungs, in which expression of APLN that inhibits PA remodeling, in LECs under hypoxia decreases. Conditioned medium collected from hypoxia-treated LECs or LECs treated with YAP activator suppresses DNA synthesis of SMCs; in contrast, an APLN receptor inhibitor inhibits the effects. **Conclusions:** These results suggest that lymphatic YAP1 signaling is necessary for maintenance of lymphatics and mediates distal PA remodeling in hypoxia-induced PH through APLN.

Abstract 034

Heme and Thrombin Disrupt Angiogenic Balance *in vitro*: Potential Driver of Placental Dysfunction in Sickle Cell Disease Pregnancies

Kylie Hutchison, Nirupama Ramadas, Ashlyn Lowery, Karissa Law, Joshua Dutton, and Erica Sparkenbaugh
Department of Pathology and Laboratory Medicine, Blood Research Center, University of North Carolina at Chapel Hill, Chapel Hill, NC

Introduction: Pregnancy in women with sickle cell disease (SCD) is high risk and marked by thrombosis, pre-eclampsia/eclampsia, increased maternal mortality, and increased fetal morbidity and mortality. SCD pregnancies result in pathologically abnormal placentas with stunted villi and reduced spiral artery remodeling which reduces placental efficiency. Trophoblasts are essential for proper placental development with the two types, villous and extravillous, forming the villi and remodeling the maternal spiral arteries, respectively. Trophoblast's main role in placental development is the production of angiogenic factors to establish a highly vascularized placenta. Angiogenic balance is vital for proper placental development and involves pro-angiogenic placental growth factor (PlGF) and anti-angiogenic soluble fms-like tyrosine kinase-1 (sFLT1) production. The sFLT1/PlGF ratio is a biomarker of angiogenic balance, and in both pre-eclampsia and pregnancy in SCD patients, an elevated sFLT1/PlGF ratio indicates an anti-angiogenic shift. The etiology behind this imbalance is unknown. We hypothesized that the prothrombotic and hemolytic environment of SCD drives this imbalance. **Methods:** Cultured human HTR8/SVneo and BeWo trophoblasts, and EA.Hy926 endothelial cells, were treated with heme (0 – 30 μ M) and human α thrombin (10 IU/mL) for 24 hours. Supernatants were collected for analysis of PlGF and sFlt1 protein by ELISA (R&D Systems). Cell lysates were collected for protein analyses. **Results:** In HTR8 trophoblasts, heme caused a dose-dependent decrease in PlGF expression, and modestly increased sFLT1 release. Thrombin had no effect on PlGF expression, yet increased sFLT1 release alone, and synergized with heme to further increase sFLT1 release. This combined effect of heme and thrombin on sFLT1 was confirmed in BeWo cells. Heme decreased PlGF and modestly increased sFLT1 expression in EA.Hy926 cells and thrombin synergized with heme to increase sFLT1 further. PAR1 inhibition with the antagonist vorapaxar prevented the thrombin-induced increase in sFLT1 in endothelial cells. **Conclusion:** This work provides insight into the direct impact excess heme and thrombin have on driving the anti-angiogenic environment of SCD placentas. Future studies will examine the effects of heme and thrombin on signaling downstream of PAR1 to clarify how the prothrombotic state of SCD contributes to placental dysfunction and the angiogenic imbalance observed.

Abstract 035

Spn42Dd Genetic Manipulation in *Drosophila* as a Model for Human SERPINE1 Deficiency

Michelle Thayer and Marit Nilsen-Hamilton

Department of Biochemistry, Biophysics, and Molecular Biology, Iowa State University, Ames IA

Introduction: Clinical studies in the Bernese Amish community have identified a rare haploinsufficient mutation in SERPINE1, encoding plasminogen activator inhibitor-1 (PAI-1), that is associated with increased longevity,

reduced cardiovascular disease, lower fasting insulin, and decreased very low-density lipoprotein (VLDL) production. However, the mechanisms linking reduced PAI-1 activity to these beneficial outcomes remain poorly defined. Rodent models are informative but resource-intensive and limited in throughput. To address this gap, we developed a *Drosophila melanogaster* model to investigate the orthologous gene Spn42Dd, a conserved serpin implicated in immune and protease regulation. **Methods:** We conducted tissue-specific knockdown of spn42dd using the GAL4/UAS system in adult muscle tissue and evaluated effects on midlife mortality and lifespan. To generate a precise genetic model of the Amish-associated SERPINE1 mutation, we used CRISPR-Cas9 with a single-stranded oligonucleotide donor to create a targeted dinucleotide addition in Spn42Dd. Phenotypic assays included automated negative geotaxis performance, survival analysis, and preliminary body composition studies. Structural homology was evaluated via AlphaFold protein modeling. **Results:** Muscle-specific spn42dd knockdown significantly delayed midlife mortality and extended lifespan relative to controls ($p < 0.01$), mirroring human PAI-1 deficiency phenotypes. Preliminary evaluation of CRISPR-generated spn42dd knockout flies indicate an enhanced climbing ability ($p < 0.05$) compared to controls. Additionally, water weight analysis indicates an optimal water to body weight ratio in mutant flies, suggesting healthy physiological states such as metabolism and/or inflammatory response. Structural modeling confirmed high similarity between human PAI-1 and Spn42Dd, supporting functional conservation. **Conclusions:** Our findings provide evidence that loss of spn42dd in *Drosophila* correlates with key features of human SERPINE1 deficiency, including improved survival and neuromuscular function. This model offers a powerful platform for dissecting conserved molecular pathways linking PAI-1 activity to longevity, metabolism, and inflammation. The approach enables a resourceful investigation of gene-environment interactions and translational aging mechanisms with broad relevance to human health.

Abstract 036

Anti-Fibrinolytic Strategies Improve Liver Regeneration and Prevent Post-Hepatectomy Liver Failure in Mice and Patients

Zhihao Li^{1,*}, Zimu Wei^{2,*}, Dafna J. Groeneveld², Amy W. Strilchuk³, Matthew J. Flick⁴, Yawen Dong¹, Vanja Podrascanin¹, Mark J. Truty¹, Michael L. Kendrick¹, Sean P. Cleary⁵, Susanne G. Warner¹, Rory L. Smoot¹, Alice Assinger⁶, Christian J. Kastrup³, Paul Karanicolas⁷, James P. Luyendyk², and Patrick P. Starlinger¹

¹Department of Surgery, Division of Hepatobiliary and Pancreas Surgery, Mayo Clinic, Rochester, MN; ²Pathobiology and Diagnostic Investigation, Michigan State University, East Lansing, MI; ³Michael Smith Laboratories, University of British Columbia, Vancouver, Canada; ⁴Department of Pathology and Laboratory Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC; ⁵Department of Surgery, University Health Network, Toronto, Canada; ⁶Center of Physiology and Pharmacology, Institute of Vascular Biology and Thrombosis Research, Medical University of Vienna, Vienna, Austria; ⁷Division of General Surgery, Sunnybrook Health Science Centre, Toronto, Canada; *Co-first authors.

Introduction: Blood coagulation and hepatic fibrin(ogen) deposition are linked to liver regeneration in mice and patients. However, longstanding experimental evidence suggests the fibrinolytic protease plasmin(ogen) promotes liver regeneration. **Methods:** We pursued experimental and clinical approaches to uncover the basis for these paradoxical observations. **Results:** Delayed recovery of liver mass after 2/3rd partial hepatectomy (PHx) in plasminogen-deficient (Plg^{-/-}) mice was not observed in mice with pharmacological siRNA (siPlg)-induced plasminogen deficiency. Surprisingly, siPlg treatment enhanced hepatocyte proliferation markers (i.e., BrdU, PCNA) and accelerated recovery of plasma albumin levels in mice after 2/3rd PHx. Plasminogen deficiency induced by siPlg also increased hepatocyte proliferation in mice after 90% PHx, an experimental setting of failed regeneration. Prior studies have shown that hepatic fibrin(ogen) deposition fails in patients that develop post-hepatectomy liver failure (PHLF) and in mice after 90% PHx. Administration of the plasminogen inhibitor tranexamic acid (TXA) increased hepatic fibrin deposition in mice in this setting. Clinically, our post-hoc analysis of the prospective randomized HeLiX trial showed perioperative TXA was associated with three-fold lower odds of PHLF (OR=0.28), which improved to eight-fold lower odds among patients with impaired baseline liver function (OR=0.13). **Conclusions:** These convergent findings suggest major therapeutic opportunity in revisiting the precise role of plasminogen in liver regeneration.

Session 9: Modulators of Disease – Microbiota, Mucus, and Intestinal Remodeling

Abstract 037

High-Fat Diet Induces Mucus-Producing Metaplasia in Gastric Epithelium of Mice

Makenna Grozis, Charulekha Packirisamy, and Amy C. Engevik

Department of Regenerative Medicine and Cell Biology, Medical University of South Carolina, Charleston, SC

Introduction: High-fat diets (HFDs) are increasingly prevalent and have been associated with a range of health disorders, including obesity, diabetes, and cancer. While the impact of HFD on the intestine has been well studied, its effects on the stomach remain poorly understood. The stomach is lined with specialized epithelial cells essential for digestion and acid secretion. Damage results in the loss of acid-producing parietal cells and the trans differentiation of chief cells into mucus-secreting metaplastic cells, a response known as gastric metaplasia. We hypothesized that HFD induces gastric damage resulting in metaplasia and chronic inflammation. **Methods and Results:** Adult male wildtype (WT) C57BL/6 mice were fed either standard chow (10% kcal from fat) or a high-fat diet (54% kcal from fat, primarily from lard) for 25 weeks. Stomachs were analyzed histologically. H&E staining revealed normal gastric architecture with abundant parietal and chief cells in chow-fed mice. In contrast, HFD-fed mice showed a marked loss of parietal cells and the appearance of metaplastic glands enriched in mucus-secreting cells. To further characterize these changes, we performed immunostaining for markers of mucus (GSII, UEA-1, MUC5AC), proliferation (Ki67), parietal cells (HK-ATPase, γ -actin), tuft cells (DCLK1), chief cells (MIST1), G cells (gastrin) and macrophages (F4/80). HFD-fed mice exhibited robust features of metaplasia, including reduced parietal and chief cell populations, increased mucus and tuft cells, elevated epithelial proliferation, an expansion of G cells, and increased macrophage infiltration. **Conclusion:** These findings demonstrate that prolonged HFD exposure induces gastric metaplasia in WT mice. While gastric metaplasia is typically associated with *Helicobacter pylori* infection, ulcers, or certain medications, our study reveals that dietary fat alone is sufficient to trigger epithelial injury and initiate a damage-repair response in the stomach. This observation underscores the stomach's sensitivity to dietary fat and suggests that chronic HFD consumption may predispose individuals to gastric cancer.

Abstract 038

High Fat Diet Creates a Pro-Inflammatory Niche Promoting Gastric Metaplasia

Charulekha Packirisamy¹, Annika Matthiesen², Pooja Pradeep², Janet Boggs², Sarah A. Dooley¹, Rachel Edens-Valentine¹, Piper McKee¹, Makenna Grozis¹, Catrina Robinson², Kristen A. Engevik¹, Melinda A. Engevik¹, and Amy C. Engevik¹

¹*Department of Regenerative Medicine and Cell Biology, Medical University of South Carolina, Charleston, SC;*

²*Department of Neurology, Medical University of South Carolina, Charleston, SC*

Introduction: Obesity prevalence in the United States has more than tripled over the past six decades, with severe obesity nearly quadrupling. High fat diet (HFD) consumption is a major contributor to this epidemic, playing a significant role in excessive weight gain and the worsening of metabolic disorders. HFD consumption disrupts multiple physiological systems, including the cardiovascular, immune, renal, and gastrointestinal systems. Although the intestinal effects of HFDs are well characterized, their impact on the stomach remains largely unexplored and this knowledge gap is increasingly relevant as fat-rich, processed diets become more common. The gastric epithelium is composed of specialized epithelial cells including mucus-secreting cells, acid-producing parietal cells, and enzyme-secreting chief cells. Loss of parietal cells or epithelial injury triggers chief cell transdifferentiation into metaplastic lineages, a reparative process that can progress to dysplasia and malignancy under chronic inflammation. We hypothesize that a HFD disrupts the gastric epithelium, resulting in increased infiltration of immune cells, including mast cells, macrophages, and ILC2s, promoting inflammation and gastric metaplasia. **Methods:** Wildtype C57BL/6J mice were maintained on either a standard chow diet (control) or a HFD for 25 weeks. Gastric tissues were analyzed by histology and immunofluorescence for epithelial composition, immune infiltration, and metaplasia-associated markers. Gastric organoids from control mice were treated with fatty acids to assess direct epithelial responses. **Results:** Chronic HFD feeding resulted in gastric mucosal thickening, parietal and chief cell loss, and immune cell infiltration. Immunostaining revealed tuft cell hyperplasia, significantly increased mast cell, macrophage and ILC2 infiltration, indicating a robust pro-inflammatory microenvironment. Metaplasia markers including CD44v9, AQP5, GSII, and phospho-ERK1/2 were upregulated in HFD-fed mice. Fatty acid-treated organoids showed increased growth in vitro, supporting a direct effect of HFD on gastric epithelial cells. **Conclusion:** HFD consumption disrupts gastric epithelial integrity,

triggers immune-mediated inflammation, and drives metaplasia. These findings highlight the stomach as a target of diet induced injury and provide a mechanistic link between modern dietary patterns and gastric carcinogenesis.

Abstract 039

V-ATPase Activity Regulates Intestinal Restitution Through Endocytic Control of Signal Transduction in *Drosophila*

Doug Terry, Liping Luo, Josh Lee, and Brian Robinson

Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, GA

Introduction: Vacuolar ATPases (V-ATPases) are highly conserved multi-subunit proton pumps that drive the progressive acidification of endosomes and lysosomes. By regulating progressive acidification of the endolysosomal pathway, V-ATPase activity impacts signaling transduction pathways both positively (e.g., internalization and activation of receptor-ligand complexes in endosomes) and negatively (e.g., degradation of pathway mediators in lysosomes). While the role of V-ATPases in human neurodegenerative diseases and cancer has been extensively studied, the requirement for these proteins in intestinal restitution remains poorly understood. Here, we use *Drosophila* to study the role of V-ATPases in regulating intestinal-injury and repair driven by excessive oxidative stress. **Methods:** Adult male *Drosophila* 2-5 days post-eclosion were fed either a vehicle diet or that same diet supplemented with 10mM paraquat to induce oxidative stress. The GAL4-UAS system was used to alter target gene expression in a cell and tissue specific manner. Intestines were dissected and analyzed by immunofluorescent staining and confocal microscopy as well as RT-PCR to study conserved signal transduction pathways. Quantification was performed using ImageJ and Prism software. **Results:** We find that RNAi driven depletion of multiple subunits of the V-ATPase complex suppressed oxidative stress-induced lethality. By contrast, depletion of the main lysosomal catabolic enzyme in *Drosophila* (Cathepsin-D) had no effect. On a cellular level, these effects map to absorptive enterocytes (ECs) of the *Drosophila* intestine. Molecular analysis of intestines following 24 hours of injury by oxidative stress compared to uninjured controls reveals increased cell death (by dCP-1 staining), increased JNK-pathway activity, and increased IMD pathway signaling reporter expression compared to uninjured controls. Furthermore, depletion of Vha44 (subunit C of the V1 complex) was sufficient to suppress the increased cell death, JNK pathway, and IMD pathway markers induced by oxidative stress in the intestine. **Conclusions:** These findings suggest that inhibition of V-ATPase activity can protect against intestinal injury caused by excessive oxidative stress. On a molecular level, we find that attenuation of endolysosomal acidification dampens pro-apoptotic JNK and IMD pathways, possibly highlighting endosomal acidification as an amplifier of excessive oxidative stress and injury.

Abstract 040

Antibiotic-Driven Microbiota Changes Disrupt Goblet Cell Function and the Mucus Barrier in Cystic Fibrosis

Anna Tingler¹, Rachel Bernard², Rachel Edens-Valentine¹, Jennifer K. Spinler^{4,5}, Thomas D. Horvath^{4,5}, Numan Oezguen^{4,5}, Lisa S. Zhang³, Anthony M. Haag^{4,5}, Amy C. Engevik¹, Daniel C. Payne⁷, Maribeth R. Nicholson³, and Melinda A. Engevik^{1,6}

¹*Department of Regenerative Medicine and Cell Biology, Medical University of South Carolina, Charleston, SC;* ²*Pediatric Gastroenterology Ochsner Medical Complex, Baton Rouge, LA;* ³*Division of Pediatric Gastroenterology, Hepatology, and Nutrition, Monroe Carell Junior Children's Hospital at Vanderbilt, Nashville TN;* ⁴*Department of Pathology, Texas Children's Hospital, Houston TX;* ⁵*Department of Pathology and Immunology, Baylor College of Medicine, Houston TX;* ⁶*Department of Microbiology and Immunology, Medical University of South Carolina, Charleston, SC;* ⁷*Division of Infectious Diseases, Cincinnati Children's Hospital Medical Center, Cincinnati, OH*

Introduction: Cystic fibrosis (CF) arises from CFTR mutations and is characterized by thick mucus, chronic respiratory infections, and frequent antibiotic use. While antibiotics are indispensable for infection control, their broader effects on the intestinal epithelium remain unclear. We hypothesized that antibiotic exposure alters gut microbial communities and reduces metabolites critical for mucus production, goblet cell function and goblet cell numbers. **Methods:** Fecal samples were collected from three groups: individuals with CF on antibiotics, individuals with CF without recent antibiotic exposure, and non-CF controls. Samples underwent 16S rRNA

sequencing and untargeted metabolomics (LC-MS/MS). Colonic biopsies were analyzed for MUC2 and goblet cell abundance by immunostaining. *In vitro*, mucus-producing intestinal cells and mouse colonic organoids were treated with antibiotics or with supernatants from stool-based bioreactors exposed to antibiotics, and mucus production was measured by qPCR and immunostaining. Mucus stimulation was also analyzed in mucus producing intestinal cell lines exposed to antibiotics via lectin staining of mucus sugars and Alcian Blue staining of acidic mucins. In parallel to the *in vitro* experiments, C57BL/6 mice received oral antibiotics or PBS to assess colonic mucus thickness. **Results:** Microbiome analysis revealed significant shifts in individuals with CF on antibiotics compared to controls and those with antibiotic exposure, including loss of *Anaerostipes*, *Ruminococcus*, *Blautia*, and *Bifidobacterium*. Metabolomics showed antibiotic-associated depletion of amino acids and metabolites that promote mucus production. Biopsies from individuals with CF on antibiotics had fewer MUC2 positive goblet cells and reduced MUC2 fluorescent staining. *In vitro*, antibiotics did not directly suppress mucus production in cells, but supernatants from antibiotic-treated bioreactors failed to stimulate MUC2. Colonic organoids, however, exhibited reduced MUC2 expression and reduced MUC2 staining under antibiotic conditions. *In vivo*, antibiotic-treated mice displayed a thinner colonic mucus layer compared to PBS controls. **Conclusions:** Antibiotic exposure in CF disrupts gut microbial composition and depletes metabolites essential for mucus and goblet cell homeostasis. These findings highlight the importance of antibiotic stewardship in CF individuals.

Session 10: Liver Metabolism and Injury

Abstract 041

The Role of β -catenin-serotonin Signaling Axis in Hepatocyte Reprogramming During Cholestatic Liver Disease

Rithwik Aggarwal, Pamela Cornuet, Matthew D. Carson, Chhavi Goel, and Kari Nejak-Bowen
Organ Pathobiology and Therapeutics Institute, Department of Pharmacology and Chemical Biology, Pittsburgh Liver Research Center, University of Pittsburgh and University of Pittsburgh Medical Center, Pittsburgh, PA

Introduction: Cholestatic liver disease is characterized by disrupted bile flow in accumulation of toxic bile in the liver. Hepatocytes and cholangiocytes arise from the same common progenitor during liver development. Prior research has shown that hepatocyte reprogramming supports hepatobiliary repair. Importantly, the Wnt/ β -catenin pathway has a key role in hepatocyte reprogramming. Serotonin is a tryptophan metabolite that is synthesized in the gut, the brain, and cholangiocytes in the liver. Within the liver, enhanced serotonin signaling has been shown to exacerbate cholestatic injury. Interestingly, crosstalk between the serotonin receptor signaling and the Wnt/ β -catenin pathway has a role in hepatocyte proliferation. Based on prior work, we hypothesize β -catenin signaling is coupled with serotonin signaling to promote hepatocyte reprogramming in cholestasis. **Methods:** Mice were administered 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) to induce cholestasis. Livers were processed for histology and RNA was isolated for qRT-PCR. Murine AML12 hepatocytes were stimulated with serotonin to evaluate the expression of cholangiocyte markers and hepatocyte reprogramming. **Results:** Cholestatic mice had an increase in the number of hepatocytes expressing the cholangiocyte marker A6. DDC treated mice had upregulation of the serotonin signaling receptors *Htr2a* and *Htr2b* as well as the serotonin synthesis enzyme *Tph2*. After stimulating AML12 mouse hepatocytes with serotonin, the expression of *Sox9*, a cholangiocyte marker, was significantly upregulated by 4.5-fold. These findings support the premise that serotonin signaling may be involved in hepatocyte-to-cholangiocyte transdifferentiation. To evaluate the role of β -catenin in serotonin signaling, AML12 cells were transfected with the constitutively active form of β -catenin. Overexpression of β -catenin enhanced serotonin receptor expression. *In vivo* mice expressing constitutively active β -catenin have increased A6+ hepatocytes. Laser capture microdissection of these A6+ cells revealed that in transgenic animals, serotonin receptor signaling was significantly upregulated versus WT A6+ hepatocytes. **Conclusions:** These findings support the premise that β -catenin activation promotes serotonin signaling, which may be key for hepatocyte reprogramming and liver repair in cholestasis. Future studies are needed to decide if the β -catenin-serotonin signaling axis is a viable therapeutic target for biliary repair in cholestasis.

Abstract 042

Zone-Specific β -Catenin Expression Is Required for Maintenance of Liver Zonation and Regeneration After Partial Hepatectomy

Prerna Chakkingal, Chang Kyung (Joanna) Kim, Minakshi Poddar, Sucha Singh, and Satdarshan P. Monga
Organ Pathobiology and Therapeutics Institute, Department of Medicine, Pittsburgh Liver Research Center, University of Pittsburgh and University of Pittsburgh Medical Center, Pittsburgh, PA

Introduction: The adult liver carries out a diverse set of vital functions, including metabolism (fat, protein, carbohydrate, xenobiotic), synthesis (bile, protein, vitamins) and detoxification. Liver zonation defines the spatial division of functions based on the location of cells along the porto-central blood flow and largely divides into three zones: zone 1 (periportal), zone 2 (midlobular), and zone 3 (pericentral). Under various disease conditions, specific zones exhibit differential vulnerabilities, ultimately leading to a disruption of normal liver zonation. Despite recent progress elucidating Wnt/ β -catenin signaling as the master regulator of liver zonation, particularly in maintaining zone 3 hepatocyte identity and function, significant gaps remain with respect to the zone-specific functions of β -catenin at baseline and after partial hepatectomy (PHx), since its upstream effectors Wnt2 and Wnt9b in endothelial cells are expressed in a decreasing gradient from zone 3 to zone 1. **Methods:** To investigate the zone-specific function of β -catenin in liver zonation and in regeneration after PHx, we generated *Gls2-Cre^{ERT2}; Ctnnb1^{fl/fl}* (*Gls2^{ΔCtnnb1}*), *Igfbp2-Cre^{ERT2}; Ctnnb1^{fl/fl}* (*Igfbp2^{ΔCtnnb1}*), and *Cyp1a2-Cre^{ERT2}; Ctnnb1^{fl/fl}* (*Cyp1a2^{ΔCtnnb1}*) mice to delete β -catenin in zone 1, 2 and 3, respectively. Deletion was induced by tamoxifen injection (100mg/kg) of 10–12-week-old mice, and livers were harvested after a 12-day washout period. **Results:** β -catenin deletion in zone 1 hepatocytes in *Gls2^{ΔCtnnb1}* showed an expansion of a midzone marker, cyclin-D1-expressing cells, although another midzone marker, IGFBP2 staining, did not appear to have changed significantly. No apparent changes in staining patterns of CYP2F and CYP2E1 (zone 1 and 3 markers, respectively) were noted. β -catenin deletion zone 2 hepatocytes in *Igfbp2^{ΔCtnnb1}* mice showed a narrower expression pattern of Cyclin-D1 and IGFBP2, indicating reduction of midzone. However, no significant changes were noted in CYP2F and CYP2E1. β -Catenin deletion in zone 3 hepatocytes in *Cyp1a2^{ΔCtnnb1}* mice resulted in loss of glutamine synthetase (GS) and CYP2E1 expression in the 1-3 cell layers around the central vein and gain of ectopic expressions of zone 1 markers E-cadherin and CYP2F2 in these cells. Interestingly, a reduced number of Cyclin-D1-expressing cells was noted in male mice but not in female mice. **Conclusion:** These data suggest that β -catenin regulates zonation of all hepatocytes across the liver lobule.

Abstract 043

The β -catenin/Liver X Receptor Axis Regulates Cholesterol Metabolism and Inflammation in Murine Cholestasis

Ridgeway Case¹, Chhavi Goel¹, Jack Drda¹, Rong Zhang¹, Silvia Liu^{2,4}, Matthew D. Carson², Joseph Locker⁴, Pamela Cornuet², Fu-Ying Qin³, Laura Molina¹, Xiaochao Ma^{3,4}, and Kari Nejak-Bowen^{2,4}

¹Department of Pharmacology and Chemical Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA; ²Organ Pathobiology and Therapeutics Institute (OPTIn), University of Pittsburgh School of Medicine, Pittsburgh, PA; ³Department of Pharmaceutical Sciences, University of Pittsburgh School of Pharmacy, Pittsburgh, PA; ⁴Pittsburgh Liver Research Center, University of Pittsburgh School of Medicine, Pittsburgh, PA

Introduction: Primary sclerosing cholangitis (PSC) can result in retention of toxic bile acids (BA) within hepatocytes leading to cell death, inflammation, and fibrosis. Lithocholic acid (LCA) is a BA associated with adverse clinical outcomes in PSC; however, studies focusing on therapeutic interventions that target this toxic BA are lacking. We hypothesize that β -catenin, which has been shown to regulate BA homeostasis, may reduce LCA-induced cholestasis. **Methods and Results:** Liver-specific β -catenin knockout mice (KO) and wild-type littermates were fed 0.6% LCA supplemented diet for 7 days. KO had a remarkable reduction in bile infarcts and hepatobiliary injury, as shown by histology and serum biochemistry. In the absence of β -catenin, xenobiotic metabolic pathways including pregnane X receptor (PXR) were activated, resulting in decreased serum hydrophobicity in KO. Additionally, loss of β -catenin increased HNF4 α occupancy of the oxysterol-activated liver X receptor (LXR) promoter in KO, resulting in the upregulation of downstream targets that regulate cholesterol metabolism and transport in hepatocytes. Conversely, LXR activation also repressed expression of inflammatory genes, such as IL-33 and ST2, which in turn decreased recruitment of disease-exacerbating type 2 innate lymphoid cells (ILC2). **Conclusions:** PXR- and LXR-mediated processes such as regulation of BA composition and cholesterol metabolism are enhanced by loss of β -catenin during LCA-induced cholestasis. Additionally,

inhibiting β -catenin suppresses the inflammatory response by downregulating the LXR/IL-33/ST2/ILC2 axis. Taken together, these data demonstrate a newly-identified β -catenin-dependent mechanism for attenuating hepatic injury in patients with cholestasis.

Abstract 044

Loss of Cholangiocyte β -catenin Supports Angiogenesis and Hepatocyte Reprogramming through NF- κ B-mediated Signaling during Murine Cholestasis

Matthew D. Carson^{1,2,3}, Jamie Fornasaglio⁴, Laura Molina⁵, Pamela Cornuet¹, Jia-Jun Liu^{1,3}, Silvia Liu^{1,2,3}, and Kari Nejak-Bowen^{1,2,3}

¹Organ Pathobiology and Therapeutics Institute, University of Pittsburgh School of Medicine, Pittsburgh, PA; ²Department of Pharmacology and Chemical Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA; ³Pittsburgh Liver Research Center, University of Pittsburgh and University of Pittsburgh Medical Center, Pittsburgh, PA; ⁴Department of Biology, Seton Hill University, Greensburg, PA; ⁵Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA

Introduction: Cholestatic liver disease arises from impaired bile flow and accumulation of the toxic bile that can lead to end-stage liver disease. Our prior work has demonstrated that hepatocyte β -catenin has a pleiotropic role in cholestatic injury, including regulating hepatocyte-to-cholangiocyte transdifferentiation. However, the role of cholangiocyte β -catenin on cholestasis pathogenesis is unknown. The purpose of this study was to define the role of cholangiocyte β -catenin on hepatobiliary injury responses in mice with cholestasis. **Methods:** Inducible-Osteopontin (OPN)-Cre- β -catenin-floxed C57BL/6 mice were used to delete β -catenin in cholangiocytes in two models of cholestasis. Mdr2 KO- β -catenin-floxed:OPN-Cre (DKO) mice were administered tamoxifen to delete β -catenin from cholangiocytes. Wild-type and cholangiocyte β -catenin KO mice were also administered a 5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) diet for four weeks. Serum was collected to evaluate liver enzymes. Livers were processed for histology, and RNA was isolated to assess injury, angiogenesis, and hepatocyte transdifferentiation. RNA-seq analysis was performed on isolated cholangiocytes. Cholangiocytes were treated with β -catenin siRNA *in vitro* to determine changes in angiogenic factors. Conditioned media from cholangiocytes were used to evaluate endothelial cell expansion *in vitro*. **Results:** Hepatobiliary injury was not exacerbated in mice lacking cholangiocyte β -catenin. Notably, cholangiocyte β -catenin KOs had fibrotic hepatic arteries and increased periportal angiogenesis versus controls. Isolated β -catenin KO cholangiocytes showed increased expression of angiogenesis pathways and genes that were associated with NF- κ B activation. Paralleling RNA-seq findings, *in vitro* silencing of cholangiocyte β -catenin increased the expression of *Vegf* and *Pdgfb*. Stimulated cholangiocyte media from these cells promoted endothelial cell proliferation, recapitulating the angiogenic phenotype found *in vivo*. Immunofluorescent staining showed β -catenin KO mice had an increased number of hepatocytes expressing cholangiocyte markers and ductular cells expressing β -catenin, indicating enhanced hepatocyte reprogramming. **Conclusion:** This work reveals a novel role of cholangiocyte β -catenin in regulating vasculature and hepatocyte reprogramming during cholestasis, which may be critical for preventing accelerated liver injury.

Abstract 045

Imaging Cytokine Profiles Reveal Hepatocyte-Specific Cytokines and Their Potential Role In Chronic Liver Disease

Shadie Shrestha, Arya Chandrashekar, Ramsey Rohner, Kamal Baral, Leah Spade, and Bilon Khambu
Department of Pathology and Laboratory Medicine, School of Medicine, Tulane University, New Orleans, LA

Introduction: Cytokines are essential mediators of inflammation, typically associated with immune cell activity. Yet, the precise origin of cytokine release – both during the early stages of chronic liver disease (CLD) and in its advanced phases marked by cytokine storms – remains poorly understood. The potential role of non-immune cells, especially hepatocytes, in driving this inflammatory response has been largely overlooked. This study aims to uncover hepatocyte-specific cytokine expression and evaluate its contribution to the pathogenesis of CLD. **Methods:** A curated list of 221 cytokines was analyzed using immunohistochemically (IHC) stained images from the Human Protein Atlas (HPA). Cytokine expression was categorized based on localization to hepatocytes, non-hepatocytes, or both. Select cytokines were validated in preclinical CLD mouse models and human liver samples (normal, NAFLD, and cirrhosis) using IHC, Western blotting, and immunofluorescence staining. **Results:** A

screening of 221 cytokines was conducted. 129 cytokines were available in the Human Protein Atlas. The 221 cytokines were classified into the following categories: interleukins, interferons, tumor necrosis factors, transforming growth factor, TGF- β , growth factors, lymphokines, miscellaneous hemopoietins, C-chemokines, CC-chemokines, CXC-chemokines, CX3C-chemokine, CXCR-chemokines, CCR-chemokines, and ACKR-chemokines. Among the 129 hepatic cytokines, 71 were expressed by hepatocytes, 33 by non-hepatocytes, and 24 by both cell types, indicating that cytokines are most prevalent in hepatocytes. Non-hepatocytes and both liver cell types expressed stable levels of certain cytokines, while non-hepatocyte expression increased modestly. Validation of BMP4, IL-17, IL-22, and FGF17 confirmed hepatocyte-specific expression in both mouse and human liver tissues. **Conclusions:** Hepatocytes are active contributors to cytokine production and may play a direct role in liver inflammation and CLD progression. Future studies will investigate the regulatory mechanisms behind hepatocyte cytokine expression and how these profiles evolve across different stages of liver disease.

Abstract 046

CYP2E1 Mediates Glyoxalase-1 Expression

Alexandra A. Tomasevich, Reagan M. Roberts, Kristina M. Stayer, Hyland C. Gonzalez, and Jessica H. Hartman
Department of Biochemistry and Molecular Biology, Medical University of South Carolina, Charleston, SC

Introduction: Cytochrome P450 isoform 2E1 (CYP2E1) is a monooxygenase enzyme that plays an important role in endogenous and xenobiotic metabolism. CYP2E1 is a leaky enzyme that is known to generate reactive oxygen species (ROS) during its catalytic cycle and is associated with the development of liver and intestinal pathologies. CYP2E1 also catalyzes the formation of toxic metabolites such as the reactive aldehyde methylglyoxal (MGO). MGO is associated with metabolic dysfunction as well as the production of advanced glycation end products (AGEs), which promote the production of ROS and contribute to cellular oxidative stress. Main sources of MGO outside of CYP2E1 activity include its formation as a glycolysis byproduct following spontaneous degradation of triose phosphate intermediate, dihydroxyacetone phosphate (DHAP), and glyceraldehyde-3-phosphate (G3P). The glyoxalase system detoxifies MGO with glyoxalase 1 (GLO1) as the rate-limiting enzyme. Further, GLO1 is downstream of nuclear factor erythroid 2-related factor 2 (Nrf2), a key transcription factor in antioxidant response. **Methods:** Mice with germline deletion of CYP2E1 (129/Sv-Cyp2e1tm1Gonz/J, CYP2E1-KO) and wild-type mice (129S1/SvImJ, WT) were used to investigate the relationship between CYP2E1 and the glyoxalase system. RNAseq was used to determine transcriptional levels of GLO1, while western blotting was used to probe protein expression of GLO1 across several tissues. Liver metabolomics data was used to investigate the metabolite S-D-lactoylglutathione which is formed by GLO1. Lastly, LC/MS techniques were used to measure MGO levels in tissues. **Results:** RNAseq in our CYP2E1-KO mice model revealed significant alterations in GLO1 metabolism. This system was investigated in various tissues such as small intestine, kidney, and heart, where an approximate 50% reduction in GLO1 protein expression was observed in CYP2E1-KO mice in all tissue types. Further, metabolomics from CYP2E1-KO livers show a decrease in S-lactoylglutathione that is consistent with the decrease in GLO1 expression. Lastly, preliminary LC/MS data has shown an increase in MGO within CYP2E1-KO livers. **Conclusions:** These findings indicate a previously unappreciated role for CYP2E1 in mitigating MGO toxicity through GLO1 expression – suggesting that CYP2E1 may exert regulatory control over the glyoxalase system and play a role in maintaining oxidative balance through Nrf2 signaling and downstream gene expression.

Session 11: Cancer Molecular Pathways and Therapeutic Strategies in Diverse Cancers

Abstract 047

Fibroblast-Mediated ECM Remodeling and Lymphangiogenic Shift in HPV-Driven Cancer Cells

Harsh Nitin Dongre^{1,2}, Lorena Larios Salazar¹, Neha Rana¹, Rammah Elnour¹, Siren Fromreide¹, Olav K. Vintermyr³, J. Silvio Gutkind⁴, Line Bjørge^{5,6}, Diane R. Bielenberg², and Daniela Elena Costea^{1,3}

¹The Gade Laboratory for Pathology and Centre for Cancer Biomarkers CCBIO, Department of Clinical Medicine, Faculty of Medicine, University of Bergen, Norway; ²Vascular Biology Program, Boston Children's Hospital, Department of Surgery, Harvard Medical School, Boston, MA; ³Department of Pathology, Haukeland University Hospital, Bergen, Norway; ⁴Moore's Cancer Centre, University of California, San Diego, La Jolla, CA; ⁵Centre for Cancer Biomarkers CCBIO, Department of Clinical Science, Faculty of Medicine, University of Bergen, Norway

Introduction: The incidence of human papillomavirus-associated (HPV+) mucosal cancers is rising, and these are characterized by a high propensity for lymph node metastasis. Metastatic spread is strongly influenced by cancer-associated fibroblasts (CAFs), who play a pivotal role in driving disease progression across various mucosal cancer types, including those of the head and neck and vulva. While recent research has begun to elucidate the role of CAFs in HPV-independent mucosal cancers, their contribution to HPV+ tumor biology remains poorly understood. **Methods:** Oral (OSCC1) and vulvar (UMSCV4) cancer cell lines were transfected with HPV E6 and E7 oncoproteins and subsequently co-cultured with carcinoma-associated fibroblasts (CAFs) derived from matched oral and vulvar mucosal tissues. To investigate transcriptional changes, bulk RNA sequencing and quantitative PCR (qPCR) were performed to identify differentially expressed genes. Protein-level alterations were assessed using multiplex cytokine profiling and western blot analysis. The impact of CAF-conditioned media on lymphangiogenesis was evaluated via Matrigel-based lymph-endothelial tube formation assays. Finally, co-cultures were implanted into immunodeficient nude mice to examine tumor growth dynamics *in vivo*. **Results:** CAFs co-cultured with HPV E6/E7-transfected cancer cells exhibited elevated gene and protein expression of hepatocyte growth factor (HGF), a key mediator of angiogenesis, compared to CAFs paired with wild-type (WT) cancer cells. In turn, E6/E7-transfected cancer cells co-cultured with CAFs showed upregulated gene and protein expression of vascular endothelial growth factor-C (VEGF-C) and Neuropilin-2, relative to their WT counterparts. Functionally, E6/E7 cells promoted the formation of more stable lymph-endothelial tube structures *in vitro* in the presence of CAFs, demonstrated enhanced invasiveness in 3D organotypic cultures, and generated larger, progressively growing tumors when co-implanted with CAFs *in vivo*, compared to WT cancer cells. **Conclusions:** CAFs enhance the invasive and lymphangiogenic potential of HPV E6/E7-transfected cancer cells through reciprocal signaling involving HGF and VEGF-C. These interactions promote aggressive tumor behavior both *in vitro* and *in vivo*, underscoring the stromal contribution to HPV+ cancer progression.

Abstract 048

Microbes, Methylation, and Motors: *Acinetobacter*'s Role in Silencing Rab8A in Colorectal Cancer

Rachel Edens-Valentine, Sarah A. Dooley, Melinda A. Engevik, and Amy C. Engevik

Department of Regenerative Medicine and Cell Biology, Medical University of South Carolina, Charleston SC

Introduction: Colorectal cancer (CRC) ranks among the most common and deadliest cancers worldwide. Previous studies demonstrate that the molecular motor Myosin 5b (MYO5B) is decreased in CRC. In normal cells, MYO5B relies on the binding partner Rab8A to successfully traffic cargo. However, the role of Rab8A in CRC remains underexplored. **Methods:** We examined RNA, methylation, and protein levels of MYO5B and Rab8A in human colon tissue, cancer cell lines, and human colonic organoids. Cells were treated with a DNA-demethylating agent to examine the role of methylation in regulating MYO5B and Rab8A levels. Additionally, we examined bacterial population correlations with Rab8A in CRC and found that *Acinetobacter*, a bacteria known to be increased in CRC patients, was negatively correlated with Rab8A expression in CRC tumors. Accordingly, we treated human colonoids and cancer cell lines with *Acinetobacter* to evaluate alterations in Rab8A *in vitro*. **Results:** We found that Rab8A was reduced at the protein and gene level in CRC compared with controls across patient and tumor phenotypes. We observed hyper-methylation of MYO5B and Rab8A in CRC and found treatment of cancer cells with a demethylating compound increased gene expression. Treatment of healthy colonoids and cancer cell lines with *Acinetobacter* increased methylation and decreased Rab8A expression. **Conclusion:** Our findings identify alterations in Rab8A in CRC and uncover a novel relationship between *Acinetobacter* and Rab8A in the colon. Our data suggests increased *Acinetobacter* may contribute to the hyper-methylation and resulting silencing of Rab8A in CRC impacting MYO5B function.

Abstract 049

Mechanistic Investigation of Mutated β -Catenin Regulation of POU2F1/OCT1 in Hepatocellular Carcinoma

FNU Shahjahan¹, Junyan Tao^{1,2}, and Satdarshan P. Monga^{1,2}

Introduction: Hepatocellular carcinoma (HCC) represents roughly 90% of all primary liver cancers and is the fourth leading cause of cancer-related mortality worldwide. Between 30 – 40% of HCC cases harbor gain-of-function mutations in the CTNNB1 gene, which encodes β -catenin, causing abnormal activation of the Wnt/ β -catenin signaling pathway. In both human HCC and mouse models, CTNNB1-driven β -catenin activation is closely associated with an immune-excluded tumor microenvironment (TME) marked by impaired T-cell recruitment and poor response to immune checkpoint inhibitors. Mutated β -catenin driven HCC mouse models mimic this immune-cold TME. However, the relationships between β -catenin and immune tolerance of tumor initiation are not fully understood. **Methods:** We analyzed liver tissue from mutated β -catenin HCC mice and wild-type controls using western blotting to assess OCT1/POU2F1 expression. We performed co-immunoprecipitation (co-IP) to test for direct interaction between mutated β -catenin and OCT1. **Results:** Western blot analysis showed no detectable OCT1 protein in livers from mutated β -catenin HCC mice compared with wild-type mice. Co-IP experiments did not reveal a direct physical interaction between mutated β -catenin and OCT1 in HCC livers, suggesting that the observed repression of OCT1 by mutated β -catenin may be indirect. **Conclusions:** Our findings indicate that mutated β -catenin may regulate OCT1/POU2F1 indirectly rather than through direct binding. We are expanding these studies by performing additional co-IP experiments to identify potential bridging proteins and by employing luciferase reporter assays to quantify OCT1 transcriptional activity in HCC cell lines with mutated β -catenin expression. Together, these approaches will clarify the mechanisms of β -catenin regulation of POU2F1 in HCC and may inform strategies to convert an immune-cold TME into an immune-hot TME.

Abstract 050

Resensitizing Therapy-resistant Anoxic Metastatic Melanoma Cells

Daryl Forney¹, Mariella Andrews², Tejaswini Kannan¹, Gustavo Untiveros², and Luigi Strizzi^{1,2}

¹College of Osteopathic Medicine, and ²College of Graduate Studies, Department of Pathology, Midwestern University, Downers Grove, IL

Introduction: Metastatic melanoma significantly reduces patient survival. Metastatic growth can outpace vascularization creating areas of extreme hypoxia or anoxia. HIF-1 α is a master transcriptional regulator of cellular survival response to hypoxia that can facilitate the selection of more stem cell-like, drug-resistant cancer cells. BRAF inhibitors (BRAFi), commonly used to treat melanoma, target cell signaling involving pERK-dependent melanoma growth. Resistance to BRAFi can lead to melanoma recurrence and poor survival. Dual specificity phosphatases (DUSP), such as DUSP6 can reduce ERK activity; moreover, DUSP6 has been shown to be upregulated by HIF-1 α during cellular responses to hypoxia. We hypothesize that HIF-1 α levels increase in metastatic melanoma cells as these adapt to the anoxic metastatic niche, which then increases DUSP6 expression, which in turn reduces ERK expression that causes resistance to BRAFi. **Methods:** Western blot (WB) was used to compare the expression of HIF-1 α , pERK, and DUSP6 in lysates from A375 melanoma cells grown in anoxia or normoxia. An MTT cell proliferation assay was used to compare the effect of the BRAFi, dabrafenib between treated normoxic and anoxic A375 cells. Lysates were collected for WB to see effects of pERK between anoxic A375 treated with the DUSP6 inhibitor, BCI and untreated control. Finally, to see if BCI can increase drug sensitivity, MTT assay was performed on anoxic A375 treated with both BCI and dabrafenib. **Results:** WB results show that anoxic A375 cells express higher levels of HIF-1 α , and lower levels of pERK compared to normoxic control cells. Dabrafenib treatment reduced cell proliferation of normoxic but not anoxic A375 cells presumably due to reduced expression of the pERK target in these cells. Inhibition of DUSP6 with BCI significantly increased pERK expression and when combined with dabrafenib significantly reduced proliferation of anoxic A375 cells. **Conclusion:** We show that melanoma cells cultured in anoxia become resistant to dabrafenib as a result of HIF1 α -associated increase in DUSP6 expression. In these anoxic cells, DUSP6 reduces pERK level, which is an important component of the signaling cascade targeted by dabrafenib. Finally, our results from the combination treatment with both BCI and dabrafenib suggest that by inhibiting DUSP6 it may be possible to re-express pERK in drug-resistant anoxic metastatic melanoma cells and reestablish sensitivity of these cells to dabrafenib.

Abstract 051

Understanding the Consequences of P2Y1 Purinergic Signaling in Colorectal Cancer

Ana G. Pettijohn, and Kristen A. Engevik

Department of Regenerative Medicine and Cell Biology, Medical University of South Carolina, Charleston SC

Introduction: Colorectal cancer (CRC) remains a leading cause of cancer-related morbidity and mortality, with a projected ~150,000 new cases in the US in 2025. Emerging evidence indicates extracellular purines and subsequent purinergic signaling play a role in cancer. Of the 15 purinergic P2 receptors, healthy gut epithelium has functional P2Y1 and P2Y2 receptors while CRC derived cell lines lack functional P2Y receptors suggesting CRC may have altered purinergic receptor profile. While purinergic receptor activation by ATP addition can inhibit cancer growth, the purinergic receptor expression and downstream consequences of purinergic receptor activation in CRC remain poorly understood. We hypothesize that gut epithelial purinergic receptors are altered in CRC and could be potential therapeutic targets. **Methods:** *In silico* analysis of RNA sequencing data from The Cancer Genome Atlas (TCGA) was used to assess changes in purinergic receptor expression in CRC tumors versus normal tissue. We performed P2Y agonist treatments in cancer derived cell lines T84 and LoVo cells expressing the calcium indicator GCaMP6s. To assess the functional consequences of P2Y1 in cancer, we transduced T84 cells to knock-in P2Y1. Live imaging microscopy of agonist treatment and migration (via scratch assay) were done to compare parental T84 and P2Y1 knock-in T84 GCaMP6s cells. **Results:** TCGA RNA-seq data analysis showed CRC tumors have significant decreases in P2Y1 compared to normal tissue. We observed significant decreases in P2Y1 regardless of patient sex, age, weight, stage or type. Regardless of P2 receptor expression, T84 and LoVo cells had weak to no calcium signaling response following P2Y activation, indicating impaired purinergic signaling. Reintroduction of P2Y1 into T84 cells led to altered morphology and growth. Interestingly, P2Y1 knock-in T84 cells exhibited delayed cell proliferation compared to parental T84 cells. Furthermore, in a scratch assay, parental T84 cells successfully exhibited full wound closure within 24 hours, while P2Y1 knock-in T84 cells failed to fully close. **Conclusion:** Collectively this data suggests that purinergic signaling downregulation may play a role in the colorectal cancer phenotype and that pharmacologic modulation of this pathway could provide a novel therapeutic strategy for CRC treatment.

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